



DOUTORAMENTO EM CIÊNCIAS FARMACÊUTICAS

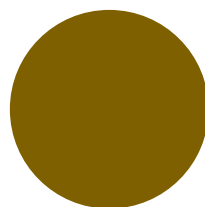
ESPECIALIZAÇÃO EM NUTRIÇÃO E QUÍMICA DOS ALIMENTOS

Encapsulation of Extracts of Plants and by-products on Lipid-based Nano/micro Carriers

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PhD Thesis co-supervision (Cotutelle) between the University of Porto and the
Shahid Beheshti University

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Shahid Beheshti University
Medicinal Plants and Drugs Research Institute

*“The knowledge of anything, since all things have causes, is not acquired or complete
unless it is known by its causes.”*

*“When you do not know the nature of the malady, leave it to nature; do not strive to
hasten matters. For either nature will bring about the cure or it will itself reveal clearly
what the malady really is.”*

Avicenna

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Abstract

Plant-derived compounds, as well as food by-products, can have a significant biological activity, but weak bioavailability or low stability may limit their effective applications. This PhD aimed to develop techniques to obtain polyphenol-enriched extracts from plants and food by-products and encapsulate them in lipid-based nano/micro carriers, with or without modifying the lipidic surface. More specifically, after samples extraction and enrichment of crude extracts based on polyphenolic compounds, encapsulation through phytosome and lipid surface modification was carried out.

This approach is justified by the large volume of by-products obtained and discarded by food and agricultural industries, leading to environmental concerns. Using food by-products and converting them into high-value products, developing and using green and ecologically correct technologies, is a challenge for all and an unavoidable attitude. The use of these by-products can have a significant impact at economic level, in waste reduction, and allows obtaining new nutrient-rich extracts or functionalized compounds, back to the worldwide market. These new products can be useful on food, pharmaceuticals or cosmetic industries. Fruits and vegetables, cereals, and coffee are major waste generators of food industries, with significant amounts of high-value phytoconstituents.

Fractionation of crude extracts by polymeric adsorbent exchange-column chromatography methods promoted their enrichment in bioactive compounds based on functionality and group categorization of phytoconstituents. The enriched fractions were then evaluated by HPLC, GC-MS, and spectrophotometry, aiming to know their main phytoconstituents. These enriched fractions can function as sources of compounds for natural supplements, food additives or drug candidates, playing a critical role in human health and safety.

Taking into account the poor bioavailability, low stability and low absorption rates of some phytoconstituents, a pertinent choice are natural-based carriers for safe and target delivery of such compounds/extracts, without forgetting their inherent properties. The lipid-based nano/microcarriers, due to their similarity to cell membranes, considerably improve the cell barrier permeation by phytoconstituents. Nevertheless, their low stability can limit their functionality.

Surface modifications of lipid-based carriers considerably improves the compounds permeability through cell barriers (permeation) and enhances the solubility and stability of phytoconstituents. Then, to develop such surface modifications in these carriers, novel

techniques should be established, based on green chemistry, to design appropriate carriers for both hydrophilic and lipophilic phytoconstituents.

In this PhD thesis, an overview of two decades of liposome surface modifications is presented, focusing phytosome recent advancements. Liposome/phytosome approaches and their bilayers diversity are discussed, and novel recent attempts are presented. Several functionalities from food and dietary supplements to drugs, genetics, and vaccination immunology were attained.

In another chapter, recent improvements to control the impact of drying techniques on morphology and texture of phytoconstituents and nutrients, controlled/sustained release, and storage stability were ascertained. It is discussed how a drying technique extends the food products shelf-life, by reducing trapped water and inhibiting microorganisms and enzymes. Moreover, recent advances in drying technologies such as hybrid drying (a combination of different drying techniques), and spray drying are introduced as promising techniques to improve the morphology and texture of dried materials, preserve them, and increase their storage time.

Natural polymers were selected to protect the lipid carriers, enhance stability of phytoconstituents, and decrease the necessary dose, due to a sustained and controlled release profile. Therefore, these putative structures could ingeniously be used for various applications, from food ingredients and supplements to treatments, chemotherapy (capsule, soft gel, serum), prevention therapies (like in vaccines against pathogens), and in beauty and skincare products (cream, shampoo, lotion, etc.).

Therefore, to follow the aim, different formulations were evaluated with special attention to nano-emulsions (lipid-based nanoparticles), phytosomes, cholesterol incorporating phytosomes and coating using natural polymers (encapsulation by spray dryer). All produced particles, in powder or emulsion, were evaluated regarding their physiochemical properties (particle size, zeta potential, surface morphology by SEM and TEM). The *in vitro* release profile was evaluated with dissolution assays and the results were analysed using mathematical models for release profile prediction, such as zero-order, first-order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas, and Weibull. Furthermore, biological assays in terms of antimicrobial and antitumoral activities were evaluated in some studies. Finally, the *in silico* molecular arrangement of phytoconstituents within the lipid bilayer was confirmed based on molecular docking.

Chapter three focuses on the formulation of functionalized phospholipidic multi-component nanoemulsions (using *Eucalyptus globulus* and *Mentha piperita* essential

oils) with a significant antibacterial, aiming to replace synthetic preservatives. The optimized formula had a particle size of 93-100 nm, confirmed by DLS and TEM images, showing high stability for 730 days. The Korsmeyer-Peppas model clarified the release of phytoconstituents. The mass transfer followed Phase I (Fickian models). The results showed a significant reduction in MIC values, with a 32-, 250-, and 15-fold activity enhance against *E. coli*, *S. aureus* and *S. enteritidis*, respectively, compared to an original mixture of essential oils. In conclusion, this multi-component phospholipid-based nanoemulsion could be a promising candidate to be used as a natural-based food preservative.

Chapter four explores *Gunnera tinctoria* based on its nutritional, antioxidant, and antitumoral properties. Higher antioxidant activity was observed in baby leaves, followed by adult leaves and inflorescences. A phenolic extract was enriched using adsorbent resin column chromatography (DiaionTM HP20LX and Relite EXA90). Antitumoral effects were observed with the enriched fractions in breast (MCF-7) and pancreatic (AsPC-1) cancer cell lines, being more pronounced in the latter. To improve protection and membrane absorption rates of phenolic compounds, nano-phytosomes and cholesterol-conjugated phytosomes coated with natural polymers were loaded with the enriched fraction. In addition, the molecular arrangement of phenolics within both types of phytosomes were studied, for the first time, by molecular docking. Polarity and molecular size were found to be key factors on the molecular arrangement of the lipid bilayer.

Chapter five focuses on coffee silverskin, a major by-product from coffee roasting industry, rich in compounds with health benefits. Silverskin extracts were enriched in caffeine and chlorogenic acid, by adsorbent column chromatography, and contents were increased 3.08 and 2.75-fold, respectively. The enriched fractions were loaded in nano-phytosomes, and these structures were conjugated with cholesterol to improve the permeation rate. Subsequently, these nano-lipid carriers were coated with different natural polymers to improve protection and compounds stability. The produced particles were evaluated regarding product yield, encapsulation efficiency, loading capacity, particle size, surface charge, and *in vitro* release. All samples exhibited anionic surface charge. FTIR and molecular docking confirmed the interactions between phytoconstituents and lipid bilayers. The best docking score observed for chlorogenic acid exhibited stronger hydrogen bonding, resulting in stronger interaction. The release mechanism fits well with Korsmeyer-Peppas and Higuchi kinetic models. Moreover, most

of the formulated particles revealed to follow the diffusion-Fick law or an anomalous diffusion.

As a conclusion, the data elicited by molecular docking demonstrated a good interaction between the phytoconstituents and the phospholipic bilayer. Moreover, the stability of lipid carriers was considerably enhanced due to natural polymers coating in acidic media, during storage, and handling. They also showed a sustained and controlled release profile, allowing a decrease in the phytoconstituents required dose.

Keywords: Encapsulation; liposome; phytosome; surface modification; drying technologies; *Gunnera tinctoria*; coffee silverskin; by-product; nanoemulsion.

Resumo

Os compostos derivados de plantas, bem como os subprodutos alimentares, podem ter uma actividade biológica significativa, mas uma fraca biodisponibilidade ou baixa estabilidade podem limitar o seu potencial de aplicação. Este doutoramento visou desenvolver técnicas para obter extratos enriquecidos com compostos fenólicos extraídos de plantas e subprodutos alimentares e encapsulá-los em nano/microtransportadores à base de lípidos, com ou sem modificação da superfície lipídica. Mais especificamente, após a extração das amostras e o enriquecimento dos extratos em compostos fenólicos, efetuou-se o seu encapsulamento através de fitosomas e modificação da superfície lipídica.

Esta abordagem justifica-se pelo grande volume de subprodutos obtidos e descartados pelas indústrias alimentares e agrícolas, o que leva a preocupações ambientais. A utilização de subprodutos alimentares e a sua conversão em produtos de elevado valor, desenvolvendo e utilizando tecnologias verdes e ecologicamente correctas, é um desafio para todos e uma atitude inevitável. A utilização destes subprodutos pode ter um impacto significativo a nível económico, na redução de resíduos, e permite a obtenção de novos extratos ricos em nutrientes ou compostos funcionalizados, trazendo-os de volta ao mercado mundial. Estes novos produtos podem ser úteis nas indústrias alimentar, farmacêutica ou cosmética. Frutas e vegetais, cereais e café são grandes produtores de resíduos nas indústrias alimentares, que apresentam quantidades significativas de fitoconstituintes de valor acrescentado.

O fracionamento de extratos brutos por métodos cromatográficos com adsorventes poliméricos promoveu o seu enriquecimento em compostos bioativos com base na funcionalidade e categorização dos grupos dos fitoconstituintes. As frações enriquecidas foram então analisadas por HPLC, GC-MS e espectrofotometria, com o objetivo de identificar os seus principais constituintes. Estas frações enriquecidas podem funcionar como fontes de compostos para suplementos naturais, aditivos alimentares ou candidatos a fármacos, desempenhando um papel crítico na saúde e segurança humana.

Tendo em conta a fraca biodisponibilidade, baixa estabilidade e baixas taxas de absorção de alguns fitoconstituintes, uma escolha pertinente são os transportadores à base de produtos naturais para a entrega segura e orientada de tais compostos/extratos, sem esquecer as suas propriedades inerentes. Os nanos /microtransportadores de base lipídica, devido à sua semelhança com as membranas celulares, melhoram

consideravelmente a permeação dos fitoconstituintes através das barreiras celulares. No entanto, a sua baixa estabilidade pode limitar a sua funcionalidade.

As modificações de superfície desses transportadores à base de lípidos melhoram consideravelmente a permeabilidade dos compostos através da barreira celular (permeação) e aumentam a sua solubilidade e estabilidade. Assim, para efetuar tais modificações de superfície, devem ser desenvolvidas novas técnicas, baseadas em química verde, de forma a obter transportadores adequados tanto para compostos hidrofílicos como lipofílicos.

Nesta tese de doutoramento, é apresentada uma visão global de duas décadas de modificações superficiais de lipossomas, focando nos recentes avanços fitossômicos. São discutidas diferentes abordagens sobre lipossomas/fitossomas e a diversidade de bicamadas e apresentados os desenvolvimentos mais recentes. Foram alcançadas várias funcionalidades, desde alimentos e suplementos alimentares até medicamentos, genética e imunologia vacinal.

Noutro capítulo, apresentam-se as melhorias recentes no controlo do impacto das técnicas de secagem na morfologia e textura dos fitoconstituintes e nutrientes, na libertação controlada/sustentada, e na estabilidade durante o armazenamento. Discute-se como uma técnica de secagem prolonga a validade dos produtos alimentares, reduzindo a água retida e inibindo os microrganismos e enzimas. Além disso, tecnologias recentes de secagem, tais como a secagem híbrida (uma combinação de diferentes técnicas de secagem) e o *spray drying*, são introduzidas como técnicas promissoras para melhorar a morfologia e a textura dos materiais secos, preservá-los e aumentar o seu tempo de armazenamento.

Diferentes polímeros naturais foram selecionados para proteger os transportadores lipídicos, aumentar a estabilidade dos fitoconstituintes e diminuir a dose necessária, devido a um perfil de libertação sustentada e controlada. Estas estruturas poderão, assim, ser utilizadas para várias aplicações, como ingredientes alimentares, suplementos, ou até para tratamentos, quimioterapia (cápsula, gel suave, soro), terapias de prevenção (como em vacinas contra agentes patogénicos), e em produtos de beleza e de cuidado da pele (creme, champô, loção, etc.).

Portanto, para seguir este objectivo, diferentes formulações foram avaliadas dedicando especial atenção às nano-emulsões (nanopartículas de base lipídica), fitosomas, fitossomas contendo colesterol e revestimentos usando polímeros naturais (encapsulamento por *spray drier*). Todas as partículas produzidas, em pó ou emulsão,

foram avaliadas quanto às suas propriedades físico-químicas (tamanho da partícula, potencial zeta, morfologia superficial por SEM e TEM). O perfil de libertação *in vitro* foi avaliado com ensaios de dissolução e os resultados foram analisados utilizando modelos matemáticos para a previsão do perfil de libertação, tais como: ordem zero, primeira ordem, Higuchi, Hixson-Crowell, Korsmeyer-Peppas e Weibull. Além disso, em alguns estudos foram realizados ensaios biológicos de atividade antimicrobiana e antitumoral. Finalmente, confirmou-se a disposição molecular *in silico* dos fitoconstituintes no interior da bicamada lipídica com base no acoplamento molecular.

O capítulo três foca-se na formulação de nanoemulsões fosfolipídicas multicomponentes funcionalizadas (utilizando óleos essenciais de *Eucalyptus globulus* e *Mentha piperita*) com actividade antibacteriana significativa, tendo como objetivo a substituição de conservantes sintéticos. A fórmula otimizada apresentou uma granulometria de 93-100 nm, confirmada por imagens de DLS e TEM, mostrando uma estabilidade elevada durante 730 dias. O modelo Koresmeyet-Pepps clarificou a libertação dos fitoconstituintes. A transferência de massa seguiu a Fase I (modelos Fickian). Os resultados mostraram uma redução significativa nos valores de MIC, com um aumento de actividade de 32, 250 e 15 vezes contra *E. coli*, *S. aureus* e *S. enteritidis*, respetivamente, em comparação com uma mistura original de óleos essenciais. Em conclusão, esta nanoemulsão multicomponente à base de fosfolípidos poderia ser um candidato promissor para ser usado como conservante alimentar de base natural.

O capítulo quatro explora a *Gunnera tinctoria* com base nas suas propriedades nutricionais, antioxidantes e antitumorais. Foi observada uma maior atividade antioxidante nas folhas bebé, seguida das folhas adultas e inflorescências. Um extrato fenólico foi enriquecido utilizando cromatografia de coluna de resina adsorvente (DiaionTM HP20LX e Relite EXA90). Foram observados efeitos antitumorais com as fracções enriquecidas nas linhas de células de cancro da mama (MCF-7) e do pâncreas (AsPC-1), sendo mais pronunciados nestas últimas. Para melhorar a proteção e as taxas de absorção dos compostos fenólicos, nanofitosomas e fitossomas conjugados com colesterol revestidos com polímeros naturais foram carregados com a fração enriquecida. Além disso, a disposição molecular dos compostos fenólicos dentro dos dois tipos de fitossomas foi estudada, pela primeira vez, com base no acoplamento molecular. Verificou-se que a polaridade e o tamanho molecular eram fatores chave na disposição molecular da bicamada lipídica

O capítulo cinco foca-se na pele de prata do café, o subproduto da indústria de torrefacção do café, rico em compostos com benefícios para a saúde. Os extratos de

pele de prata foram enriquecidos por cromatografia em coluna adsorvente, tendo em conta os teores de cafeína e ácido clorogénico, obtendo-se aumentos de 3,08 e 2,75 vezes, respetivamente. As frações enriquecidas foram carregadas em nanofitosomas, e estas estruturas foram conjugadas com colesterol para melhorar a taxa de permeação. Subsequentemente, estes nanotransportadores lipídicos foram revestidos com diferentes polímeros naturais para melhorar a proteção e a estabilidade dos compostos. As partículas produzidas foram avaliadas relativamente ao rendimento do produto, eficiência do encapsulamento, capacidade de carga, tamanho das partículas, carga superficial e libertação *in vitro*. Todas as amostras exibiram carga superficial aniónica. As interações entre os fitoconstituintes e a bicamada lipídica foram confirmadas por FTIR e acoplamento molecular. O melhor valor de ancoragem foi observado para o ácido clorogénico que exibiu uma ligação de hidrogénio mais forte, resultando numa interação mais forte. O mecanismo de libertação corresponde aos modelos cinéticos Korsmeyer-Pepps e Higuchi kinetic. Além disso, a maioria das partículas formuladas revelaram seguir a Lei de Fick ou uma difusão anómala.

Como conclusão, os dados obtidos através do acoplamento molecular demonstraram uma boa interação entre os fitoconstituintes e a bicamada fosfolípica. Além disso, a estabilidade dos transportadores lipídicos foi consideravelmente reforçada devido ao revestimento de polímeros naturais, em meios ácido, e durante o armazenamento e manuseamento. Também mostraram um perfil de libertação sustentada e controlada, permitindo uma diminuição da dose necessária de fitoconstituintes.

Palavras-chave: Encapsulação; lipossoma; fitossoma; modificação da superfície; tecnologias de secagem; *Gunnera tinctoria*; pele de prata do café; subproduto; nanoemulsão.

چکیده

صنایع غذایی، کشاورزی، و مشتقات گیاهی منبع تولید حجم وسیعی از زباله های جهان را به خود اختصاص می دهند . استفاده مناسب از زباله های جانبی محصولات غذایی و تبدیل به محصولات غذایی و دارویی با کیفیت بالا توسط روش های وابسته به شمی سبز یکی از نیازهای اخیر روز دنیا به شمار می رود . پروژه دکترا فوق با هدف توسعه تکنیک های نوین به منظور تولید عصاره غنی شده پلی فنول از گیاهان و مواد غذایی و کپسوله کردن آن ها را در ساختار نانو/میکرو حامل های لیپیدی و در نهایت پوشش ساختارهای فیتوزمی توسط پلیمرهای زیستی انجام شد.

از این رو، حجم زیادی از محصولات جانبی به دست آمده و از مواد غذایی و صنایع کشاورزی که منجر به نگرانی های زیست محیطی می شود توجه و در فرم محصول غذایی با کیفیت بالا وارد بازار جهانی می شود. استفاده از محصولات فرعی مواد غذایی (زباله های غذایی) و تبدیل آنها به محصولات با ارزش بالا، توسعه و استفاده از فن آوری های سبز و زیست محیطی، یک چالش برای همه و نگرشی اجتناب ناپذیر است. استفاده از این محصولات جانبی می تواند تاثیر قابل توجهی در سطح اقتصادی و کاهش ضایعات داشته باشد روش فوق اجازه می دهد تا عصاره های غنی از مواد مغذی جدید با ترکیبات کاربردی و جدید به بازار جهانی بازگردانند شود. این محصولات جدید می تواند در مواد غذایی، دارویی یا صنایع آرایشی و بهداشتی مفید باشد .

میوه ها، سبزیجات، دانه ها، قهوه و غلات به عنوان دومین تامین کننده بزرگ زباله های غذایی در بردارنده غلظت بالایی از ترکیبات طبیعی فعال با ارزش دارویی و غذایی بالایی هستند . غنی سازی عصاره حاصل از زباله های غذایی بر مبنای نوع و غلظت ترکیبات موثره توسط تکنیک های کروماتوگرافی بر پایه پلیمرهای جذب سطحی، شرايطی را فراهم می کند که ترکیبات موثره حاصل از بازیافت زباله های غذایی در فرم قابل ارائه و با کیفیت بسیار بالا، دوز و اثرات جانبی کمتر در فرم محصولی جدید به بازار جهانی ارائه شود . بدین منظور عصاره تام این ترکیبات بر اساس نوع ماده موثره و نوع محصول نهایی غنی شده و توسط تکنیک های معمول HPLC, GC_MS و آزمون های عصاره تام فنی، و فلاونوئیدی مورد بررسی قرار گرفت. عصاره غنی شده ترکیبات موثره گیاهی و غذایی را میتوان به عنوان منبع مناسب غذایی و یا ماده اولیه دارویی در صنایع مختلف دارو، مکمل های خوراکی و افزونده های غذایی در نظر گرفت.

با در نظر گرفتن زیست فراهمی، شدت جذب و پایداری بسیار کم ترکیبات طبیعی، استفاده از حامل های با منبع طبیعی و زیست پذیر با اعضای بدن به منظور دارورسانی هوشمند ضروری و لازم می باشد . نانو /میکرو حامل های لیپیدی به علت شباهت های قابل توجه بین غشاهای سلولی به طور قابل ملاحظه ای به افزایش فراهمی زیستی و جذب (نفوذ) ترکیبات طبیعی کمک می کند. بدین منظور به عنوان روشی نوین در فرمولاسیون ترکیبات طبیعی استفاده می شود

بدلیل پایین بودن پایداری حامل های لیپیدی و اصلاح سطح و ساختار این حامل ها، روشی مناسب به منظور افزایش تاثیر پذیری این ترکیبات ارائه شد . اصلاح ساختار حامل های لیپیدی بر اساس تکنیک انکپسولاسیون و بر مبنای شیمی سبزی برای هردو دسته ترکیبات آبگریز و آبدوست استفاده می شود . اصلاح حامل های لیپیدی باعث پایداری ترکیبات طبیعی در محیط شیمیایی بدن و همچنین در انبارداری و عرضه می شود.

اصلاح حامل های لیپیدی (پوشش ساختارهای فیتوزمی توسط پلیمرهای زیستی) به طور قابل توجهی باعث افزایش نفوذپذیری ترکیبات از طریق موانع سلولی (نفوذ) و افزایش حلالیت و پایداری ترکیبات می باشد. بدین منظور تکنیک های جدید بر اساس شیمی سبز برای هر دو دسته ترکیبات آب دوست و آب گریز پایه گذاری شد.

در مقاله مروری اول، اصلاح سطح لیپوزوم دو دهه گذشته و با تمرکز بر پیشرفت های اخیر فیتوزوم (لیپوزم بر پایه ترکیبات طبیعی) مورد بررسی قرار گرفت . در این پژوهش ساختارهای اصلاح شده بحث می شود . علاوه بر این، کاربرد

این ساختارها در مواد غذایی، دارویی، و مکمل‌های غذایی مورد بحث و بررسی قرار گرفت. همچنین کاربرد ساختارهای فوق در صنایع دارویی، ترکیبات طبیعی، ژن درمانی و واکسیناسیون و ایمنی مورد مطالعه قرار گرفت.

در مقاله مروری دوم، مطالعات اخیر بر روی تأثیرات سیستم‌های خشک کن صنعتی بر کیفیت محصول غذایی از لحاظ شکل ظاهری، تخلخل، رهائش و آزاد سازی کنترل‌شده و پایداری در انبارداری و عرضه مورد بررسی قرار گرفت. از این رو چگونگی تأثیرات دستگاه خشک کن به کیفیت و نیمه عمر محصول نهایی، از طریق کاهش رطوبت باقی مانده در ماده غذایی بررسی شد. با این حال از تکنولوژی‌های اخیر صنایع خشک کن‌های غذایی به عنوان مثال: خشک کن هیبریدی (ترکیب چند نوع دستگاه خشک کن) و خشک کن پاششی، به عنوان روش های قطعی با عملکرد بالا به منظور بهبود ساختار و کیفیت در محصولات غذایی یاد شد.

و در نهایت به نقش پلیمرهای طبیعی بر سلامت انسان و نقش اساسی این ترکیبات در فرایندهای دارورسانی نوین (بالخصوص انکپسولاسیون توسط خشک کن پاششی) بحث شد. ترکیبات مناسب و پرکاربرد در انواع فرمولاسیون‌های دارویی و همچنین به عنوان پوشش در حامل‌های لیپیدی شناخته می‌شوند. پلیمرهای طبیعی به عنوان ساختارهای با پتانسیل بالا در صنایع غذایی، دارویی، مکمل‌های غذایی و دارویی به منظور ساخت کپسول، کپسول‌های ژلاتینی، سرم و ... استفاده می‌شود. همچنین، این ترکیبات در فرمولاسیون های داروهای وابسته در پیشگیری، درمان و حتی واکسیناسیون علیه پاتوژن‌های انسانی و محصولات مراقبت از پوست و مو و زیبایی مانند کرم، شامپو، لوسیون و غیره به طور عمده استفاده می‌شوند.

بنابراین، بر مبنای تعارف لحاظ شده در بالا فرمولاسیون‌های مختلف با کاربرد غذایی و دارویی مثل نانوامولسیون، نانو ذرات لیپیدی پوشش داده شده توسط پلیمرهای طبیعی (فیتوزم و فیتوزم اصلاح شده توسط کلسترول) ساخته شده. نانو/ میکرو ذرات ساخته شده بر اساس خصوصیات فیزیوشیمیایی از قبیل اندازه ذره، بار سطح، مورفولوژی سطح مورد بررسی قرار گرفت. رهائش ترکیبات طبیعی در شرایط *In-vitro* بررسی و نمودارهای حاصل توسط مدل‌های سینتیکی ریاضیاتی همچون سینتیک نوع صفر، نوع یک، و مدل‌های ریاضی هیگوچی، هیکسون وال، کورسمایر و ویبال مورد بررسی قرار گرفت. و در آخر قرارگیری ترکیبات طبیعی در ساختار لیپیدی برا اولین بار توسط نرم افزار مولکولار داکینگ مورد آنالیز و بررسی قرار گرفت.

فصل سوم بر مبنای نانوذرات چند جزء ی فسفولیپیدی با استفاده از اسانس‌های اکالیپتوس و نعناع فلفلی با خاصیت ضد باکتری قابل توجه، با هدف جایگزینی با نگهدارنده‌های مصنوعی فرموله شد. فرمول بهینه شده دارای اندازه ذرات 93-100 نانومتر بود که توسط DLS و تصاویر TEM تایید شد. ذرات ساخته شده دارای پایداری بالا 730 روز بوده است. انتشار ترکیبات فعال از درون کپسول‌ها توسط مدل کورسمایر-پیس بر اساس انتقال جرم فاز I (مدل های فیکیان) توضیح داده شد. نتایج نشان داد که کاهش قابل توجهی در مقادیر MIC، با افزایش فعالیت 32، 250 و 15 برابر نسبت به *E. coli*، *S. aureus* و *S. enteritidis* نسبت به مخلوطی اولیه از روغنهای ضروری، نشان داد. در نتیجه، نانوامولسیون فوق فرموله شده مبتنی بر فسفولیپید چند جزء ی می تواند یک نامزد امیدوار کننده به عنوان یک نگهدارنده غذایی با پایه طبیعی باشد.

فصل چهارم خواص تغذیه‌ای، آنتی اکسیدان و ضد توموری *Gunnera tinctoria* را بررسی می کند. براساس مطالعات انجام شده، فعالیت آنتی اکسیدانی بالاتر در برگ های نوزاد مشاهده شد، به دنبال آن برگ‌های بالغ و گل آذین. عصاره فنی با استفاده از کروماتوگرافی ستونبیر مبنای رزین جاذب *Diaion™ HP20LX* و *EXA90 Relite* غنی شده بود. اثرات ضد توموری بالایی در فرکشن‌های غنی‌شده در سلول‌های سرطانی پستان (MCF-7) و بالخصوص پانکراس (ASPC-1) مشاهده شد. برای بهبود حفاظت و میزان جذب غشایی ترکیبات فنی، نانو فیتوزوم‌ها و فیتوزوم‌های اصلاح شده توسط کلسترول توسط پلیمرهای طبیعی مورد استفاده قرار گرفتند. علاوه بر این، برهم کنش هیدروژنی بین ترکیبات فنی در هر

دو نوع فیتوزوم برای اولین بار توسط مولکولار داکینگ مورد مطالعه قرار گرفت. قطبش و اندازه مولکولی عامل اصلی در ترتیب مولکولی دو لایه لیپید شناسایی شد.

فصل پنجم بر سیلور اسکین قهوه غنی از ترکیبات طبیعی فعال تمرکز می‌کند. عصاره سیلوراسکین بر مبنای کافئین و اسید کلروژنیک با استفاده از کروماتوگرافی جذب سطحی غنی شده است به ترتیب 3.08 و 2.75 برابر افزایش یافت. نانو فیتوزوم ها بر اساس عصاره غنی بارگیری شد. ساختارهای فیتوزمی توسط کلسترول اصلاح شده‌اند. پس از آن، این حامل‌های نانو لیپیدی با پلیمرهای مختلف طبیعی پوشش داده شدند تا ثبات و پایداری ترکیبات را بهبود بخشد. ذرات تولید شده در مورد عملکرد محصول، بازده کیسوله سازی، ظرفیت بارگیری، اندازه ذرات، شارژ سطح و رهایش کنترل شده مورد بررسی قرار گرفت. تمام نمونه‌ها شارژ سطحی آنیونی را نشان دادند. مولکولار داکینگ و FTIR تعاملات بین فیتوزم و ترکیبات فعال را تایید کردند. بهترین درجه اتصال برای اسید کلروژنیک مشاهده شده نشان دهنده پیوند هیدروژنی قوی‌تر بوده که منجر به تعامل قوی تر با لایه لیپیدی شد. مکانیسم رهایش به خوبی با مدل‌های کورسمایر-پیپس و هیگچی تفسیر شد. علاوه بر این، رهایش اکثر ذرات فرموله شده توسط قانون انتشار فیکیان و یا انتشار پلیمری پیروی شد.

کلیدواژه ها: انکپسولاسیون، لیپوزم، فیتوزم، اصلاح ساختار سطح، سیستم‌های خشک‌کن، *Gunnera tinctoria*،

سیلوراسکین، نانوامولسیون.

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Abbreviations

A

AD	Atopic dermatitis
AML	Myeloid leukemia
AOs	Antisense oligonucleotides
AsPC-1	Pancreatic cancer
A	Heat transfer surface area (m ²)
A _p	Internal cross-field of vial section (cm ²)

B

BBI	Biomolecular & Biomedical Imaging
B-F127	F127 triblock copolymer
BMDM	Bone marrow-derived macrophages
BTCI	Bowman-Birk protease inhibitors
BV	Baculovirus

C

CFL	Cholesterol-free liposome
CFP	Cholesterol-free phytosome
CE	Catechin equivalents
CHL	Cholesterol
Chl.A	Chlorogenic acid
CLICG	Chitosan-coated liposome loaded with indocyanine green
CMC	Critical micellar concentration
COPD	Chronic obstructive pulmonary disease
CPD	Cycle-butane pyrimidine dimers
CPP	Cell-penetrating peptide
CTSL	Conventional thermal sensitive liposome
C _D	Drag coefficient
C _p	Specific heat of food sample/heat capacity in Jkg ⁻¹ K ⁻¹)
C _{pa}	Constant specific heat pressure (J/kgK)

D

DAPI	4',6-diamidino-2-phenylindole dihydrochloride
DCF-GuT	Discarded fraction of GuT
DCP	Dicetyl phosphate
DDA	Dimethyldioctadecylammonium
DDAB	Dimethyl dioctadecyl ammonium bromide
DDP	Dimethyldioctadecylammonium
Di/ DI	Diaion™ HP20LX
DMPC	1,2-dimyristoyl-sn-glycero-3-phosphocholine
DMRIE	1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide
DOGS	Dioctadecylaminoglycyl-spermine
DOPE	Dioleoyl-phosphatidylethanolamine/1,2-dioleoyl-sn-3-phosphoethanolamine
DOTAP	1,2-dioleoyl-3-trimethylammonium propane
DOTMA	2,3-bis[oley]oxipropyl-trimethylammonium chloride
DOPG	1,2-dioleoyl-sn-glycero-3-phospho- <i>rac</i> -1-glycerol
DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
DSC	Differential scanning calorimetry
DOX	Doxorubicin

D_{av}	Density of vapor
D_K	Knudsen diffusion
D	Diffusion coefficient
D_p	Penetration depth
d	Equivalent diameter
d_{fp}	Mean free path
d_p	Particles/droplet diameter (m)
d_s	Characteristic diameter in (m)
E	
ECC	Enhance on caffeine content.
EE	Encapsulation Efficiency
EChI	Enhance on chlorogenic content,
E-GuT	Enriched fraction of GuT
EGaL	Enhance on gallic acid content.
EO	Essential oils
E	Electrical strength
F	
FID	Flame ionization detector
FRAP	Ferric reducing antioxidant power
FTIR	Fourier-transform infrared spectroscopy analyses
FSE	Ferrous sulfate equivalents
G	
GAE	Gallic acid equivalents
GaL.A	Galic acid
GC-MS	Gas chromatography-mass spectrometry
GuT	<i>G. tinctoria</i> adult leaves
GuT ₁	Hydroethanolic solvent of <i>G. tinctoria</i>
GuT ₁ -Di ₁ -Malto	Enriched fraction of <i>G. tinctoria</i> -Maltodextrin
GuT ₁ -Di ₁ -PE	Enriched fraction of <i>G. tinctoria</i> -Pectin
GuT ₁ -Di ₁ -PE/Stch	Enriched fraction of <i>G. tinctoria</i> -Pectin/starch
GuT ₁ -Di ₁ -Stch	Enriched fraction of <i>G. tinctoria</i> -Starch
GuT ₂	Ultrasonication with water of <i>G. tinctoria</i>
g	Gravity acceleration (ms ⁻²)
H	
HA	Hemagglutinin
HA	Hyaluronic acid
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid
HHP	High hydrostatic pressure
HLB	Hydrophilic-lipophilic balance
HLP	Hybrid liposome
HLMC	Hydroxypropyl methylcellulose
HPC	Hard protein corona
HPLC	High-performance liquid chromatography
H _{Ev}	Evaporation Heat transfer
H _{tcond}	Heat transfer by conduction
H _t	Heat transfer
H _{tconv}	Heat transfer by convection
H _{trad}	Heat transfer by radiation

H_e	Total heat efficiency
H_{ti}	Total input energy (heat)
ΔH_s	Heat transfer of ice sublimation (cal/g)
ΔH_v	Invisible evaporation heat presented in (J/kg)
H_{tm}	Heat transfer based on manometric Temperature
H_a	Humidity of drying air
H_s	Drying air saturation humidity (kg/kg air)
H_{ab}	Efficient heat transfer (h_{ab})
K_{Ht}	Heat transfer coefficient
Δh_{EV}	latent heat of evaporation (J/kg)
ΔH_{vap}	Heat of vaporization
I	
ICG	Indocyanine green
IL-10	Interleukin 10
IR	Infrared radiation
i_b	Ion mobility
J	
J	loss of solvent rate
K	
K	Rate of evaporation
k_a	Thermal conductivity of drying medium in (W(mK) ⁻¹)
k^∞	Thermal conductivity (W(mK) ⁻¹)
K_c	Mass transfer coefficient
k_{ice}	Ice thermal conductivity presented in (W/mk°)
k_{um}	Constant unit of measurement
L	
LC	Lecithin
LC	Loading Capacity
LM	Leukocyte-mimetic
LNP	Lipid-based nanoparticles
LP	Liposome/Phosome
LUV	Large unilamellar micelle
L_T	Thickness/ thickness of particle bed (m)
L	Belt length (m)
M	
MCN	Multi-component nanoemulsions
MCF-7	Breast cancer
MHB	Mueller hinton broth
MIC	Minimum inhibitory concentration
MLV	Multilamellar
mTECs	Mouse tumor endothelial cells
MVL	Multivesicular
MVV	Multivesicular vesicles
MUV	Medium sized unilamellar vesicles
MW	Microwave
M_c	Total moisture content/mass transfer
M_l	Mass of liquid (circulated in system)/ mass of solvent

m_i	Mass of the particles/droplet (sample i)
m_{dm}	Mass of dried material (kg)
M_R	Moisture ratio (g)
M_e	Equilibrium dried moisture (%)
M_0	Initial dried moisture (g)
M_t	Moisture content (dried) (g)
M_{ti}	Mass transfer-rate of Ice sublimation (g/hr) per vial
M_d	Final dry content (g)
M_i	basic wet content (g)
M_p	Mass factor per unit (kg/h)
M_a	Air molar mass 29kg/kmol
M_{ts}	Mass transfer from the surface to material (kg/s m ² bar)
M_{wm}	Moisture content of water to material (kg)
M_{pd}	Droplet/particle momentum (kg.m/s)
M_{mw}	Molar weight of the solvent
M	Molecular mass
M_{tv}	Mass transfer of vapor
Mw	molecular weight
N	
NA	Neuraminidase
NADH	Nicotinamide adenine dinucleotide
NEs	Nanoemulsion,
NLC	Nanostructured-lipid carrier
NICNDAC	N-dimethyl acrylamide
NP	Natural polymer
N-polymer	Natural polymer
NRR	Novel redox-responsive
NRR-LDX	Multidrug resistant nano-micellar particles
n	drying exponent
n_i	Number of ion per unit (ion density)
$n_{v,sur}$	The water content generated within sample which Could remove by diffusion and convention in border condition (mol/m ² s)
$n_{w,sur}$	Water content could remove with the same condition from the border (mol/m ² s)
O	
OLV	Oligolamellar vesicles
P	
PC-104	1,3-bis-(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane
PCS	Photon correlation spectroscopy
PCs	Phenolic compounds
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PH	Phosphatidylcholine,
Ph/Chl-GuT ₁ -Di ₁	Phytosome/Cholestrol-enriched fraction of <i>G. tinctoria</i>
Ph-GuT ₁ -Di ₁	Phytosome-Enriched fraction of <i>G. tinctoria</i>
Ph/Chl-GuT ₁ -Di ₁ -Malto	Phytosome/Cholestrol-enriched fraction of <i>G. tinctoria-Maltodextrin</i>
Ph/Chl-GuT ₁ -Di ₁ -PE	Phytosome/Cholestrol-enriched fraction of <i>G. tinctoria-Pectin</i>
Ph/Chl-GuT ₁ -Di ₁ -PE/Stch	Phytosome/Cholestrol-enriched fraction of <i>G. tinctoria-Pectin/starch</i>
Ph/Chl-GuT ₁ -Di ₁ -Stch	Phytosome/Cholestrol-enriched fraction of <i>G. tinctoria-starch</i>

Ph-GuT ₁ -Di ₁ -Malto	Phytosome-Enriched fraction of <i>G. tinctoria</i> -Maltodextrin
Ph-GuT ₁ -Di ₁ -PE	Phytosome-Enriched fraction of <i>G. tinctoria</i> - Pectin
Ph-GuT ₁ -Di ₁ -PE/Stch	Phytosome-Enriched fraction of <i>G. tinctoria</i> -Pectin/Starch
Ph-GuT ₁ -Di ₁ -Stch	Phytosome-Enriched fraction of <i>G. tinctoria</i> -starch
PH/CHL-SIDI	Phytosome/cholesterol- Enriched fraction of Silveskin
PH/CHL-SIDI-GA	Phytosome/Cholesterol-Enriched Silveskin-Gum Arabic
PH/CHL-SIDI-GA/STC	Phytosome/Cholesterol-Enriched Silveskin-Gum Arabic/Starch
PH/CHL-SIDI-MA	Phytosome/Cholesterol-Enriched Silveskin-Maltodextrin
PH/CHL-SIDI-PE	Phytosome/Cholesterol-Enriched Silveskin-Pectin
PH/CHL-SIDI-PE/STC	Phytosome/Cholesterol-Enriched Silveskin-Pectin/Starch
PH/CHL-SIDI-STC	Phytosome/Cholesterol-Enriched Silveskin-Starch
PH-SIDI	Phytosome-Enriched fraction of Silveskin
PH-SIDI-GA	Phytosome-Enriched Silveskin-Lecithin-Gum Arabic
PH-SIDI-GA/STC	Phytosome-Enriched Silveskin-Lecithin-Gum Arabic/Starch
PH-SIDI-MA	Phytosome-Enriched Silveskin-Lecithin-Maltodextrin
PH-SIDI-PE	Phytosome-Enriched Silveskin-Lecithin-Pectin
PH -SIDI-STC	Phytosome-Enriched Silveskin-Lecithin-Starch
PH-SIDI-PE/STC	Phytosome-Enriched Silveskin-Lecithin-Pectin/Starch
PNE	Phospholipid-based nanoemulsion
P _∞	Atmospheric vapor pressure of the solvent (equal to zero)
P _s	Solvent concentration at the nozzle surface
∇P	Pressure gradient within the vapour pressure of Water at the surface and the partial pressure in the air
ΔP	Pressure drop
P _{ice}	Ice vapor pressure presented in (Torr) at sublimation temperature
P _{sub}	Water vapour pressure at sublimation (N/m ²)
P _c	Water vapour pressure at condenser temperature (N/m ²)
Pr	Prandtl number related to continuous phase (C _p μ/k _∞)
Q	
Q	Power/ energy
Q _R	Radio Frequency power conversed to thermal Energy presented (W/m ³)
Q _{ev}	Moisture energy evaporation
Q _{sp}	Total sensible heating
q	Quantity of the electrical charge belong to each ion
R	
RF	Radio frequency
Re/RE	Releite EXA90
RESS	Supercritical solutions
RRNH	Redox-responsive-nano-hybrid
RSL	Reversible surface liposome
R _{ps}	Total of normalized dry layer and stopper Resistance presented in (cm ² Torr.hr/g)
R	Universal gas constant is equal to 8.3144 (J/mol)
R _d	Drying rate
R _t	Current nozzle radius
S	
SA	Scavenging ability
SC	Sodium cholate

SDC	Sodium deoxycholate
SEM	Scanning electron microscop
SF	Synovial fluid
SLE	N-(3-hexadecyloxy-2-hydroxypropyl)-N-2-hydroxyethyl hexadecanamide
SLN	Solid-lipid nanoparticle
SI	Silverskin by-products
SI1	Hydroethanolic maceration
SI2	Ultrasonication extraction
SIDI	Enriched silverskin
SIDI-GA	Enriched Silveskin-Gum Arabic
SIDI-GA/STC	Enriched Silveskin-Gum Arabic/Starch
SIDI-MA	Enriched Silveskin-Maltodextrin
SIDI-PE	Enriched Silveskin-Pectin
SIDI-STC	Enriched Silveskin-Starch
SIDI-PE/STC	Enriched Silveskin-Pectin/Starch
SPC	Protein corona
SUV	Small unilamellar micelle
STC	Sodium taurocholate
S _g	Gas saturation (dimensionless)
S _w	Water saturation (dimensionless)
Sh _{eff}	Effective Sherwood number
T	
TCA	Trichloroacetic acid
TE	Trolox equivalents
TEM	Transmission electron microscopy
TFC	Total flavonoids content
TPC	Total phenolics content
TSL	Thermal sensitive liposome
T	Temperature
T _i	Inlet temperature
T _s	Surface/plate temperature
T _{st}	Steam temperature
T _f	Bulk fluid temperature
T	Temperature of drying air
T _s	Bottom surface air temperature (°C/k - depended on equation)
T [∞]	Surrounding air temperature (°C)
T _{sub}	Sublimation temperature (°C)
T _{i,s}	Surface temperature of the droplet (K)
T _{g,∞}	Temperature of external dried droplet layer (K)
t	Time (the value depend to equation and mention on text)
Δt	Average temperature of chamber from gas and particles
U	
U	Diffusion coefficient of cumulate vapor (m ² /s)
V	
v	Water vapor of working air
V	Volume/ specific volume

Y	
Y	Height of the substance (m)

W	
W_t	Residual mass (g)
W_g	Dried powder mass (g)

Greek Nomenclature

α	Thermal diffusivity (m^2/s)
β	Volumetric thermal expansion coefficient (K^{-1})
β_{cr}	Dried layer constant permeability (kg/s m bar)
λ_0	Free space wavelength
λ_L	Moisture evaporation constant equal to 2336 kJ/kg
λ	Invisible sublimation of the ice presented in (g/KgK)
λ_y	Specific heat capacity thermal conductivity in ($W.m^{-1}.K^{-1}$)
δ	Dissipation factor
δ_t	Thermal emissivity
f	Frequency
μ_v	Dynamic viscosity in (Pa.s)
μ	Viscosity (Pa)
μ_{ak}	Air kinetic viscosity (m^2/s)
φ	Shape factor
φ_p	Porosity
$\partial T/\partial t$	Rate of heat ($^{\circ}C/s$)
ρ	Density (kgm^{-3})
ρ_i	Density of sample i
ρ_p	Density of particles
ρ_g	Density of acceleration of gravity
ρ_v	Ionic conduction power per unit
ρ_{av}	Density of vapor(kg/m ³)
ρ_a	Mass density of air (kg/m ³)
$\rho_{v,amb}$	Vapor density of the ambient air
v	Velocity (ms^{-1})
v_s	Superficial velocity (ms^{-1})
v_{steam}	Steam velocity (ms^{-1})
v_v	vapour velocity (ms^{-1})
ε	Void fraction of bed
$\acute{\varepsilon}$	Constant of relative permittivity (relative dielectric)
$\acute{\varepsilon}$	Loss factor
σ_{rad}	Stefan-Boltzmann constant

Note

The submitted or published papers keep the original format, adapted to the body of the thesis, according to the specific rules of each journal. Thus, variations will be found in the different articles throughout the chapters, especially concerning the structure, references, classification, and the used language.

All the references not included in the publications, like those used in the General Scope section, are presented at the end of the respective section (as indicated in the index), according to the Vancouver style, as recommended by the “formatting standards for Master's Dissertations and Doctoral Thesis” established by the Faculty of Pharmacy of the University of Porto.

PART I | THEORETICAL SECTION

General scope

Nature is an important source of active phytoconstituents with significant pharmacological properties (1). In addition, food and agricultural industries produce large amounts of wastes which encourage the development of novel aspects concerning their recycling by converting them into high-value products through eco-friendly and green technologies. Indeed, certain food-processing industries, such as fruits, vegetables, seeds, tea, cereals, or coffee, among others, are main generators of wastes that present a high value of financial perspective (2).

Therefore, finding effective and safe carriers to deliver specific compounds in an accurate pathway, simultaneously reducing their side effects by using distinguished scientific developments is a major aim. Micro/nanoparticles are a relevant tool for the delivery of phytoconstituents/drugs to the target cells to treat a variety of disorders, or even for chemotherapy. In the last decades, encapsulation was one of the most distinguished developments in the field of micro/nanoparticles, with relevant purposes on pharmacology, medicine (3, 4), cosmetics and personal care, food industry, chemistry, and agriculture, among other fields (5, 6).

The main encapsulation reason in all industries remains in protecting the active ingredients from oxidation or deactivation due to reactions in the environment. Moreover, in pharmaceutical and food industries, to enhance the bioavailability of natural compounds in human body, especially in gastric media, is also an important aim. Furthermore, a controlled and sustained release also decreases the potential toxicity of drugs/phytoconstituents, reduces the dosage, and increases particles stability during storage and handling. Encapsulation also increases the possibility of applications, like the conversion of liquids into solids (powder), separation of incompatible materials, masking organoleptic properties like color, taste, odor, and safe handling of toxic materials (7). Eventually, it can be a coating technique to trap a material within another. The core includes the active ingredients (e.g. nutrient, drugs, fragrances, biocides, vitamins...), while the shell, composed of different natural polymers, isolates and protects the nucleus. Polysaccharides, proteins, lipids and their derivatives are natural polymers that can be used to achieve different objectives (3, 8). Consequently, two main factors must be considered: first, the suitability of the surface material, according to the target cell; second, the release time that allows the maximum absorption yield and efficiency.

Lipid-based nanoparticles (LNP) protect the active constituents with a lipid structure in the outer layer as the main protection site, which improve the performance of poorly water-soluble/lipid-soluble bioactive compounds, allowing, in general, high drug loading capacity, high encapsulation efficiency, long-term stability, and may improve bioavailability, stability, acceptability, as well as safety of food systems (9).

Moreover, LNP overcome permeation of drugs through intercellular and transcellular pathways, two different routes to permeate drugs/phytoconstituents through cells or a skin layer (10). Above all, LNP with special attention on liposomes, phytosomes and nanoemulsions, can represent a revolution on target delivery of drugs/phytoconstituents, both *in vitro* and *in vivo* (11).

Liposomes and phytosomes (L/P), natural-based liposomes, are natural phospholipid-based drug carriers, with a considerable similarity to human cell membranes. Such feature allows delivering active polar components to targeted cells, since phospholipids surround a hydrophilic compound through specific interactions, facilitating its transference through the cell membrane (12).

Biopolymer coated L/P are developed to enhance the positive features of natural carriers and improve the protection of the lipid structure, especially in gastric media, storage and handling, and to enhance release time, resulting on better functionality (13). It is noteworthy that chemical bonds between the liposomal layer and the biopolymer are not necessary. They could get shape individually or in complex, depending on the preparation method. The schematic arrangement of the natural polymeric coating lipid bilayer will be discussed further in this dissertation. The biopolymer-coated liposomal layer could decrease/increase the particle size (according to the desired function) and definitely improves homogeneity (similar distribution) of the particle size. In sum, biopolymers are used to protect the structural stability of the phospholipid bilayer and to control the drug release rate (13, 14). The final particles could be obtained using different drying techniques, but a special attention will be given to spray drying.

Drying is a critical unit operation on fabrication of particles and physical food preservation/stabilization of products and it occurs when the solvent (liquid) is vaporized by heat supplying to fresh material. The evaporation of liquids trapped in the surface is faster and needs less energy. Oppositely, the water trapped in the inner layer needs to be transferred to the outer layer to be eliminated, requiring more energy to be carried out. Drying a large amount of material and, at the same time, preserve its physicochemical and biological properties and its morphological texture, is still a

challenging task. Nowadays, different approaches are available, but spray drying is the most popular drying system in food, pharmaceutical, chemical, and biochemical industries. It converts droplets into low bulk density dried particles/capsules/powders by spraying a liquid phase into a hot drying chamber applied by a conduction heat supplier (typically air), which causes rapid evaporation and solidification. Although spray drying is considered a one-step process, it can be divided into four phases: i) atomization of input feed; ii) droplets mix (spraying gas (air /neutral nitrogen)) and free/coated moisture; iii) spraying the mixture, air and droplet to nozzle; and iv) product recovery (15).

In terms of performance, in this dissertation, fractionation of crude extracts using polymeric adsorbent resin column chromatography was performed to promote the extracts enrichment in bioactive compounds based on functionality and group categorization. In this dissertation, a special attention will be given to phenolic compounds. The enriched fraction provides an efficient outcome to multifold the phytoconstituents concentration, resulting on higher biological activities at low dosage and reduced side effects (16).

Therefore, a wise consideration on different market-related aspects, including the comprehensive procedures for extraction and characterization of natural and biologically active components and formulations based on environmentally safe chemistry, can provide controllable and safe products.

References

1. Gowe C. Review on potential use of fruit and vegetables by-products as a valuable source of natural food additives. *Food Science and Quality Management*. 2015;45:47-61.
2. Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades from 01/1981 to 09/2019. *Journal of Natural Products*. 2020;83(3):770-803.
3. Ye C, Chi H. A review of recent progress in drug and protein encapsulation: Approaches, applications and challenges. *Materials Science and Engineering: C*. 2018;83:233-46.
4. Sharma D, Ali AAE, Trivedi LR. An Updated Review on: Liposomes as drug delivery system. *PharmaTutor*. 2018;6(2):50-62.
5. Jafari SM. Biopolymer Nanostructures for Food Encapsulation Purposes: Volume 1 in the Nanoencapsulation in the Food Industry Series: Academic Press; 2019.

6. Vejan P, Khadiran T, Abdullah R, Ismail S, Dadrasnia A. Encapsulation of plant growth promoting Rhizobacteria—prospects and potential in agricultural sector: a review. *Journal of Plant Nutrition*. 2019;42(19):2600-23.
7. Suganya V, Anuradha V. Microencapsulation and nanoencapsulation: a review. *Int J Pharm Clin Res*. 2017;9(3):233-9.
8. Shishir MRI, Xie L, Sun C, Zheng X, Chen W. Advances in micro and nano-encapsulation of bioactive compounds using biopolymer and lipid-based transporters. *Trends in Food Science & Technology*. 2018;78:34-60.
9. Tamjidi F, Shahedi M, Varshosaz J, Nasirpour A. Nanostructured lipid carriers (NLC): A potential delivery system for bioactive food molecules. *Innovative Food Science & Emerging Technologies*. 2013;19:29-43.
10. Szunerits S, Boukherroub R. Heat: a highly efficient skin enhancer for transdermal drug delivery. *Frontiers in bioengineering and biotechnology*. 2018;6:15.
11. Fathi M, Mozafari MR, Mohebbi M. Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends in food science & technology*. 2012;23(1):13-27.
12. Bozzuto G, Molinari A. Liposomes as nanomedical devices. *International journal of nanomedicine*. 2015;10:975.
13. Pal K, Banerjee I, Sarkar P, Kim D, Deng W-P, Dubey NK, et al. *Biopolymer-Based Formulations: Biomedical and Food Applications*: Elsevier; 2020.
14. Yousefi AT, Ikeda S, Mahmood MR, Rouhi J, Yousefi HT. Encapsulation technology based on coacervation method to control release of manufactured nano particles. *World Applied Sciences Journal*. 2012;17(4):524-31.
15. Delshadi R, Bahrami A, Tafti AG, Barba FJ, Williams LL. Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. *Trends in Food Science & Technology*. 2020.
16. Fathi F, Nejad Ebrahimi S. Investigation of Physiochemical Properties Nanophytosome obtained from of Polyphenolic Enrich fraction of *Satureja khuzistanica* by Freeze-drying. *Nashrieh Shimi va Mohandesi Shimi Iran*. 2021.

Thesis organization and outline

Objective

General objective

The main objective of this work was to obtain enriched fractions with biologically active components of natural sources and formulate an innovative delivery product based on environmentally safe chemistry, in a controllable and safe way.

Specific objectives

- To characterize and evaluate the enrichment efficiency of natural sources (plants or food by-products) regarding their group characterization.
- To encapsulate of enriched fractions through lipid-based nano-microcarriers with special attention given to liposome, phytosome, and nano-emulsion.
- To modify phytosome surfaces by incorporating cholesterol into the lipid bilayer in order to improve stability.
- To encapsulate (coating) lipid-based nano-microcarriers (phytosome and cholesterol-containing phytosome) using natural polymers to protect the lipid bilayer-phytoconstituents, improve their stability, and control release.
- To evaluate the physiochemical properties of nano/-micro particles/capsules.
- To assess biological properties of enriched fractions and active phytoconstituents.

The current dissertation is organized in two main parts:

Part I - Theoretical section

This section includes two chapters corresponding to scientific review articles that introduce the main idea of thesis dissertation and contextualize the work.

Chapter 1: A New Approach on Target Delivery: A Review of Two Decades of Micro/Nano-Liposome/Phytosome Modification

Faezeh Fathi, Samad Nejad Ebrahimi, Alireza Fathi, Reza Mohsenian kouchaksaraee, Rita Carneiro Alves, and Maria Beatriz Prior Pinto Oliveira.

This chapter contains experimental unpublished data and contained comprehensive information about liposomes, a main subgroup of lipid-based nanoparticles (LNP) known as an efficient drug carriers due to its substantial cell membrane similarity. This study aims to overview two decades of liposome surface modification focusing on recent advancements with notable regards to phytosome (phytoconstituents liposomal backbone). It discusses liposome/phytosome approaches concerning their bilayers diversity and compiles numerous functionalities focusing on novel recent attempts to treat a broad range of human disorders. Moreover, it discuss functionalities from food/dietary supplements to drugs, phytoconstituents, as well as their potential for gene and vaccination immunology.

Chapter 2: Emerging on Recent Drying technique on Food Safety and Quality: A Review

Faezeh Fathi, Samad N. Ebrahimi, Luís Carlos Matos, M. Beatriz P.P. Oliveira, Rita C. Alves.

*This chapter contains experimental unpublished data (Published 2022-Comprehensive Reviews in Food Science and Food Safety (CRFSFS) - Reviews and Trends) and contains some key topics of recent drying techniques and their applications in food industry, also focusing on recent improvements on drying techniques to maintain the optimized texture of active phytoconstituents and nutrients, their release, and storage stability. It also explores recent efficient improvements on drying technology that produces high quality and low-cost final products, compared to conventional methods. Despite the recent advances in drying technologies, hybrid drying (a combination of different drying techniques), and spray drying (drying with the help of encapsulation techniques) are still promising techniques in food industries. Nevertheless, optimizing a drying technique and using an appropriate drying agent should also provide a promising solution to preserve, for instance, probiotic bacteria and antimicrobial preservers.

Part II - Experimental section.

- I. This section includes three chapters written in the form of research articles containing the experimental results (two submitted and one published);

Chapter 3: Antimicrobial Multi-Component Lipid-Based Nanoemulsion of *Eucalyptus globulus* and *Mentha piperita* as Natural Preservative

Faezeh Fathi, Samad N. Ebrahimi, Peyman Salehi, Atefeh Safari, Zeinab Shahsavari, Javad Hadian, Luís Carlos Matos, Rita C. Alves, Beatriz B. P. Oliveira.

*This chapter contains experimental unpublished data (Published 2021 - Journal of Dispersion Science and Technology) and describes the chemical composition of Peppermint and Eucalyptus essential oils, the development of a nanoemulsion (lipid-based) with more than two years of stability and evaluation of antibacterial activity.

Chapter 4: Exploring *Gunnera tinctoria*: from Nutritional and Anti-tumoral Properties to Phytosome Development Following Structural Arrangement Based on Molecular Docking

Faezeh Fathi, Samad N. Ebrahimi, Ana I. G. Valadão, Nelson Andrade, Anabela S.G. Costa, Cláudia Silva, Alireza Fathi, Peyman Salehi, Fátima Martel, Rita C. Alves, M. Beatriz P.P. Oliveira.

*This chapter contains experimental published data (Published 2021 – Molecules). It describes the nutritional profile of the plant, the fabrication of phytosomes and modified phytosomes coated with natural polymers, the physiochemical evaluation of the nano-micro particles and the *in silico* structural arrangement of phytoconstituents of *Gunnera tinctoria* within a lipid bilayer based on molecular docking.

Chapter 5: Formulation of Coffee Silverskin Lipid-based Nano-carriers Coating by Natural Polymer: Physiochemical Properties, *In-Vitro* Release Mechanism, and *In-Silico* Molecular Modeling

Faezeh Fathi, Samad N. Ebrahimi, João A. V. Prior, Susana M. L. Machado, Reza Mohsenian Kouchaksaraee, M. Beatriz P.P. Oliveira*, Rita C. Alves.*

*This chapter contains experimental unpublished data (Published 2022 - Pharmaceutics) and describes fractions enrichment with caffeine (purine alkaloid) and phenolic compounds, fabrication of phytosomes and modified phytosomes coated with natural polymers, the evaluation of physiochemical properties of nano-micro particles, the *in vitro* release mechanism and the *in silico* structural arrangement of phytoconstituents from coffee silverskin within a lipid bilayer based on molecular docking.

Chapter 1

A New Approach on Target Delivery: A Review of Two Decades of
Micro/ Nano-Liposome/Phytosome Modification

A NEW APPROACH ON TARGET DELIVERY: A REVIEW ON TWO DECADES OF MICRO/NANO-LIPOSOME/PHYTOSOME MODIFICATION

Faezeh Fathi, Samad N. Ebrahimi, Alireza Fathi, Reza Mohsenian kouchaksaraee, Rita C. Alves, and M. Beatriz P. P. Oliveira*

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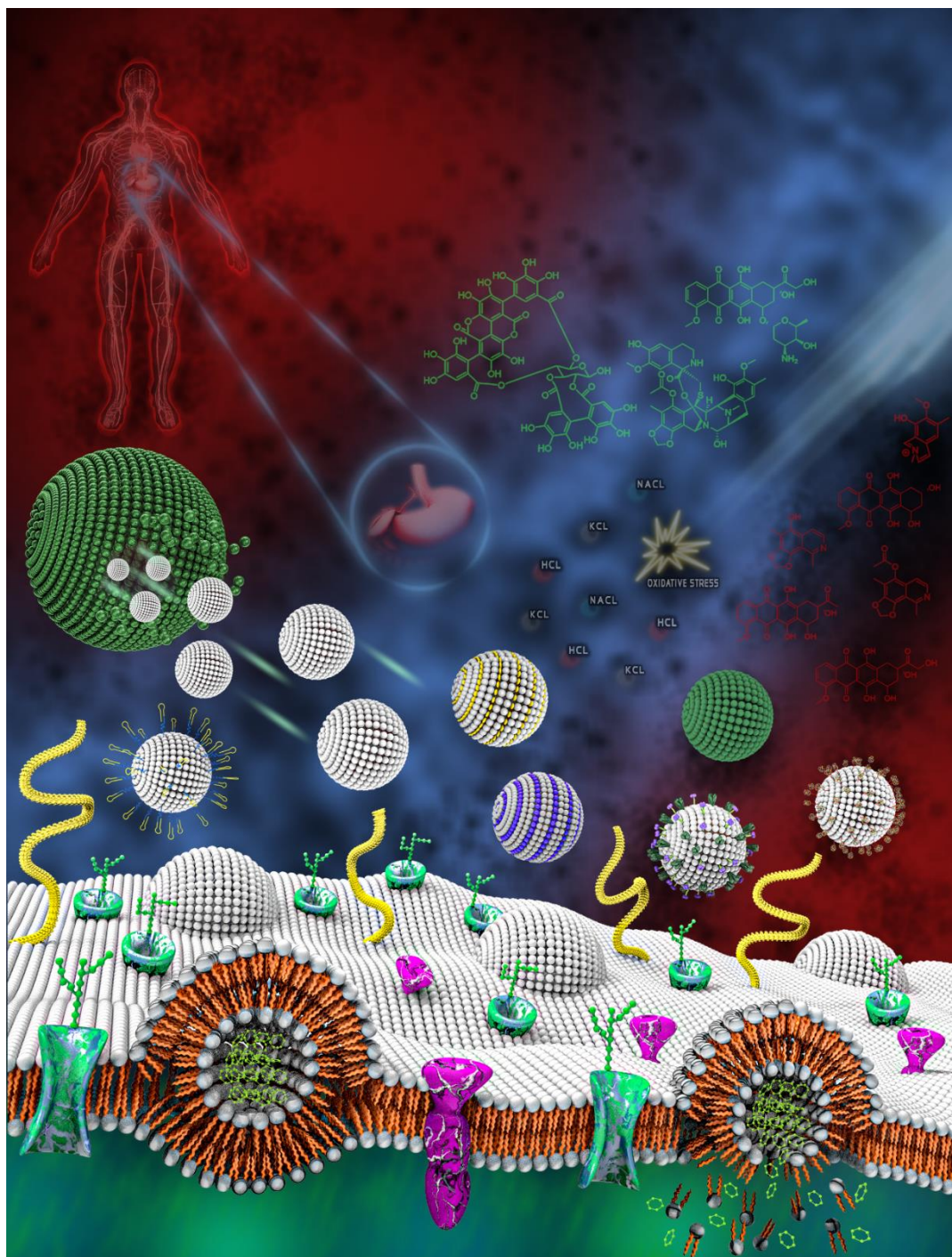
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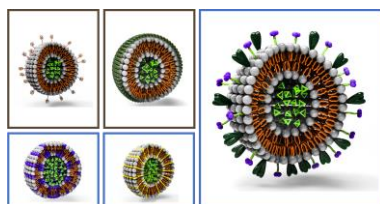
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1-1. Cover Page



ToC Figures



1.2. Abstract

Liposomes are one of the main subgroups of lipid-based nanoparticles (LNP) known as efficient drug carriers due to its substantial cell membrane similarity. This practical lipid-based bilayer significantly improves drugs permeability through a transdermal route, approved as a possible way to target delivery of phytoconstituents, drugs, enzymatic agents, RNA, DNA, and vaccine immunology. This study aims to overview two decades of liposome surface modification focusing on recent advancements with a notable regard to phytosome (phytoconstituents liposomal backbone). It discusses liposome/phytosome approaches concerning their bilayers diversity and compiles numerous functionalities focusing on novel recent attempts to treat a broad range of human disorders. Furthermore, various functionalities from food and dietary supplements to drugs, phytoconstituents, gene, and vaccination immunology were discussed.

Keywords: liposome, phytosome, virosome, lipid-based carrier, surface modification, drug delivery, gene delivery.

1.3. Main Text

1.3.1. Introduction

Effective or not, toxic or not, safe or safe at optimized dosages, sustained, controlled or direct release, these are simple questions that became a challenge for the scientific community in what concerns to drug/phytoconstituents target delivery. Therefore, finding effective and safe carriers to deliver certain compounds in an accurate pathway, simultaneously reducing their side effects by using distinguished scientific developments is the aim. Micro/nanoparticles are a considerable issue for the delivery of phytoconstituents/drugs to the target cells to treat a variety of dysfunctions, as well as chemotherapy. In the last decades, encapsulation was one of the most distinguished developments in the field of micro/nanoparticles, with relevant purposes on pharmacology, medicine,^[1,2] cosmetics and personal care, food industry, chemistry, and agriculture.^[3-6] Moreover, it is also generally used in several applications, including, aromatherapy, nutraceuticals, agrochemicals, oil and gas biocides, paints and colorants, adhesives and coatings, paper industry, electronics and imaging systems.

The principal encapsulation reasons in all industries are to protect the active

ingredients from oxidation or deactivation, due to reactions in the environment. In pharmaceutical and food industries, the aim is to enhance the bioavailability of the natural products in human body, especially in gastric media and blood. Furthermore, the controlled and sustained release decreases the potential toxicity of drugs/phytoconstituents, reduces the dosage, and increases particles stability during storage and handling. Long lasting, targeted release, improved adhesion, penetration, and triggered release are optimized by changes in environmental pH and temperature. Other advantages of encapsulation are the increase of putative applications, like the conversion of liquids into solids (powder), separation of incompatible materials, masking organoleptic properties like color, taste, odor, and safe handling of toxic materials.^[7] Eventually, encapsulation can be a coating technique to trap a material within another. The core includes the active ingredients, for example, drugs, fragrances, biocides, vitamins, etc., while the shell, composed by different natural polymers, isolates and protects the nucleus. Polysaccharides, proteins, lipids and their derivatives are natural polymers with several possibilities to attain the objectives.^[1,8] Consequently, two main factors must be taken into account: first, the suitability of surface material, according to target cells features; second, a release time allowing the maximum yield and efficiency.

1.3.2. Lipid-based Nanoparticles

Lipid-based nanoparticles (LNP) constitute a well-known biodegradable and nontoxic delivery process that includes five major subgroups, taking into account the lipid matrix composition. Solid-lipid nanoparticle (SLN), nanostructured-lipid carrier (NLC), nanoemulsion, micro/nano suspension, nano liposome/phytosome (L/P) are examples of that.^[9–13] Lipid carriers entrap a wide range of bioactive sensitive structures for a targeted delivery via cell barrier or skin absorption. These structures have the ability to encapsulate both hydrophilic and lipophilic compounds and enhance the efficiency of active components to treat or prevent several disorders. They are commonly used to enhance the solubility and stability of drug components in acidic media, to eliminate immunogenicity of drug-carrier complexes, to solubilize both hydrophobic and hydrophilic drugs and to decrease the constituent circulation constraints in the serum media.^[11,14]

The LNP protect active constituents with a lipid structure in the outer layer as principal protection site, which improve the performance of poorly water-soluble/lipid-soluble bioactive compounds, allowing in general high drug loading capacity, high encapsulation efficiency, long-term stability, and may improve bioavailability and stability, acceptability as a safety of food systems.^[15–16] Overall, LNP could enhance the bioavailability,

solubility, and improve a total performance of both lipophilic and hydrophilic bioactive components. These structures provide a long-term stability and favor the delivery to target sites.^[17] The LNP overcome permeation of drugs through intercellular and transcellular pathways, two different routes to permeate drugs/phytoconstituents through a cell or skin layer.

The intercellular route explains the permeation of short-chain compounds among the cells, and the transcellular route explains the diffusion of long-chain compounds through protein-filled cells.^[18] Concluding, LNP and related structures began a proved revolution on target delivery of drugs/phytoconstituents, both *in vitro* and *in vivo*.^[9]

1.3.2.1. Liposome Technology

Liposome is a natural phospholipid-based drug carrier with a considerable similarity to human cell membranes. Such feature allows delivering active polar components to targeted cells (**Figure 1**). These super-molecular carriers protect bioactive compounds in an internal polar nucleus, surrounded by phospholipid bilayers in aqueous media. Liposome is a mechanical combination between phospholipids and bioactive compounds in micelle-shaped structures, separating the inner water phase from the outer one. Phospholipids surround a hydrophilic compound by one or multi-lipophilic external layers to facilitate its transference through a cell membrane. In this case, no chemical bonds occur between the active ingredient and the phospholipid layer. Instead, liposome creates a physical interaction between the phospholipid and the hydrophilic compound.^[19] Nowadays, liposomes are efficient structures not only used to deliver pharmaceutical compounds to treat several human disorders, from skin to cancer therapy, but also used as diagnostic imaging (radiolabeled agent) to increase a treatment planning. As liposome has a flexible structure, it can be used in various functionalities from therapeutics to diagnostic and consequently to enhance the possibility of targeted delivery according to desired purposes.^[20]

1.3.2.2. Phytosome Technology

Phytosome is a novel targeted delivery carrier, characterized by specific bonding between phospholipids and hydrophilic drugs, with possible distinctive configurations. In other words, phytosome is a non-polar career for efficient delivery of bioactive components or natural drugs through a cell membrane (**Figure 1**). It is formed by the coupling via hydrogen bond between a polar site of the phospholipid (choline site) and a polar section of the bioactive compound (OH, NH groups). In a better words, the

lipophilic part of the phospholipid gets the shape around the polar site and covers the active ingredient in a circle shape like a round capsule.^[21,22]

The most significant differences between liposome and phytosome are the chemical bonds between phospholipids and the active component, being the phytosome more specialized and practical. The molar ratio between phospholipids and the bioactive compounds is 1:1 or 2:1 depending directly to the substrate and influencing considerably the transference of the active components to a specific site. Therefore, it could be concluded that, phytosome provide better structural ratio, less toxicity, more bioavailability and flexibility compared to other LNPs. Phytosome can decrease the efficient drug doses, as well as their toxicity for human cells and organs. These structures are interesting practical features used in a wide range of applications from cosmetic products to pharmaceutical drugs. They are also suitable carriers for nutrients as well as dietary supplements. As a flexible process, it can improve the bioavailability of natural compounds in the human body, and protect natural sensitive structures against environmental conditions, like pH changes, light degradation, and ROS action. It is also a suitable carrier during the absorption process.^[23,24]

Concluding, several advantages of these technologies (L/P) were demonstrated by a new approach on surface modification.^[25] Comprehensive references to L/P increased its application by cosmetic and skin-care industry, as well as with an increased development in pharmaceuticals, food industry, dietary supplements, and also in agriculture.

1.3.2.3. Lipid Carriers in Liposome/Phytosome

Soybean lecithin, egg lecithin, phosphatidylcholine, phosphatidylserine, cholesterol, and their derivatives are the leading groups of lipid carriers used in lipid-based nanoparticles, especially in L/P structures.^[9,26,27] Lipid carriers show excellent features to encapsulate both hydrophobic and hydrophilic natural active compounds. Low toxicity, emulsification ability, wettability, encapsulation features, film formation ability, biodegradability, biocompatibility, excellent stability, high protection, and high bioavailability are characteristics that make them efficient carriers in the current issue. Lecithin is a mixture of phospholipids and fat, obtained at industrial level from egg or soybean. It is an emulsifier, carrier or neutral filler in different products for pharmaceutical, cosmetic or food industry applications. Phosphatidylcholine and phosphatidylserine are the primary components of lecithin, used as a part or as a pure powder for the mentioned applications.^[26,28,29] Cholesterol is a natural compound of animal cells, originating a stronger bilayer in LNP. It modulates size and morphology when incorporated to lipid

bilayer, as well as the permeability of micelle, and membrane fluidity. It must take into account that in some structures cholesterol can decrease permeability, as well. Although lipid carriers improve the permeability of drugs or bioactive components, they need some markers to achieve more accurate delivery profiles.^[26,30]

Glycoproteins and antigens are two groups of markers used in the L/P bilayers. Glycoproteins are sugar attached protein structures that enhance the stability of the lipid bilayer, acting as a smart receptor for the target delivery.^[31] Antigens also act as natural markers in the surface of the lipid bilayer, to be recognized by target cells, or could be considered as active ingredient and loaded inside a core. The design of a lipid-based vaccine with specific antigens to the targeting cell, could be a promising technique not only with high immunologic efficiency, but also with a high targeting efficiency.^[32] L/P surface modification is generally performed using a combination of lipid carriers, biopolymers and several types of markers, issues discuss in this review.

1.3.2.4. Differences between Liposome/Phytosome

Liposome/phytosome have several similarities, but also considerable differences. Phytosome is a liposome with specific chemical bonds between drug/extract and phospholipid (Figure 1).^[22,23] Besides, liposomes are micelle type structures without any organization (the active constituent is entrapped in the phospholipid micelle).^[23] In phytosome a particular active component links to phospholipids at different molar ratios (1:1, 1:2, or at least 1:5) depending on the substrate type and formation method. In liposome, the active constituent is surrounded by the phospholipids in micelle form around at 1:10 - 1:15 molar ratio. Therefore, phytosome reveals a lower protection of entrapped phyto-structural compounds, due to molar ratio compared to liposome (Figure 1).^[23,33]

Phytosome has a particle size lower than 600 nm. Liposome can have a wide range of sizes, from few (around 70) nm to hundred μm (depending on the structure morphology).^[33,34] Liposome is able to get shape in buffer solutions without any organic solvent,^[24] but to create the chemical bonds in phytosome structures aprotic solvents are needed.^[35] In other hand, the efficient programmable absorption of phytosome depends on the type of lipid carrier and chemical bonds. Finally, phytosome is structurally more stable than liposome due to its chemical bonds.^[23]

1.3.2.5. Size Characterization of Liposome/Phytosome

L/P carriers shape and morphology depend of the number of layers, the condition of each layer, the specific place for entrapping drugs in each layer, all directly influenced by particle size distribution. The former information explains the layer features, monolayer or bilayer, with or without semi-covalent bonds (hydrogen-bond) between lipid layer and active compounds.^[33,36] In general, L/P configuration results from the preparation method (hydration/rotation or ultra-sonication step), drying procedures, and phospholipid amount as well as active component features. Therefore, the particle size is a consequence of one or a combination of these steps. According to the number of bilayers, liposomes are multi-, oligo- or unilamellar. Examples of unilamellar liposomes, with particle sizes from 25 nm to 2 μ m, are: SUV (small unilamellar micelle shaped vesicle, size of 50-70 nm), LUV (large unilamellar micelle shaped vesicle), and GUV (giant unilamellar micelle shaped vesicle) with sizes from more than 200 nm to micrometers. They have different sizes not different layers. Another categorization of liposome vesicles considers the different multilamellar and multivesicular structures (Figure 1). In the case of multilamellar (MLV) ones, the liposome has several layers in sequence, one after another, ranging from the smallest inside to the largest outside, with sizes around micrometers. Multivesicular (MVL) is an arrangement of several non-homogeneous liposome shapes inside one giant layer without any specific order in size. The various particles with different sizes are gathering inside another, constituting a giant lipid bilayer.^[33,37,38]

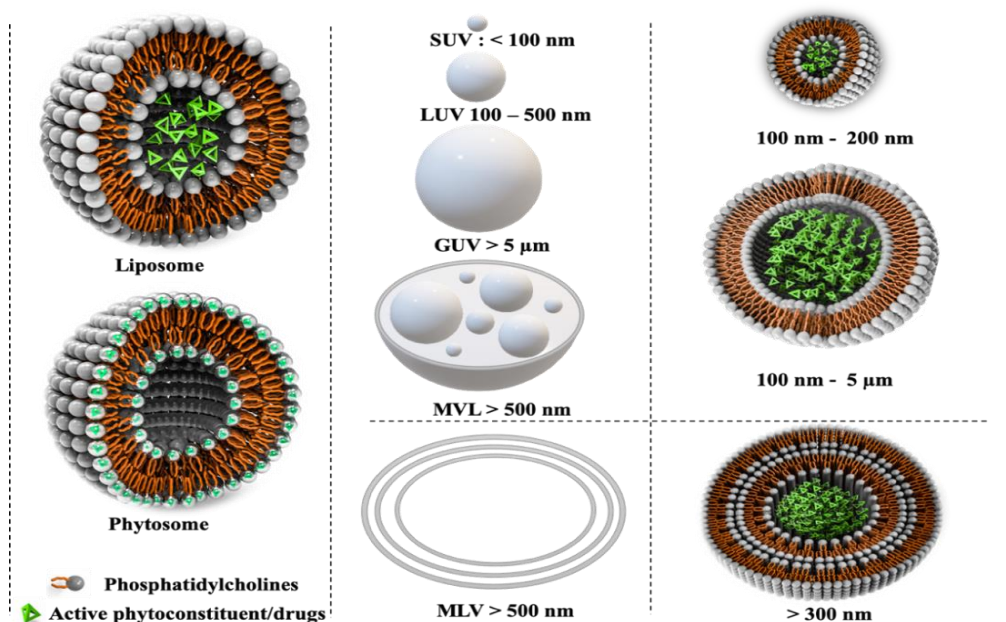


Figure 1. Main differences between liposome and phytosome, and differences in size and bilayer of L/P. SUV- small unilamellar micelle shaped vesicle; LUV- large unilamellar micelle shaped vesicle; GUV- giant unilamellar micelle shaped vesicle; MLV- multilamellar, and MVL- multivesicular. Briefly SUV < LUV < GUV < MLV $\approx$ MVL.

According to Jesorka and Orwar (2008),^[39] the preparation method affects the lipid bilayer produced. Thin-film hydration method run by evaporation-dried, spray-dried or lyophilized lipid material produces MLV. When subjected to mechanical agitation or sonication the particles change to GUV, LUV, and SUV. Meanwhile, they clarified that high-energy sonic fragmentation, extrusion, high-pressure homogenization, and solvent injection methods produce SUV; freeze-thaw cycling, swelling in non-electrolytes, de-/rehydration, extrusion, detergent dialysis and reverse evaporation produce LUV; and de-/rehydration, electro-formation solid-film hydration, and detergent dialysis produce GUV.^[33] Furthermore, more definition was available regarding the structural arrangement of liposome formation such as; OLV (Oligolamellar vesicles), MUV (Medium sized unilamellar vesicles), MVV (Multivesicular vesicles) which is not very common.^[39]

1.3.2.6. Size Reducing Agents

Conjugation of natural biopolymers such as polysaccharides and proteins may alter the particle size and completely change the particle morphology. Adding a biopolymer on the surface of the lipid-based particles could be another approach in the field of particle size distribution, namely, to decrease or increase its size. Casein, a dairy protein,^[40] and starch (in case of coating layer)^[41] could be incorporated on liposome bilayer by a thin-film hydration method can be a good example of size reducing agent. For instance, a considerable decrease in particle size occurred due to the incorporation of the casein on liposome bilayer. The hydrophobic interaction between casein chains provides an electrostatic repulsion with the lipid bilayer and causes electrostatic stability in between. In the other hand, the electrostatic stability provides a rigid cluster between the lipid chain and casein chain, making them more hydrophobic. Such interaction provides a stable and small particle. This stable nano particle provides higher release time and stability as reported.^[42]

1.3.2.7. Liposome/Phytosome Advantages

Besides the mentioned differences and similarities between liposome/phytosome technologies, the specific use of these structures presents a wide range of advantages. The following lines try to show most of the positive properties of these structures.

1.3.2.7.1. In the Case of Liposome

1. It improves the efficiency and bioavailability of drugs^[9,26] as well as provoke the intracellular delivery of drug/phytoconstituent.^[43]
2. It improves significantly drug stability compared to other encapsulation techniques.^[26,43]

3. Flexible, biodegradable, safe and immunogenic structure.^[26]
4. It decreases considerably the toxicity of several anticancer drugs like amphotericin B and Taxol.^[26]
5. Site-specific target cells for active phytoconstituents delivery.^[26,44]
6. It improves the solubility of amphiphilic and lipophilic drugs.^[44]
7. It could be optimized release time and profile and provide sustained and delayed release mechanism.^[9,26,44]
8. Flexible carriers used for different structures like antibiotics, genes, anesthetics, and insulin.^[9,26,33,44]
9. Structure suitable for cosmetics^[26] and food products.^[44]
10. It could have a practical, safe and efficient large-scale production.^[26]
11. Phosphatidylserine, one of the main components of lecithin, has the ability to pass through a human brain barrier due to its similar structure and nourish nerve cell membranes and myelin (Dual function).^[45]

1.3.2.7.2. In the Case of Phytosome

1. It improves the bioavailability of natural lipid insoluble phytoconstituents through the cell membrane (transdermal drug delivery).^[46,47]
2. Target specific due to molecular-level association; one or two phosphatidylcholine responsible for each drug molecule (Improve stability).^[46,48,49]
3. Flexible, biodegradable and safe structure.^[46,48]
4. It improves the absorption and maintains the pharmacological activities of the active ingredient.^[48,49]
5. Maximum drug entrapped efficiency^[48] with a minimum dosage and side effects.^[23]
6. Phosphatidylcholine presents a dual function: it is a lipid carrier along with a synergic hepatoprotective effect when a hepatoprotective agent is an active part (on core).^[23,46]
7. Dual-function carrier, nourishing the skin membrane.^[48]
8. Stable profile, due to the chemical bonds between phosphatidylcholine and phytoconstituents.^[23]
9. Considerable similarity with the cell membrane and lipid carriers, enhancing the permeability.^[23,47,49]
10. Transdermal permeability: clinically proved as an adequate structure for a cosmetic product.^[23,47,49]
11. Phospholipids, present in phytosome particle, act as external nutrients besides

the carrier function.^[23,48]

12. Improved absorption on intestinal lumen barriers compared to other delivery carriers.^[48]
13. Enhances the bile solubility of plant extracts.^[23]
14. Programmable liver targeting due to direct absorption of phosphatidylcholine in liver cells.
15. Low-cost preparation and low amount of active ingredient needed.
16. Significant therapeutic benefits due to increase bioavailability of phytoconstituents.^[23]
17. High stability of phytoconstituents due to established chemical bonds.^[49]
18. Protective composition against digestive enzymes and bacteria in the acidic gastric environment.^[49]
19. Non-invasive structure, adequate for immediate commercialization of accurate targeted delivery.^[23]
20. Phosphatidylserine, one of the main components of lecithin, has ability to pass through a human brain barrier due to its similar structure and nourish a nerve cell membranes and myelin, (dual function).^[45]

1.3.2.8. Liposome/ Phytosome Disadvantages

1. The release of the active ingredient from the particle occurs in a short time, not desired on wide range of functionalities. As example, fast releases time is good for nasal, serum, and cream formulations.
2. The particle size is mostly large or with a wide distribution; therefore, another preparation method or size reducing agent are needed to adjust the size.
3. Most of the preparation techniques use an organic solvent that may remain in the final product.
4. Generally, phospholipids are thermal sensitive structures, and some methods need high temperatures to L/P formation.^[50]

1.3.2.9. Preparation Methods

For drug loading on capsules three different types of method can be followed: passive drug loading, active drug loading, and modified active loading. In the case of passive drug loading, a mixture of a saturated drug solution and biopolymers is stirred until a thin film get shape. The thin film is kept overnight under vacuum or in anhydrous conditions at room temperature.^[51] In turn, active drug loading is based on surface target absorption: the carriers are prepared, and the drugs poured on their surface.

The saturated surface of carriers get shape by a general drying technique.^[52] The modified active loading is similar to the active loading technique, with a simple modification due to no-solvent evaporation, following the active loading procedures.^[51] Active loading and modified active loading use target compounds or antibodies as critical surface modification factors to programmed drug delivery. The targeted sign can be attached to the bilayer surface by three ways: direct link to the surface; direct link to the polyethylene glycol (PEG) cover (indirectly coupled to the end part of PEG); and linked to the capsule surface via PEG.^[51,52] Table 1 describes the L/P techniques from the initial drug loading method, clarifying the procedures, features, and applications of each method to the delivery process. This table shows twenty-three different categories, grouped in six main methods (Mechanical, Solvent Dispersion, Detergent removal, Supercritical fluids, Solvent evaporation, and Anti-solvent precipitation). Besides, Table 2 highlights four different techniques generally used in the drying of L/P particles. Furthermore, several papers reported the nano-spray drying technique as the most efficient when reduced particle size is required and lyophilization as the most effective to enhance the total yield. Wen and co-workers set up a new preparation technique, named rapid expansion of supercritical solutions (RESS).^[53] According to the authors, this technique is suitable for encapsulation of essential oils (EOs), and was applied to encapsulate the EO of *Atractylodes macrocephala* Koidz rhizome. This rhizome is traditionally used in Chinese traditional medicine for digestive and liver disorders, as anticarcinogenic and also as food flavor.^[54] Supercritical solutions (RESS) introduced as promising effective methods for encapsulation of different EOs, applies them directly into the sealed reactor. CO₂ gas changes to the liquid form and is mixed with ethanol, lipids, and EOs at controlled temperature and pressure. Then the mixture is hydrated with a buffer solution by a syringe pump. After that, CO₂ releases from the apparatus and the residue is collected and freeze-dried. Liposome loaded with an EO (L-EO) by RESS technique revealed a high loading capacity, stability, and a homogeneous double layer. The obtained results with L-EO demonstrated the RESS technique as a promising procedure to entrap a multi-component and better than other preparation techniques.^[53]

Table 1. Liposome/Phytosome Preparation Methods for both powder and liquid forms.

Method	Type	Application	Features	Ref
Mechanical methods	Thin lipid film hydration, antisolvent precipitation (natural swelling method/ Rotary evaporation process)	Liposome/Phytosome	Passive	[34,55,56]
	Thin layer hydration	Phytosome	Passive	[34,57]
	Membrane extrusion	Liposome	Passive	[34]
	French pressure cell (extrusion)	Liposome	Passive (Mostly SUV)	[34,44,58]
	Polycarbonate filters at low or medium pressure (extrusion)	Liposome	Passive (OLV, LUV)	[34,44,58]
	Micro-emulsification	Liposome	Passive	[44]
	Sonication (Bath and Probe)	Liposome/Phytosome	Passive	[34,44]
	Vortex or handshaking of phospholipid dispersions	Liposome	Passive (MLV)	[58]
	High pressure Homogenization	Liposome	Passive (Mostly SUV)	[58]
	Ultrasonic Irritation	Liposome	Passive (SUV at small size)	[56,58]
Solvent Dispersion methods	Bubbling Gas	Liposome	Passive (BSV)	[58]
	Reverse phase evaporation	Liposome/Phytosome	Passive	[34,44,56]
	Injection method	Liposome	Passive (LUV)	[34,44,56,58]
	Ether injection Ethanol injection			
	Phospholipid complexation	Liposome/ Phytosome	Active	[59,60]
Detergent removal method	Slow Dialysis	Liposome	Passive (LUV, OLV, MLV)	[34,44,58]
	Size exclusion chromatography	Liposome	Passive	[34]
	Gel-permeation chromatography	Liposome	ND	[44]
	Fast Dilution	Liposome	Passive (MLV, OLV, LUV, SUV)	[34,58]
Supercritical fluids		Phytosome	Passive	[34,55]
Solvent ether-injection		Phytosome	Passive	[56,58]
Anti-solvent precipitation		Phytosome	Passive	[34,61]
Freeze-thawed		Liposome	ND	[44]

*Feature (passive/active) was concluded based on preparation method. [44] ND; Not detected.

*Liposomes were classified regarding the preparation methods as follow; REV; Single/oligolamellar prepared by reverse phase evaporation, MLV/REV; Multilamellar prepared by reverse phase evaporation, SPLV; Stable plurilamellar, FATMLV; Frozen and thawed MLV vesicles, VET; Lamellar made by extrusion, FPV; Lamellar made by french press, DRV; Dehydration-rehydration, BSV; Bubblesomesa.[39]

Table 2. Drying techniques generally used for preparation of Liposome/Phytosome powders.

Method	Application	Condition	Ref
Salting out method (non-solvent precipitation)	Liposome/Phytosome	Dioxane, Acetone/Hexane	[49,62,63]
Lyophilization	Liposome/Phytosome	Dried the solvent in vacuum at -80 °C	[49,62,64]
Spray drying	Liposome/Phytosome	Dried at critical temperature (100 -150 °C) along with biopolymer	[49,64]
Solvent evaporation method (vacuum evaporator)	Liposome/Phytosome	Not specific condition	[21,58]

1.3.2.10. Evaluation Techniques

Different techniques can be used to evaluate the structural and superficial features of L/P, being spectrometry the most accurate one. Particle size is the most critical element in L/P evaluation. Indeed, size characterization also provides information about the type of particles present, estimated shapes, morphology, and homogeneity. Size and Zeta potential are two factors, along

with others, that can be measured with dynamic light scattering laser granulometry (coulter counter), photon correlation spectroscopy (PCS), or any type of equipment specialized in particle size measurement at micro and nanoscales.^[65]

Entrapment efficiency of drugs or loading capacity could be evaluated by ultracentrifugation of an accurate amount of particles dissolved on an appropriate solvent (in which the active ingredient is soluble and the cover carrier insoluble). Then the absorbance by UV spectroscopy or the surface peak by high-performance liquid chromatography (HPLC) were measured. Transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray powder diffraction (XRD) are other techniques that also give informations about the above mentioned features. TEM is typically used to analyse the particles (micelle/capsule) shape, stability in solution or emulsion, either on micro or nanoscale. SEM images are commonly used to analyse particles-containing powders. Although SEM and TEM provide an accurate information about the particle size, they do not give any specific information about the chemical bonds in phytosome structure (contrarily to XRD images). ¹H-NMR, ¹³C-NMR, FTIR and HPLC-ELSD are general evaluation methods that also provide particular information about chemical bonds and differences between the L/P structures.^[65,66] Table 3 describes with details the most relevant techniques to characterize L/P particles.

Table 3. Evaluation Methods to confirm the presence of a Liposome/Phytosome structure.

Evaluation Method	Type	How it to process	Ref
FT-IR	Phytosome	A chemical reaction occurs between the hydroxyl group of the phytoconstituents and the polar (choline) head of the phospholipid; a specific broad peak appears in the spectrum after the interaction.	[67]
It might be different according to the structures. The specific peak could be recognized by simple comparison between the spectra of phytoconstituents or phospholipids with a phytosome.			
TLC	Liposome/ phytosome	It could be possible with an appropriate mixture of solvents, which prevents the opening of lipidic bilayer or breaking a chemical bond between phospholipid and active ingredient on the silica surface. The difference in retention time indicates/confirmed the generation of a new complex termed as phytosome.	[67]
Differential scanning calorimetry (DSC)	Phytosome	Comparison between the melting point of pure compounds, and encapsulated compounds. Differences in 2θ angle of a pure compound, phospholipid, and encapsulation structure revealed the availability of these structures. Generally, the peak belonging to active ingredient must be eliminated and a partial part of phospholipid peak remain with appearing a new peak.	[67]
Apparent differences in peak behavior, change in melting point, and peak temperature results to be considered. The crystalline shape of encapsulated form of active ingredients can affect on melting point. Finding the difference in the melting point could help the evaluation based on DSC.			
Thermal gravimetric analysis (TGA)	phytosome	Use for a sample in a solid phase; TGA analysis could recognize thermal changes due the chemical bond between the active ingredient and phospholipid, which affects the thermal activity of the new structure.	[67,68]
Solid-solid transitions, loss of solvent, fusion, glass transitions, and decomposition are thermal activities used for phytosome evaluation.			
¹ H NMR (¹³ C-NMR, ³¹ P-NMR)	Liposome/ phytosome	H-bond from the chemical reaction between the hydroxyl group of phytoconstituents, and the polar part (choline and phosphate ion) of phospholipid provide specific peak on ¹ H-NMR. ¹ H-NMR shows the availability of the non-polar tail of phospholipid. The result from ¹ H-NMR revealed no interaction between the tail of the phospholipid were occurred. Additionally, it revealed a specific shield around the polar part corresponding to the N-methyl of choline, which confirm the formation of phytosome. H-bond belong to a phytosome peak might be around 8ppm on ¹ H NMR spectra. The H-bond can have specific chemical shift due to phytoconstituents.	[68-72]
¹³ C-NMR, ³¹ P-NMR could not give specific data about chemical bonds; they could provide an idea about the existence of a phosphate group, the number and type of carbons. ¹ H NMR might be a fast and straight technique for the evaluation of drugs in L/P structures.			
X-ray diffraction (XRD)	Phytosome	XRD demonstrate a lack or partial crystalline peaks of phytoconstituents, revealing the existence of entrapped structure in phospholipid. XRD revealed reduction in crystallinity of phytoconstituents in availability of phytosome (The intensity of phytoconstituents peak after encapsulation by phytosome significantly decreases and the intensity of phospholipid peak after encapsulation significantly increases).	[66]
Only crystalline phytoconstituents and un-entrapped phytoconstituents are detectable. These structures remained unprotected in solution.			

Table 3. Continued. Evaluation Methods to confirm the presence of a Liposome/Phytosome structure

Evaluation Method	Type	How it to process	Ref
SEM	Liposome/ phytosome	SEM image could show a spherical surface without any impurity of active ingredient on the surface of Liposome/phytosome	[66]
The image could not show the specific vision of phospholipid or active ingredients, but the uniform shape of phospholipid covers the active ingredient in the surface of phospholipid are detectable.			
TEM	Liposome/ phytosome	The particle size distribution investigated by TEM pictures. The existence of a particle might reveal the availability of particles.	[66]
Fluorescence spectrum measurement	Liposome/ phytosome	Fluorescence agent used for labeling of liposome. Check behavior of the encapsulated particle, based on the labeling agent.	[73]
Rhodamine B used as fluorescence agent			
Photon Correlation Spectroscopy (PCS)	Liposome/ phytosome	Generally, provide information about the existence of particle, size and polydispersity of particle size. It might not provide suitable information about the difference between liposome/phytosome and structural information.	[65]
HPLC/UV-VIS	Liposome/ phytosome	The new specific peak or any changes in retention time of phytoconstituents/ drugs in high-performance liquid chromatography shows the existence of new structure coupled with chemical bonds. The decomposition of phytosome structure in the HPLC column is unavoidable, which causes decrease in a peak intense or lack of existence of new peak.	[21,74]
HPTLC	Phytosome	It could be possible with an appropriate mixture of solvents to prevent the bilayer opening	[67]
Electron spin resonance (ESR)	Liposome	Used to determine the chemical composition based on spin labeling technique according to different spectroscopic techniques.	[75,76]
Electron paramagnetic resonance spectroscopy (EPR); A method for material recognition with unpaired electrons. The fundamental theories of EPR are like nuclear magnetic resonance (NMR).			
Tissue-Clearing Techniques	Liposome	Optical tissue-clearing is a combination of fluorescent-labeling and fluorescent proteins methods	[77]
Visualize a three-dimensional distribution of liposome in human body especially in lung.			

1.3.3. New approaches

Taking into account the knowledge about lipid carriers, this section presents recent challenges in the modification of L/P surface, as well as practical examples of more accurate delivery. Almost thirty new approaches in the L/P area are discussed below, in terms of variety of lipid layer and surface modification. They were established to improve the efficiency of these formulations and to achieve different functionalities, being the origin of a new generation of L/P. In fact, based on literature, there are several types of lipid layer as well as various modifications to be applied (e.g. cationic, anionic, emulsion, PEGylating, corona protein, redox responsive, etc.) resulting in numerous liposomal surface modifications.^[58] Chart 1 briefly classify new approaches in terms of lipid layer variety and surface modification, giving a quick overview regarding this variety of liposome.

Liposome		
Variety of liposome lipidic layer		Variety of liposome coating
Cationic Liposome; Cationic Lipid; (DOTMA, DDAB, and DOTAP)	Ethosome; Alcoholic non-toxic Phosphatidylcholine	Biopolymer Conjugated Liposome; natural polymer
Anionic Liposome; Phosphatidic Acid; (DOPG)	Sphingosome; Sphingolipids	Aptamosome; Aptamer- Liposome
Archaeosome; Bilayer Etheric-based	Niosome; Non-ionic Small Sphere	Protein corona-Liposome; Protein corona
Cubosome; Self-assembled Liquid Crystalline	Ufasome; Unsaturated Fatty Acids	Virosome; Proteo-liposome/virus-like Particle
Lipogel; Microgel, Hydrogel, Thermogel, etc.	Cryptosome; Poloxamer (Non-ionic Triblock Copolymer)	Enzymosome; Enzymatic Agent Coating Liposome
Ceresosome; Ceramic or Silica-based Layer	Pharmacosome; Nano-micelle lipidic layer	Vesosome; Multilayer Liposome
Emulsome; Liposomal Layer in form of Emulsion	Colloidosome; Carboxylated polystyrene latex	PEGylated Liposome; PEG400, PEG600, PEG1000
PH-sensitive Liposome; PH-sensitive Polymer or pH-sensitive Phospholipid; DOPE		Transfersome; Edge Activators
Novel Redox-responsive Liposome; Novel Redox-responsive (NRR)		Cholesterol-free Liposome
Pseudo Ceramide liposome; Pseudo-ceramides or ceramide precursors		Photosome; Photolyase (Bacterial Enzyme)
Thermo-Sensitive Liposome; Thermal Sensitive Liposome (TSL)		
Temperature-responsive Reversible Liposome RSL (Reversible Surface Liposome); NICNDAC		
Hybrid Liposome		

Chart 1. Numerous liposome structures reported in literature are characterized by a variety of lipid layers and surface modifications.

1.3.3.1. Cationic Liposome

Cationic liposome is a subgroup of liposome prepared with a cationic (charged) lipid group (amphiphilic lipids) or neutrally charged lipids. 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide [DMRIE], 2,3-bis[oleyl]oxipropyl-trimethylammonium chloride (DOTMA), dimethyl dioctadecyl ammonium bromide (DDAB), 1,2-dioleoyl-3-trimethylammonium propane (DOTAP), dioleoyl-phosphatidylethanolamine (DOPE), dioctadecylaminoglycyl-spermine (DOGS), 1,2-dioleoyl-sn-3-phosphoethanolamine (DOPE) and dimethyldioctadecylammonium (DDA) are examples of synthesised cationic lipid carrier used in liposome structures.^[78,79] Cationic liposome is formed by a positive head group and a hydrophobic lipid group. The positive charged lipid could have a good interaction with the negatively charged cell membranes, increasing the transfer rate from plasma to cell membranes. Cationic liposomes are mainly used for gene delivery, including DNA and RNA in cell cultures.^[78–80] There are also a designed application of cationic liposome to treat cervical cancer, an abnormal growth of cervix cells that could expand to other organs.^[81]

A benzimidazolylquinoxaline structure, synthesised with a benzimidazole and quinoxaline pharmacophores, was designed as anticancer agent to improve there bioavailability. The synthesized structure was loaded on PEGylated liposomes produced with cholesterol and L- α -phosphatidylcholine, modified by thin film hydration method with arginine and 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) as cationic agent. The formed particles, with almost 100 nm and negative zeta potential, were stabilized at room temperature. It showed an effective anti-tumoral effect *in vitro*. 2-benzimidazolylquinoxaline-loaded by PEGylated arginine liposomes demonstrated 16% cell viability at the concentration of 0.15 mg/ml, and less than 40% toxicity against normal human liver cells at the range between the lowest concentration (0.015 mg/ml) and the highest (5 mg/ml).^[80]

In another study, polyethyleneimine (PEI) was used as a cationic polymer to modify the liposome surface of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) by thin film hydration method. A significant increase on calcein penetration (a dye used to reveal some localizations in cell imaging applications) through the cells membrane was verified. The authors also demonstrated the essential role of the polymer size in the DMPC-PEI interaction and the direct impact of the molecular weight on the transfer of the particle through cell membranes in a gene delivery field.^[82]

Other cationic lipid used to design an efficient liposome on Biomolecular & Biomedical Imaging (BBI) is DOTAP (1,2-dioleoyl-3-trimethylammonium propane). DOTAP-liposome was loaded with two Bowman-Birk protease inhibitors (BTCL, Black-eyed pea Trypsin and Chymotrypsin Inhibitor) by the reversed phase evaporation technique, followed by freeze-drying. It was possible to entrap 100 μ M of BTCL on liposome without affect it protease inhibition activity. The results revealed a multifaceted potentiality of the cationic-liposome, with a positive impact on protein delivery in a BBI biomedical application. It also seems an interesting idea for further investigation on delivery of BBI biomedical functionalities.^[83]

In other research, a size-adjustable cationic liposome was produced with different cationic lipids such as DOTAP, dimethyldioctadecylammonium (DDP) and DOPE, and loaded with marrow-derived macrophages. Cationic liposomes size was less than 750 nm due to the control of the aqueous buffer's volume and concentration on preparation procedures. In *in vitro* investigation occurred the cellular uptake of small particles (less than 500 nm) and the liposome played a similar performance in what concerns to percentage and fluorescence intensity on bone marrow-derived macrophages (BMDM). In other hand, an *in vivo* investigation showed a liposome particle less than 50 nm modify the clearance rates from the injection site and increase accumulation to the draining lymphatics.^[84]

Other example is the cationic liposome-stearylamine proved to be an effective anti-virus carrier through a brain membrane. Indocarbocyanine, a cationic dye (1,1'-dioctadecyl-3,3,3',3'-tetra-methylindocarbocyanine perchlorate, DiI), was retained in the liposomal lipid bilayers formed by combination of dicetyl phosphate (DCP) and cholesterol. The mechanism of action revealed the cationic stearylamine-liposome was directly linked to a cell membrane, blocking the penetration channel of the baculovirus (BV) and suppressed it infectivity in several mammalian cell lines, including A549 cells by more than 80%. So, stearylamine-liposome inhibit the growth of BV infection and herpes simplex virus type 1 showing antiviral effectiveness similar to acyclovir. The authors also proved that cholesterol could improve the probability of the liposome binding to the cell membranes.^[85]

In order to compare the behavior of such structures, three liposome categories for oral application (anionic, cationic, and pegylated liposomes) were designed and verified their efficiency on drug loading, through a epithelial monolayer (a minimum dosage with a maximum efficiency). The cationic liposome was transported almost ~50% through tripler epithelial culture models. It was proved liposome modification could be an

excellent strategy to optimize integral transport and cellular uptake of the active ingredient across the cells.^[86]

1.3.3.2. Anionic Liposome

The anionic liposome is another surface modification used often for macromolecules transferring since it helps to overcome several restrictions on the target delivery. Thereby, the anionic liposome improves stability, decreases particle aggregation, and enhances endocytosis in solution, comparatively to the cationic one. Anionic liposome headgroup and the phosphate group of macromolecules like DNA and RNA have the same negative charge, which causes a repulsion force between them. Consequently, anionic liposomes are not as efficient as cationic or neutral ones in gene delivery. Lipid compounds normally used in anionic liposome are phosphatidic acid, 1,2-dioleoyl-sn-glycero-3-phospho-*rac*-1-glycerol (DOPG), phosphatidylglycerol and phosphatidylserine.^[87,88] *In vivo* and *in vitro* studies proved the significant extension on adenovirus-mediated gene expression regarding the airway epithelia when used a combination of anionic liposomes with adenovirus for therapeutic gene treatment. Furthermore, immune-protection properties of anionic liposomes were proved in this studies, as well.^[89]

Anionic liposome proved to enhance the delivery of DNA into mammalian cells with less toxicity. The liposomal layer is a complex between anionic lipid (DOPG), zwitterionic lipid (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine, DOPE) and DNA molecules encoding green fluorescence protein (GFP) with Ca^{2+} ions. *In vitro* studies demonstrated the lower toxicity of anionic liposome compared with cationic one, and a safe delivery and efficient transfection of DNA. It was also reported that anionic liposome coupled to the Ca^{2+} complex could be a promising candidate on gene delivery.^[90]

An anionic liposome for lymphatic targeting was designed with phosphatidylserine, applying microfluidic techniques. Drug accumulation in target cells increased 10-fold due to the use of microfluidic techniques to entrap loaded proteins in liposomes. Considerable retention of liposomal layers and entrapped antigens (protein) was observed from anionic lymphatic liposome. Herein, anionic lymphatic liposome proved to be a suitable carrier not only for vaccination and cancer therapy, but also antigen protein delivery.^[91]

1.3.3.3. Liposome/Phytosome Emulsion (Emulsome)

Most of researchers prefer to develop L/P (totally encapsulated particle) in powder

form. Nevertheless, liposome is also frequently prepared in the emulsion form. In fact, L/P emulsion is a common technique applied to a wide range of active components, from drugs to clinically approved natural compounds (especially lipophilic ones), and vaccines. For this purpose, it is possible to produce an oil in water (O/W) emulsion for lipophilic compounds by supercritical-assisted technique with high protection of the active components and maintaining up to 99% of their biological activities.^[92] Therefore, liposome emulsion becomes a different strategy for the delivery of oily formulations. Furthermore, some researchers name L/P emulsions as *Emulsomes*. The lipids of the liposomal structure, like lecithin and cholesterol, stabilize the emulsion in form of liposome, covered by a solid lipid layer. These structures are a liposomal emulsion, dispersed in an aqueous solution with a solid-lipid bilayer obtained by sonication.^[93] Ucisik et al. introduced an especial solid-lipid liposomal structure, with a protein and glycoprotein called *S-layer coated emulsome*, which provides three independent layers: a solid-lipid inner layer, a liposomal layer and a crystalline protein lattice covered layer. This structure aimed a specific protection of drugs or active constituents to act, without any side effect, against various diseases like viral and fungal infections or to be efficiently used in cancer, autoimmunity, and dermal therapies.^[94]

Resveratrol encapsulation by liposome emulsion is other example in this area. To prepare a stable nano-sized particle, with high efficiency and considerable antioxidant activity, a resveratrol solution was homogenized and sonicated. *In vivo* tests revealed a positive impact of the resveratrol in liposome emulsion, limiting the neointimal hyperplasia on arterial injury rats.^[95] Other example is a silybin-emulsome, designed by thin film dispersion. This structure showed to be efficient on target delivery *in vitro* and *in vivo* on rat intravenous injection. A considerable increase on accumulation of silybin in target cells (almost 2 - 2.5 fold) compared to unprotected silybin was obtained. Silybin-emulsomes attain the objective to sustain release about eight hours after an initial burst release *in vitro*.^[96]

1.3.3.4. Biopolymer Conjugated Liposome/Phytosome

Biopolymer conjugated L/P is a new combination of different biopolymers coating lipid bilayer to enhance the positive features of natural carriers and improve the protection of the lipid structure. This combination makes possible to obtain better encapsulation functionalities and enhance release times.^[97] It is noteworthy that chemical bonds between the liposomal layer and the biopolymer is not necessary. They could get shape individually or in complex, depending on the preparation

method. Figure 2 shows, in detail, the structures that can be formed by a biopolymer conjugated L/P. The biopolymer external layer plays a vital role to increase the delivery efficiency due to improvements in bioavailability, stability, release time and mechanism. The biopolymer coated liposomal layer could decrease/increase particle size (due to function) and definitely improve homogeneity (similar distribution) in the particle size. In sum, biopolymers are used to protect the structural stability of the phospholipid bilayer and to control drug release rate.^[97,98] The most popular biopolymers as encapsulating agents on target delivery are polysaccharides, proteins and lipids. They could be used in combination or individually based on preparation technique. Polysaccharide-based biopolymers include eight main groups: starch, cellulose, pectin, chitosan, alginate, gums, sodium tripolyphosphate and sucrose.^[99] Each group have several subgroups; as example, starch comprises dextrin, maltodextrin, β -cyclodextrin, modified starch, and hydroxypropyl cyclodextrin.^[100,101]

Protein-based is another group of common co-encapsulation (co-carrier) agents such as whey protein, wheat gluten, milk powder, milk, soybean, caseins, gelatin, soy protein, and cereal protein.^[102,103] The last group of natural biopolymers is lipid-based biopolymers described in the Section 2.3. For instance, gum arabic, whey protein, and chitosan are active biopolymers used to modify the surface of liposomes loaded with catechins (a main bioactive ingredient of *Camellia sinensis*-green tea^[104,105]) aiming to enhance the liposome features. The results showed an excellent improvement on stability of liposome-biopolymer structure, compared with the conventional ones.^[106] The tricarbo-cyanine dye (ICG, Indocyanine green) is used for diagnostic processes, like cardiac output or liver function, ophthalmic angiography, and also for photo-controlled target delivery. The conjugation in a Chitosan-coated liposome loaded with indocyanine green (CLICG) enhances the structural feature, improving the functionality comparatively to pure ICG. After clinical investigation, ICG and CLICG were recognized as suitable for melanoma photodynamic therapy (PDT) due to their penetration through melanoma cells. Chitosan-coated liposome showed a significant increase of ICG by skin permeation of near 150 nm size particles (between 150 - 50 nm permeate through *stratum corneum*, while less than 50 nm could enter the underlying layers of tissue). Also CLICG facilitated the cellular uptake and skin permeation of ICG as well as provided stable particles from an unstable photosensitizer.^[107]

Chitosan was also used to modify the surface of a phytosome loaded with curcumin. The authors proved that this structure had better oral absorption compared to a conventional phytosome. Chitosan-modified phytosome showed better bioavailability,

stability, and appropriate release time and profile.^[108] Cyclodextrin conjugated liposome loaded by quercetin provided a long-storage stability (almost one year), enhanced stability in simulated gastric media and improved protection against UV rays.^[109] Zein, a protein based biopolymer, was used as liposome shape modifier by electrostatic interaction between lipid layer and zein hydrolysates. The authors verified a considerable change on the stability of the active ingredient, in the nucleus of liposome, due to the zein protection.^[110]

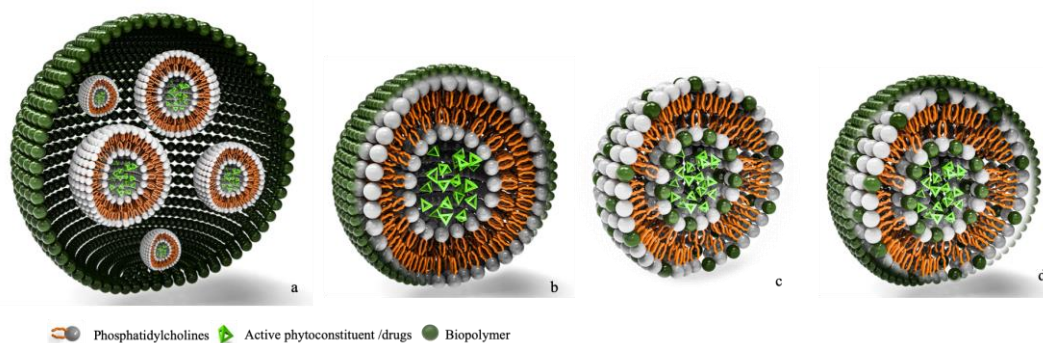


Figure 2. The different conjugated L/P, also called biopolymer coupled L/P, are influenced by various conditions such as preparation method; amount, type and feature of biopolymer; time, intensity, and power of sonication.

Regarding Figure 2, biopolymer conjugated L/P is shaped based on four categories that directly affect size, shape, and capsule layer. (a) - structure resulting from L/P aggregation and the biopolymer layer covers the small L / P groups (in different sizes). (b) - structure resulting when the L/P, small or big, is individually covered by the biopolymer. (c) - structure resulting if the biopolymer in the sonication step is added to the mixture of L/P and directly adjusted on the backbone of the original L/P structure. (d) - the structure occurring when (c) is covered by a second layer of the biopolymer.

Moreover, the occurrence of all these structures is probable during the preparation condition. In conclusion, by optimizing the preparation method, it is possible to reduce the formation of (a), but it is not possible to total elimination. In addition, by adding the biopolymer in the last step of the preparation method, it could be possible to reduce (c) formation, but it is impossible to eliminate it, as well.

1.3.3.5. PEGylated Liposome

PEGylation is a technique used to improve the efficiency of the liposome structure and can use several types of PEG, like PEG400, PEG600, PEG1000 or any type of synthesized or functionalized PEG derivatives. They can be incorporated into the

liposomal layer, according to different strategies, like the synthesis of a PEG phospholipid conjugate^[111] or PEG as the main ingredient of the liposomal layer. Typically, the PEGylated-liposome is obtained through combination of cholesterol and PEG derivatives by thin-layer hydration and post-insertion, followed by extrusion. Post-insertion procedures were, indeed, announced as a suitable technique to formulate PEGylated-liposome structures.^[112] PEGylated-liposome is able to encapsulate various drugs, like doxorubicin and arginine-glycine-aspartic acid, preventing significantly the toxicity, due to the optimised dosage decrease, whereas improve its anticancer activity.^[113]

Ciprofloxacin loaded on a PEGylated azide-modified liposome, obtained by thin-film hydration, was also reported as effective for programmable target delivery. This structure delivers the antibiotic, enhancing its anticancer activity in minimum dosages.^[114]

Several researches also reported the design of modified liposomes with functionalized PEG and indocyanine green (ICG) as the light sensitizing or Light-triggered delivery, not only to stabilize the functionality of core material, but also to improve storage stability and release content. They consider this procedure as a new approach to formulate structures for medical diagnostic using PEGylated-liposome.^[115,116]

A PEGylated liposome functionalized with ammonium bicarbonate was synthesized to enhance the intracellular delivery of loaded anticancer agents and increase the stability of the structure in the chemotherapy blood environment. The presence of an imine bond (pH sensitive) provides a specific binding between imine group and the cell target. Therefore, carbon dioxide production occurs when the imine group joins to the target and the liposome opens directly on the surface of the cell, favoring largely the release of doxorubicin intracellularly in a cell culture. The lack of ability to control the endosomal escape as well as the intracellular traffic via cellular uptake, could be considered important disadvantages of PEGylated delivery systems.^[117]

1.3.3.6. Temperature-responsive Reversible Liposome/ Thermo-Sensitive Liposome

Thermal sensitive liposome (TSL) is another natural vehicle typically used as a drug delivery carrier, not only able to catch active compounds but also to deliver encapsulated agents upon moderate hyperthermia. The TSL, in response to a change of temperature in a mild-hyperthermia range, possesses an appropriate release profile of the encapsulated drugs. Moreover, the TSL prevents premature leakage of an encapsulated

drug at physiological temperatures. Based on the release mechanism of the drug, upon heating, thermal-sensitive liposome can be further classified as conventional thermal sensitive liposome (CTSL) or lysolipid incorporated thermal-sensitive liposome.^[118,119] Pluronic F127, a protein-based detergent, is an example of a thermo-sensitive agent used to cover liposome surface by condensation reaction. An appropriate release, due to temperature changing around target cells was observed from encapsulated cinnamon by Pluronic F127 coupled to liposome.^[118] Iron oxide was encapsulated in thermo-sensitive nano-vehicles with carboxyfluorescein as a model drug carrier. The nano-vehicle proved to deliver the drug in the target cells in a thermo-sensitive media.^[119]

Reversible surface liposome (RSL) structure is another novel temperature-responsive reversible technique. A temperature-sensitive polymer introduced by Nemoto for the first time, constituted by repeated units of N-isopropylacrylamide-co-N, N-dimethyl acrylamide (NICNDAC), has the capacity to reverse the surface properties with temperature changes. NICNDAC is a rigid intracellular lipid-based temperature-responsive carriers potent for different delivery systems.^[120]

1.3.3.7. Novel Redox-responsive Liposome

Reduction-responsive is a cross-linker able to join to liposome. The novel redox-responsive (NRR) structure contains two hydrophilic head groups, similar or different, connected by a hydrophobic linkage. NRR structures are prepared with amphiphilic-synthesized polymers. These structures are typically used for delivery of positive charged drugs, like enzymes and genes (SiRNA and DNA).^[121,122]

They are used to “enhance permeability and retention” in cancer or gene therapy.^[123] Another application reported is the NRR coupled with liposome (NRR-L) as a particular and accurate technique to deliver a therapeutic agent to the target cell, improving bioavailability in the field of cancer therapy.^[124] Doxorubicin loaded to multidrug resistant nano-micellar particles (NRR-LDX) showed a significant improvement in liposome efficiency in this area. It also accelerated the release time and had a positive effect on the disappearance of multidrug resistance cancer cells.^[125]

Other approach was the NRR-L as a carrier loaded with paclitaxel (a diterpenoid alkaloid with antitumor properties, from the bark of *Taxus Brevifolia*.^[126]). The results demonstrated a high loading capacity and efficient anti-proliferative activity of NRR-paclitaxel and a considerable improvement on paclitaxel reduction triggered

release.^[127]

1.3.3.8. pH-sensitive Liposome

pH-sensitive liposome was designed to overcome the penetration of large size or hydrophilic capsules by acid-triggered destabilization. This family of liposome was prepared with a synthesized sensitive lipid, which is not stable to pH media changes. Although the mechanism of pH-sensitive carrier still unclear, these structures need to be stable in serum but unstable in the cancer cell membrane. So, the capsule opens and the entrapped content is released on the targeted media by pH changing.^[128] The pH-sensitive liposomes have a backbone of a pH-sensitive polymer or pH-sensitive phospholipid, with a polyelectrolyte group containing an ionizable functional group, allowing the programmable drug release especially on acidic media.^[129] The ionic group is affected by the pH medium, changing the shape and morphology of the liposome structure, according to the media, the pH-sensitive polymers or the phospholipid in the structure.^[130]

Hyaluronic acid (HA, Hyaluronan) is naturally present in human skin to trap water and keep skin fresh and alive, being used for some therapeutic effects. It was encapsulated in a pH-sensitive liposome with dioleoylphosphatidylethanolamine (DOPE), by thin-film hydration, aiming an effective impact on pancreatic cancer. *In vitro* experimental data revealed that the HA-pH-sensitive liposome enhanced cytotoxicity and increased cellular penetration. This action allows to overcome gemcitabine resistance by CD44 expression, a receptor for HA and drug transporters, and endosome escape aiming to have an efficient intracellular delivery.^[131]

Antisense oligonucleotides (AOs) are another example of powerful medicines in the field of gene/cancer therapy, as well as viral infections. In contrast, AOs are not stable in the *in vitro* media. Antisense-pH-sensitive liposome releases the core material in response to acidic media within the endosomal pattern while remaining stable in plasma. Therefore, pH-sensitive liposome loaded with AOs proved to be promising carriers to increase the cytoplasmic delivery of oligonucleotides after endocytosis.^[132]

Liposome coated with folate is another approach in this area. Some researchers evaluated the impact of folate in the biostability improvement and efficiency of liposome in cancer therapy. Doxorubicin was loaded on liposome coupled with folate by two different ways: PEGylated and pH-sensitive liposome. The pH-sensitive liposome approach showed to deliver effectively doxorubicin (DOX) to tumors cells, and simultaneously reduced the toxic effects. The results showed more than 60% of growth

inhibition in cancer cells, cytotoxicity activity, and a considerable reduction of pulmonary metastasis foci.^[133]

1.3.3.9. Ethosome

Ethosome is a new alcoholic (prepared with ethanol) carrier, non-toxic and flexible, for amphipathic drugs delivery (Figure 3). It has an appropriate structure to couple to hormones, cannabinoids, proteins and also polyphenolic compounds. The high penetration efficiency is a distinctive of these structures comparatively to the conventional liposome structure.^[134] The main composition of ethosomes includes phosphatidylethanolamine, phosphatidylcholine, ethanol and water, as the liposome structure. The typical preparation procedures are the same used for liposome, with some modifications in the hydration step that combines buffer solution and different percentages of ethanol.^[134,135,136]

An ethosome loaded with a salicylic acid derivative demonstrated to be efficient as transdermal carrier for delivery of the drug, improving its analgesic and anti-inflammatory activity.^[137] High skin penetration rates and safety of this carrier in transferring drugs into the pilosebaceous unit of the scalp to treat androgenic alopecia was reported.^[138]

1.3.3.10. Transfersome

Transfersome is an ultra-flexible liposome structure that significantly enhance drugs permeation through skin (Figure 3). The transfersome with a trademark of IDEA AG, Munich, Germany, has a specific structure, including an external layer that passes directly through the *stratum corneum* cells layer via an osmotic gradient.^[139] The typical preparation methods are the same used for liposome with some hydration modifications. In the case of transfersome, hydration steps occur by combination of buffer solutions and different percentages of edge activators like sodium cholate (SC), sodium deoxycholate (SDC), or sodium taurocholate (STC). The presence of SDC in some transfersome structures enhance the permeability, safety, and speed of compounds delivery, comparatively to other edge activators. It does not only deliver drugs to the targeted cells, but also pass through the lipid bilayer to specific organ cells, due to hydrostatic force. Therefore, this specific deformable-high-flexible liposome structure enhances the *in vitro* and *in vivo* skin delivery of a wide range of active ingredients/drugs.^[140] For instance, dexamethasone encapsulated according to transfersome technique proved to have a significant transdermal effect compared to conventional liposome.^[141] Diclofenac-transfersome capsule revealed a drug

availability in skin culture 10-fold higher than conventional liposome. Therefore, the meloxicam-loaded transfersome demonstrated to be a powerful delivery carrier with a high *in vitro* skin permeability.^[142] In addition, meloxicam-loaded cationic-transfersome showed a high permeability efficiency in *stratum corneum* compared to normal transfersome. The efficient change on skin permeability, suggests this structure can be a suitable candidate for an effective delivery of meloxicam through the skin.^[143]

1.3.3.11. Niosome

Niosome is a liposomal non-ionic small sphere with a double layer that improves stability (higher storage time) and bioavailability (Figure 3).^[144,145] At the surface, a non-ionic surfactant replaces the phospholipid layer present in liposome.^[145] Tween 60, tween 80, span 60 and span 80 are non-ionic surfactants typically used in niosome surface structures, in combination with cholesterol or phosphatidylcholine and cholesterol.^[145,146,147] If this structure is not hydrated takes the denomination of Proniosome. Sonication, micellar solution, micro fluidization, thin-film hydration, reversed phase evaporation, ether injection and trans membrane pH-gradient are typical methods used for niosome preparation.^[144]

Niosome has higher drug loading efficiency, higher stability (due to the surfactant-based surface), higher drug compatibility, and better pharmacological and biological activities, compared to the conventional liposome.^[145,148] Vancomycin and acyclovir encapsulated as niosome proved better physical and chemical compatibilities to keep antibacterial activities comparatively to unprotected form (almost 48 hours). Therefore, niosome could prevent drug incompatibilities, with a good therapeutic outcome in clinical trials. Besides to improve considerably the stability, it reduces adverse interactions and hospitalization periods.^[149] High entrapped efficiency of nanosized niosome was also reported regarding microfluid technique.^[150]

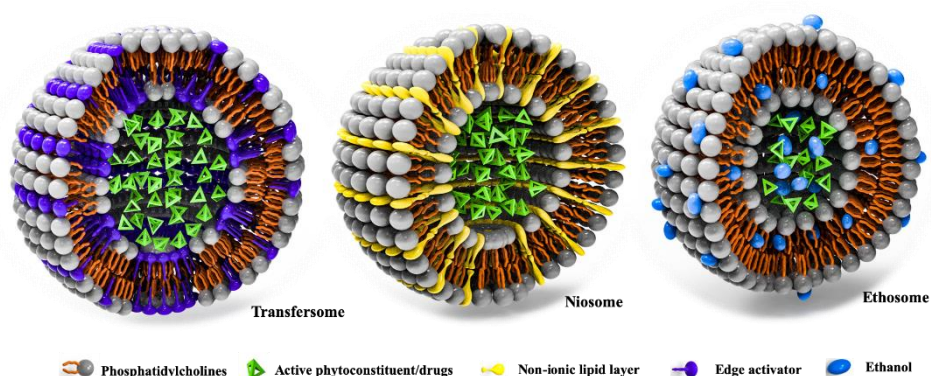


Figure 3. Liposome surface modifications based on type of liposomal bilayer.

1.3.3.12. Sphingosome

Sphingosome is a liposomal bilayer vesicle that contain sphingolipids entrapping bioactive compounds. It is another vesicular bilayer (liposome-backbone), patented since 1996.^[151] The most distinctive feature among liposome and sphingosome is the use of sphingolipids (natural/synthetic) in the liposomal structure, instead of the typical phospholipids with an aqueous content surrounded by the lipid bilayer. Sphingosome could cover a critical problem of liposome and their subgroups, like enhancing the entrapped capacity of drugs, especially for anti-cancer compounds. It improves the stability against acidic environments and increases release time on cell media (*in vivo* circulation time). It may also be a suitable carrier for chemotherapeutic agents, biological macromolecules or diagnostic agents due to flexibility in size and structural composition. These structures are much more efficient when conjugated with cholesterol, in a molar ratio between 75:25 and 55:45.^[152]

1.3.3.13. Protein Corona-Liposome

Protein corona is a structure with a dynamic amino acid sequence conjugated to a liposome, used to monitor the liposome action *in vivo* and to predict the strategic mechanism of the lipid-based nanoparticle. This structure allows benefiting of the thermodynamic and kinetic features of protein conjugated to surface of nanoparticles like liposome. Thereby, the efficiency of protein corona to modify a surface of liposome was proved, not only as a diagnostic monitoring agent but also as a novel carrier. Inclusively, the functional efficiency of protein/peptide conjugated nanoparticle improve the surface stability and accessibility of the liposomal structure, as well as the structural orientation, effects proved in diagnostic analysis. Thereby, protein corona, joined at an external double-layer in the surface of nano/micro particles, also could act as a marker to enhance their adsorption to target cells.^[153] Protein corona couples to a nanoparticle surface via protein-protein or protein-particle interaction (Figure 4). The modification of the protein sequence arrangement and concentration affect the particle functionality, promoting specific pharmacological purposes, distribution, and toxicity.^[153,154] Protein corona have two main sub-groups, hard protein corona (HPC) and soft Protein corona (SPC), according to the affinity of protein bonds to the nanoparticle surface. HPC has a substantial direct interaction on the nanoparticle surface. In contrast, SPC has a reversible interaction that can change or renew along the time. Albumin, fibrinogen and immunoglobulins are proteins generally used on a corona sequence.^[154,155] Moreover, PEGylated liposome coated by protein corona proved to be a promising strategy to control protein adsorption, affording

tailored cellular nanoparticles uptake for vaccine delivery. The interaction of this protein with liposome surface could occur by hydrogen bonds, solvation forces, and Van der Waals interactions.^[156] The interaction of protein corona and liposome, as well as the effect of protein corona in liposome function, were proved by a specific adhesion in the surface of liposome on tumor cells, as well as inherent macrophages permeation. The presence of such protein in the liposome structure improves the capacity of target delivery.^[157]

Colapicchioni and collaborators formulated an HPC nanoparticle and studied how plasma collected from breast, gastric and pancreatic patients could alter the corona structure and composition. The results revealed that different disorders affected differently protein corona of liposome. They also demonstrated that corona-protein-liposome is not only efficient in the early diagnosis of cancer, but also could be used as a promising candidate for the target delivery of nanomedicines.^[158]

In another study, protein corona was applied on a surface of PEGylated-liposome (stealth liposome) aiming a targeted nanomedicine delivery. No changes in the backbone of PEGylated-liposome during functionalization with corona protein was verified. Small doses of PEGylated-liposome functionalized by protein corona improved the efficiency of delivery, as demonstrated in *vitro* studies in fetal bovine serum and *in vivo* in the bloodstream of mice. Non-targeted and antibody-targeted HPC-liposome did not change the liposome structure and functionality.^[159]

Protein corona coupled to PEGylated-liposome was evaluated by shear stress in another study. The surface of PEGylated liposomes, modified by protein corona, produces a complex biomolecular layer, with a backbone of protein corona. It was verified that the corona of PEGylated liposomes could be considerably distinct from the one formed on static fluid. Therefore, the effect of shear stress on the corona protein modified liposome is not easy to predict, due to various arrangement of corona protein in liposome or PEGylated liposome. Although reported that the strategy to evaluate shear stress do not allow a correct and logical answer on protein corona function, a significant difference in static fluid form was obtained, allowing to find the specific point of shear stress on the formation of protein corona on the surface of PEGylated-liposome.^[160]

1.3.3.14. Pseudo Ceramide Liposome

Ceramides are essential lipids from the membranes of *stratum corneum* cells, being fundamental barriers to moisture-retention skin functions.^[161] Pseudo-ceramides or ceramide precursors are synthetic lipids, used generally in dermocosmetics, in skincare

and antiaging treatments to improve skin elasticity, smooth out the wrinkles, or even improve hair softness. 1,3-bis-(N-(2-hydroxyethyl)-palmitoylamino)-2-hydroxypropane (PC-104) and N-(3-hexadecyloxy-2-hydroxypropyl)-N-2-hydroxyethyl hexadecanamide (SLE) are pseudo-ceramide examples, with similar functional properties.^[162,163] Regarding the formation of ceramides, two groups are involved (a polar and a non-polar), a desirable feature to assemble liposome structures.^[162] This double function structure coupled to liposome aims to enhance the active ingredient permeability, becoming a structure to be considered for dermatology functions. Despite the various advantages of ceramide-based particles, these molecules in water-based solutions immediately catch each other to develop ceramide molecular crystals, causing instability on the formulation and a serious limitation to broaden their formulations. For this purpose, pseudo-ceramides-liposome formulated by combination of pseudo-ceramides, fatty acids and cholesterol as a biocompatible controllable membrane stability was designed to overcome a wide range of skin disorders, to repair the skin barrier, and to improve the active ingredient bioavailability, aiming to be used in target delivery, dermatology as well as cosmetics and skincare products.^[164,165]

A baicalein-pseudo-ceramide-liposome, formulated with synthesized pseudo-ceramides, is a natural antioxidant and anticancer agent to overcome disadvantages of synthesized ceramides (pseudo-ceramides) and to improve the natural properties and stability of the compound. A considerable enhance was reported on the stability of baicalein loaded on synthesized ceramide compared to natural one. A better delivery performance was also verified with the synthesized pseudo-ceramides-liposome.^[166]

1.3.3.15. Aptamer-Liposome (Aptamosome)

Aptamer is a peptide sequence from DNA or RNA, with a specific performance proved in both *in vivo* and *in vitro* media (Figure 4).^[167,168,169] It promotes the linkage in specific cells, expressing specific proteins with higher specificity for controlled target delivery in cancer cells.

A specific binding between the amino acids (amine or sulfhydryl groups) and lipids allows the suitable linkage to synthesize the referred structure.^[170] Targeted aptamer-liposome loaded with doxorubicin was formulated to attain the cell nucleus for treatment of breast cancer. The structure successfully passed the cell membrane, linked to nucleolin and successfully released doxorubicin and absolutely could inhibit

the growth of cancer cells.^[167]

A cationic aptamer-liposome was designed and loaded with natural antibiotics (doxorubicin and tobramycin) and a specific DNA sequence at a ratio of 1:15 doxorubicin via liposome. The loading capacity was enhanced 6-fold comparatively to tobramycin loaded in liposome. It was proved that the aptamer-based structure could act as a multifunctional or multicomponent drug vehicle.^[171] In conclusion, the aptamer-liposome considerably eliminates side effects, significantly reduces the drug toxicity either on minimum dosage, and enhances the specific targeted delivery with a considerable high load efficiency.^[172] Specific sequence of DNA-aptamer-liposome was effective *in vivo* on mouse tumor endothelial cells (mTECs). The results revealed a positive impact of aptamer-liposome in the delivery of anti-angiogenesis agents to overcome the tumor.^[173]

1.3.3.16. Ceresosome

Ceresosome (bicelles) is a ceramic or silica-based layer used for coating liposome for a broad range of biological applications, from diagnostic to therapeutic activities, functioning as organic-inorganic nano-hybrid (polyorganosiloxane) carrier.^[174,175] It is a spherical ceramic-lipid layer, synthesized by intramolecular hydrogen interactions between the polar group of the phospholipid and the silicon site of silica or a connector between lipid chain and silicon site, covering a polar group in nucleus.^[175,176] Ceresosome, was also called a *cell-friendly drug carrier* by Kikuchi and Yasuhara.^[175]

A redox-responsive-nano-hybrid (RRNH) ceresosome was developed by the ethanol injection method, with detachable disulfide bonds as connector unit of the triethoxysilyl head and the hydrophobic alkyl double chain. RRNH-ceresosome showed a high loading efficiency, high morphological stability and no toxicity on non-tumor cells (human embryonic kidney 293 cells), revealing a high biocompatibility on human cells. Furthermore, 50% inhibition for 48 hours was obtained against human hepatoma SMMC-7721 cells and breast cancer MCF-7 cells *in vitro*. The higher morphological stability, compared with conventional liposome, was observed due to the hydrogen detachable binding through the important sites of ceresosome structure.^[177]

1.3.3.17. Ufasome

Ufasome is a liposomal vesicular delivery carrier formulated with unsaturated fatty acids, aiming the protection of the bioactive compounds. The fatty acid tails and cholesterol covers the bioactive compounds by various bilayers. In general, liposome follow a *tail-to-tail* shape, but ufasome has a molecular *side-by-side* shape pattern in

the bilayer membrane. Linoleic acid and semi-synthesized derivatives are typical ufasome carriers. This structure is a pH-limited liposomal carrier (pH 7-9) with good stability, long and sustained release time and a profile directly dependent on the fatty acid type, concentration of cholesterol, type of buffer solution, concentration of lipoxygenase, and presence of divalent cations.^[178,179] Therefore, it is a flexible drug carrier, without need to be coated by natural polymer or protected by cross-linker, especially as food additives in food product industries.^[178]

1.3.3.18. Pharmacosome

Pharmacosome is the most similar liposomal structure to phytosome due to the chemical bonds between the lipid part and the bioactive compounds. They are nano-micelle type particles constituted by a polar group with an active hydrogen site to join to the non-polar site of phospholipids.^[180] It is a powerful active drug loading vesicular carrier, with enhanced stability, increased bioavailability and permeability of drugs. This technique could be used for both synthetic drugs and natural phytoconstituents.^[181,182]

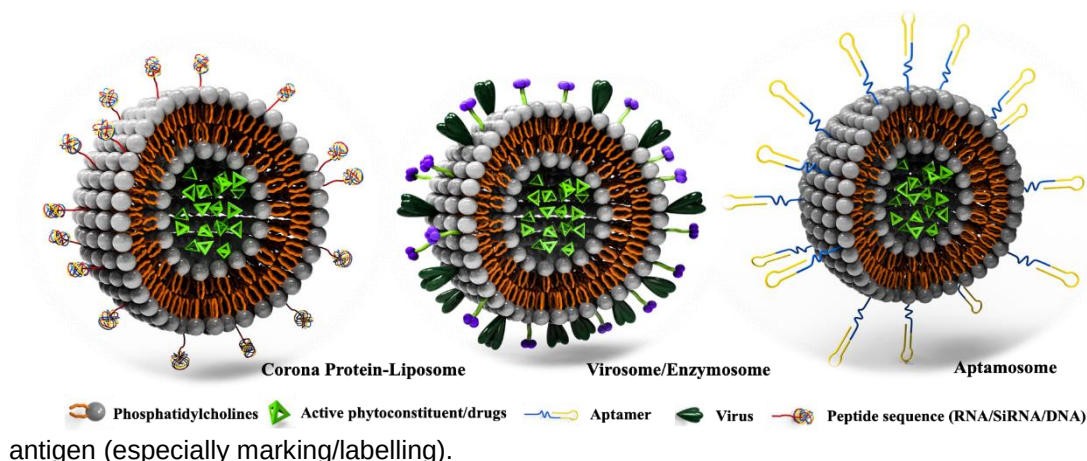
1.3.3.19. Virosome

Virosome (proteo-liposome/virus-like particle) is another liposomal vehicle suitable for vaccines against viral macromolecules (Figure 4). Virosome is a unilamellar phospholipid covered by antigen, glycoprotein, or special protein sequences (hemagglutinin, HA; and neuraminidase, NA) containing reconstituted virus (normally influenza), requiring the genetic material of the native virus suitable for drug and especially vaccine delivery.^[183] Specific labeling of virosome allows delivering the vaccine directly into the target cells. Virosome is predominantly used for delivery of influenza virus, being reported as homogeneous nano-scale particle with high entrapment efficiency, biocompatibility, biodegradable and non-toxic.^[184] Their carrier function covers the positive effects of placing the antigen into a leading delivery structure. Immune-stimulating features is an adjuvant function of virosome, stimulating specific immunity without non-specific inflammation. Virosome was reported as powerful candidate for multi-component dual functional vehicle, acting as a carrier and adjuvant on target delivery systems.^[185] Due to similarity of influenza to Covid-19 virus, the following technique might be a promising technique for delivery of Covid-19 vaccines.

1.3.3.20. Enzymosome

Enzymosome is an enzymatic site-specific target liposomal carrier, aiming to have enzymatic activity (Figure 4).^[186] A specific covalent bond is established between the liposome surface and the enzymatic agent. Indeed, a direct acylation and a physical absorption/adsorption, followed by a general encapsulation technique, allows obtaining the enzymatic liposome structure.^[187] An enzymosome was used as adjuvant to treat arthritis in an *in vivo* model. The enzymatic surface-exposed liposome shows the possibility to formulate a modified liposome with enzymatic activity. The covalent bond between fatty acid and enzyme originates a hydrophobic enzymatic surface that promotes the conjugation of an enzyme to surface of liposome, keeping the catalytic activity. A PEGylated enzymatic liposome was the best way to deliver the enzyme into inflammation site and transfer it smoothly into inflammation sites. Also, the PEGylated enzymatic liposome provides a sustained release time with a considerable enhance on enzymatic dosage into inflamed areas, increasing the anti-inflammation activity.^[188]

Figure 4. Liposome surface modifications based on peptide, protein, gene (DNA/RNA) or



1.3.3.21. Cryptosome

Cryptosome, also known as stealth liposome or ninja liposome, has a major part of its surface with PEGylated liposomal structure. The stealth liposome is covered by non-ionic triblock copolymer (Poloxamer) to get a micellar shape.^[189,190] Aiswarya and coworkers reported cryptosome as a powerful structure to overcome critical disadvantages of liposomal structures. Indeed, they found that cryptosome could improve the short-time stability of liposome, increase release time, and significantly decrease the fast metabolism of drug/active ingredient encapsulated by liposome.^[190]

1.3.3.22. Colloidosome

Dinsmore and co-workers introduced colloidosome for the first time in 2002. It is a

colloidal particle prepared with carboxylated polystyrene latex, that cover W/O or O/W structures.^[191] The arrangement of colloidosome requires a mechanism for shell coating at the interface, to convert the emulsion precursor into an energetic particle that can sustain the W/O or O/W removal. Polystyrene latex particles should protect the core material in a covering homogeneous monolayer.^[192]

1.3.3.23. Photosome

Photosome is a remarkable type of liposome with a bacterial enzyme, called Photolyase, in their surface. Photolyase is able to regenerate the cycle-butane pyrimidine dimers (CPD) in eukaryotic cells originated by UVB.^[193]

1.3.3.24. Vesosome

Vesosome is a multilayer liposomal structure arranged by liposome layer inside another liposome structure. Although GUVs are giant unilamellar micelle shaped vesicles, vesosome is another giant liposomal structure. According to Huisoo and co-workers, vesosome is a suitable technique for serum application with a most uniform nano-size structure, fabricated by continuous flow microcentrifugation and proved by fluorescence microscopy.^[193] Multi-liposome layer (vesosome) loaded by doxorubicin could avoid the fast release of the entrapped drug and avoid any unwanted interaction during time periods. The potent anti-tumor activity of doxorubicin-vesosome against HeLa tumor-bearing nude mice as well as optimized release time was confirmed.^[194]

1.3.3.25. Archaeosome

Archaeosome is a bilayer etheric-based liposomal structure containing archaea bacterial-etheric lipids in their composition. It is a cholesterol-free structure which acts as a thermo-adaptive agent.^[195,196] Lipid film hydration, sonication, extrusion and detergent dialysis were the most popular preparation methods of archaeosome. These structures are more stable than other liposome structures, with a long-time *in vitro* stability (almost one or two years).^[195] According to Benvegna and co-workers, the type of archaeosome directly depends on the microbial origin of the extracted lipid used in liposomal surface. These authors also revealed the high stability of these specified structures against the typical instability of nano/micro encapsulated particles.^[196]

1.3.3.26. Cubosome

The next generation of smart lipid-based structures are cubosome, asymmetrical

cubic-shaped particles within apparent cubic frames. In another explanation, cubosome is a self-assembled liquid crystalline particle, with a proper ratio of water and surfactant, affording unique features. Cubosome is a biocompatible drug carrier, due to its high bioadhesion, thermodynamic stability, important controlled release, and suitable for hydrophilic, hydrophobic or amphiphilic drugs, active ingredients, and protein. 2-monoolein (oleoylglycerol) or stearyl glycerol and other similar structures are typically used as carriers in liposomal cubosome.^[197] This structure gets shape by reversed continuous cubic phase by amphiphilic lipids, which is of interest for various applications, especially smart drug delivery, biosensors and membrane proteins.^[198]

1.3.3.27. Lipogel (Microgel, Hydrogel, Thermogel , etc.)

Another approach in this field is concerned to design gel-liposome structures. Microgel, gel-filled vesicles, gel core liposome, hydrogel and nano-lipogels are different kinds of Lipogel coatings membrane.^[199] Microgel compounds are water-swollen structures coupled to conjugate hydrophilic homopolymers. These structures play an important role due to the sensitive deformation of drugs/active ingredient by pH changes, ionic bonds, microgel features, and temperature. Regarding acidic, polar, or non-polar functional groups, different functions are introduced, based on the different chemical functionalities.^[200] Sodium hyaluronate microgel and lecithin originated a microgel-liposome structure to enhance the delivery of a drug to specific sites, in specific conditions. The microgel-liposome was able to keep water in the skin, and hydrate it during a long period.^[201] It was reported as a promising carrier to transfer a peptide antimicrobial agent to a target cell, over a week, due to pH tolerance.^[202] This is the case of doxorubicin loaded on temperature sensitive microgel-liposome that revealed the inherent potential of microgel to decrease drug toxicity in high dosage and was able to monitor drugs transfer in diagnostic technologies.^[203] Also, a microgel-liposome designed by a microfluidic method was able to enhance gel viscosity, increase release time, and stability.^[204]

The incorporation of chitosan on hydrogel liposomes provided a high-entrapped efficiency between 77-81 %, improved the gel's texture properties and the mucoadhesiveness in *ex vivo* conditions, allowed a sustained and controllable release and showed no toxicity on vaginal cell. In general, hydrogel-liposome coated by chitosan and loaded with resveratrol and epicatechin shows a high anti-inflammatory and antimicrobial activities compared to vitamins C and E, along with longer retention time at the vaginal site.^[205]

Hydrogel-liposome is also used in cell cultures and cell therapy. Therefore *Modified xanthan gum–liposome-hydrogel using phosphatidylethanolamine as a cross-linker* showed to be an excellent cross-linker on target drug delivery for cell therapy.^[206]

L-carnosine loaded on phytosome coated with hyaluronic acid as microgel agent was encapsulated by a rapid solvent evaporation technique, resulting in a significant decrease in the surface tension of the structure. Comparing with a pure phytosome, phytosome-microgel had a programmable release time in the target cells.^[207]

A highest entrapped efficiency >99%, optimized particle size (51.66-650.67 nm) and high drug release in two hours (77.61-99.78 %) were showed by phytosome-thermogel loaded by an anti-obesity agent. A considerable reduction on body weight, adipose tissue weight and slight systemic effect on the lipid profile were observed from *in vivo* assay on male albino rats.^[208] Gelatinized-core liposome was formulated, based on it high corneal penetration affinity for treatment of glaucoma disorder, to improve the drug penetration through a corneal barrier, especially for hydrophilic drugs. This structure considerably enhances the entrapped efficiency up to 50% and improves sustained drug release compared to simple liposome. As a result, gelatinized-core liposome improves successfully the encapsulation of hydrophilic drugs and overcome the penetration of such a drugs with a suitable functionality on pharmaceutical industry.^[209]

1.3.3.28. Cholesterol-free Liposome

Although cholesterol is one of the main components of L/P structures, some researchers believed in cholesterol-free liposome (CFL)/ phytosome (CFP). They reported that L/P prepared with cholesterol are not very efficient in the permeability/delivery of some drugs. Therefore, they developed a cholesterol-free structure to enhance knowledge about this powerful carrier.^[210] Also, a liposome structure modified with rhamnolipids (a biosurfactant), and designed by the ethanol injection method, clarified the advantages of cholesterol-free liposome comparatively to conventional ones. The results showed decreasing particle size and increasing homogeneity, optimized stability and released profiles from experimental data.^[211]

1.3.3.29. Hybrid Liposome

Hybrid means the combination of two or more factors to provide an individual result. The factors could be the preparation method and carriers or the combination of both.

For example, hybrid liposome (HLP) is a vesicular and micellar molecule designed by a combination of two or more types of liposome structures or preparation techniques to provide unique trustable delivery carrier (Chart. 1). HLP-indocyanine green as anti-tumor agent for the treatment of colorectal cancer was designed with L- α -dimyristoyl-phosphatidylcholine, polyoxyethylene 20, and sorbitan monolaurate by sonication. A considerable decrease in tumor size, in the cecal section, was observed by histological analysis, after the intravenous administration of HLP -indocyanine green *in vivo*. Finally, it was proved the effectiveness of HLP as theranostic agent in patients with colorectal cancer, primarily through photodynamic therapy.^[212]

A metal HLP nanoparticle was designed as a programmable stable carrier. The HLP metal nanoparticle has better colloidal stability and endocytosis efficiency than the one without liposome, *in vivo*.^[213] Acetazolamide and hydroxypropyl methylcellulose (HPLMC) were loaded in a HLP for glaucoma treatment. The results showed a 30.25-fold increase in the bioavailability and absorption of HPMC with a long-term release in rabbit eyes.^[214]

Bowel cancer or colorectal cancer is the third most common diagnosed malignancy and the second leading cause of cancer death. HLP loaded with anti-tumor agents were tested to block the abnormal growth of bowel cancer. The *in vitro* results showed a considerable growth inhibition on a human colon adenocarcinoma cell line (human colorectal cancer cells *WiDr*).^[215]

1.3.4. Function of Liposome/Phytosome in Pharmaceuticals

The novel developments on drug delivery, regarding L/P, led to an enhancement of particles efficiency. Indeed, the scientific revolution on modification of surface layers contributed to eliminate delivery issues, simultaneously enhancing particles stability and improving release time. As a result, these techniques are highlighted as promising candidates for pharmaceutical applications. Since, L/P structures were developed as a skin permeation technique (mention on section 2.7.). These structures proved to cover a wide range of applications, for example in the fields of cancer, gene, infection and cardiovascular therapies. Meanwhile, in the following section some practical functions of L/P in pharmaceuticals are presented and discussed.

1.3.4.1. Cancer Therapy

As mentioned, sensitive toxic drugs, synthetic or natural, could be encapsulated on L/P structures, aiming a targeted delivery on cancer therapy due to substantial

advantages mention on section 2.7.1. (1, 2, 4, and 5) and 2.7.2. (1, 4, 8, and 15). For instance, 5-Fluorouracil, a drug clinically approved to treat colorectal cancer, is an example of such application. When encapsulated with apigenin (with dual synergic function), via a LUV structure, prepared by thin-film hydration method, a synergistic effect on colorectal cancer treatment was observed, with an excellent cytotoxicity effect on the cell lines. The *in vivo* and *in vitro* tests revealed a significant decrease in cancer cell growth and an enhancing apoptotic potential, demonstrating a positive clinical application of this dual-drug liposome structure.^[216]

A cationic liposome, prepared by a thin-film hydration method with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) or 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), loaded with voacamine helped to overcome the doxorubicin resistance of osteosarcoma cells. The concentration and type of glycerol-lipids directly affect the absorption mechanism of voacamine combined with doxorubicin, reversing the cell resistance.^[217] The result is the acceptance of cationic liposome (section 3.1.)

GLUT5 is a specialized fructose receptor on the small intestine, favoring the transfer of fructose through the enterocyte. A surface modified liposome by fructose and a peptide was formulated for target delivery to treat a breast cancer. This fructose/peptide double modified liposome loaded with paclitaxel has a strong inhibition effect against breast cancer without any toxicity, regarding biopolymer coating marked by GLUT5 (fructose receptor) as a target labeling.^[218]

Phytosome is another excellent candidate to entrap drugs for anticancer evaluation. Celastrol, a natural quinone (pentacyclic triterpenoid) from *Tripterygium wilfordii* and *Celastrus regelii*, presents potent anticancer, anti-inflammatory, antiobesity and autoimmune activity.^[219–221] *In vivo* tests of phytosome loaded by celastrol in rabbits, a considerable improvement on its bioavailability on cancer therapy was shown due to phytosome layer protection and improved drug permeation.^[222]

In other research, curcumin loaded on phytosome was used as a gemcitabine subsidiary for pancreatic cancer. The synergistic effect of both compounds improved the safety and the bioavailability in phases I and II of clinical trials.^[223]

The phytosome permeability against the most aggressive and abnormal brain tumor growth, evaluated *in vivo*, showed a positive outcome on mouse-GBM GL261 cells (improve drug permeation). It was suggested phytosome as a *stable and safe alternative strategy* for delivery of bioactive compounds functionalized to cancer therapy.^[224] A combination of biotin and F127 triblock copolymer (B-F127), via

covalent bond, conjugated with liposome surface improved the delivery of erythrosine-decyl ester and inhibited abnormal growth of cancer cells. B-F127 showed high loading capacity, no cytotoxic effects, slow-release time, and high stability in fibroblasts from a *Mus musculus* cell line.^[225]

1.3.4.2. Inhalation Therapy

Asthma, rhinitis, chronic obstructive pulmonary disease (COPD) and byssinosis are some examples of respiratory disorders that play a critical role in human life. The effect of L/P on the treatment of inhalation-dependent diseases was proved by several studies due to main inherent functionality of L/P mention on section 2.7.1. (1- 4) and 2.7.2. (1, 3, 4, 8, and 9). This is the case of boswellia acid that has poor solubility and, consequently, low absorption in the human body. A significant uptake of a boswellia-phytosome was effective in reducing patient asthmatic symptoms.^[226] Gingerol is another candidate to treat inhalation disorders due to analgesic, anti-inflammatory, antibacterial, antimicrobial, antifungal, antiviral and antiparasitic activities.^[227] ^[228] Suitable treatment of bronchiectasis is another challenge for drug delivery systems. Tran and co-workers combined a lipid-transmembrane pH gradient with ciprofloxacin encapsulated on liposome structure to treat a bronchiectasis infection. They reported a suitable release time and appropriate drug concentration on target cells obtained by dynamic loading.^[227] Chitosan-modified phytosome loaded by gingerol showed considerable release and oral absorption. Furthermore, the positive effect of modified phytosome on respiratory infections as well as antioxidant activity was demonstrated *in vitro* tests.^[229]

1.3.4.3. Brain Disorders

Blood-brain barrier is the most selective human cell barrier due to the vital function of the brain. Passing through brain barrier without any side effect is an important challenge to drug delivery. Phosphatidylserine, a main component of lecithin, has a critical ability as brain nutrient, and play a vital role in human brain biochemistry and physiology. Therefore, it could play a dual functionality for delivery of phytoconstituent/drugs to target brain cells (explained and cited on section 2.7.1. (10) and 2.7.2. (19). By the way, the effect of various structures was studied on brain disorders. For instance, L/P could be suitable and powerful carriers able to transfer a bioactive compound through the blood-brain barrier in a safe and manageable mode. Stable glutathione-liposome, prepared by thin-film hydration and extrusion, was able to pass through a brain membrane easily. Glutathione could protect the brain against oxidative stress without any related side effect.^[230]

1.3.4.4. Alzheimer Therapy

Suitable carriers are still needed to delivery anti-Alzheimer agents through the blood-brain barrier in a targeted, safe and effective way. Brain barrier is the main specific-site for Alzheimer therapy, and the liposome structure became more efficient (explanation on section 4.3.) Several studies were developed to transfer an anti-Alzheimer agent via a L/P technique. α -mangostin has high anti-Alzheimer, anti-inflammatory, antioxidant and anti-tumor activities. It is a polyphenolic xanthone (xanthonoid) obtained from *Garcinia mangostana*.^[231,232] The permeability of α -mangostin through the blood-brain barrier has been a challenge for decades. *In vitro* studies revealed liposome loaded with α -mangostin increased its accessibility to brain, and *in vivo* tests confirmed the availability of α -mangostin on target brain cells, confirming the efficient impact of liposome to deliver phytoconstituents/drugs to brain cells.^[232]

1.3.4.5. Diabetes Therapy

Berberine has a considerable pharmacological functionality on treatment/prevention of diabetes, comparatively to synthetic drugs. A berberine-phytosome was produced to overcome the low oral bioavailability and gastrointestinal absorption, obtaining a considerable enhancement on these properties. This structure has high entrapment efficiency (more than 85%), nanoscale particle size, negative surface charge, and showed a considerable increase on oral bioavailability (almost 3-fold) compared to previous investigation. Furthermore, *in vivo* berberine-phytosome suppress the fasting glucose levels and improve the hyperlipidemia metabolism of diabetic mice. As result, berberine-phytosome proved to be a suitable candidate for anti-diabetic drug.^[233]

1.3.4.6. Weight-loss Therapy

Several risk factors cause obesity, and as consequence of that, different disorders such as chronic kidney disease, diabetes and cardiovascular diseases can occur. Anti-obesity phytoconstituents functionalized on prevention, treatment or weight maintenance after significant weight loss, become nowadays one of the challenging factors. L/P improves the bioavailability and permeation of phytoconstituents and provides an efficient pharmacological effect. Regarding the issue, the combination of green tea and piperine as weight maintenance compounds after diet (anti-obesity and weight maintenance), via phytosome carriers, aimed to increase their permeation, as well as monitor the related impact on the human body. Greenselected phytosome

(caffeine-free) enhances the bioavailability and permeation of phytoconstituents, as well as stimulate thermogenesis, which may have a clinical potential for the maintenance of weight, after intentional weight loss. The authors reported the necessity of more studies to confirm the effects and to optimize the dosage of Greenselect Phytosome to achieve the expected results.^[234]

Liposome loaded by Interleukin 10 (IL-10), a therapeutic anti-inflammatory component, causes high affinity of macrophages in obesity-induced inflammation. Furthermore, the structure showed considerable anti-obesity and anti-inflammatory effects on obese mice, due to the potent macrophage targeting ability.^[235]

1.3.4.7. Infection Therapy

CAL02, a broad-spectrum antitoxin agent in clinical-stage, is active against the most predominant infection factors such as gram-positive and gram-negative bacteria, in hospital-acquired pneumonia, ventilator-associated pneumonia, community-acquired pneumonia and intra-abdominal infections,^[236] with a reference to section 3.19. Therefore, an anti-infective lipid bilayer (liposome) functionalized and targeted against deadly infections, aimed to improve the efficiency of antibiotics and their controlled release (providing enough time to the bactericidal effect appeared), decrease the illness duration, avoid side effects and significantly reduce the development of the systemic disease.^[237] As demonstrated, liposome can be a suitable carrier to deliver antibiotics for treatment of diverse infections, in a more effective manner.

1.3.4.8. Cardiovascular Therapy

Cardiovascular disorders include any symptom of the vascular organs (heart and blood vessels) not acting appropriately. The L/P could enhance the activity of cardiovascular agents due to permeation through a cell membrane improvement and drugs toxicity reduction (section 2.7.1. points 1, 2, and 5, and 2.7.2. points 1 and 8). Meanwhile a *Ginkgo biloba*-phytosome was designed, by film hydration method, to prevent oxidative damage and inhibit lipid membrane peroxidation, affecting the cardiovascular function. To study the effect of such structure 85 mg/kg of isoproterenol was administered in rats to induce cardiotoxicity, by myocardial infarction, with myocardial necrosis. Considerable myocardial necrosis, depletion of endogenous antioxidants and a significant rise of marker enzymes were observed in serum levels from isoproterenol-treated rats compared with normal rats. *G. biloba*-phytosome showed cardioprotective activity, with low levels of serum enzymes and lipid peroxidation and increased levels of endogenous antioxidants.^[238]

1.3.4.9. Depression Therapy

Depression is a common mental health disorder that causes lack of motivation and interest. Brain barrier is the main site-specific for antidepressants, and a liposome can be more efficient as explained in section 4.3. For this purpose, *Annona muricata* L. is cited as containing several compounds with antidepressant, anti-inflammatory and antitumoral activities.^[238,239] Regarding antidepressant activity, an aqueous extract of *A. muricata* showed to be a strong hydrogen peroxide scavenger and a moderate monoamine oxidase inhibitor. L/P structures aiming to protect such extract from gastric environment and improve its permeability across the blood-brain barrier were produced, including cholesterol and phosphatidylcholine, and functionalized with a peptide ligand. Phytosome showed lower toxicity in hCMEC/D3 cells, but more binding efficiency, enzyme inhibition and scavenging activity. Liposome particle in *in vitro* assay was more stable, with slow-release time, and appropriate for depression therapy. The presence of cholesterol improved the permeability through a cell membrane. Eventually, phytosome enhanced the bioavailability of polyphenolic enriched extract of *A. muricata*, revealing to be a serious candidate for further *in vivo* assay.^[240]

1.3.4.10. Eye Disorders

The eye is another critical organ that requires attention. A timolol maleate liposomal hydrogel was formulated to enhance permeability of this compound through precorneal region, achieving a more effective therapeutic treatment of glaucoma. An *in vivo* assay on rabbit showed that this hydrogel structure significantly improved the bioavailability and retention of timolol maleate on precorneal eye region. Moreover, no eye irritation was observed, along seven days of monitorization, and a decrease in the intraocular pressure was verified.^[241]

1.3.4.11. Liver Disorders

Phosphatidylcholine, the main component of lecithin, has hepatoprotective properties and is typically used as a lipid layer. Considering this, it could play a vital role in delivery of hepatoprotective agents as well as provide a synergic effect (section 2.7.). In this line, silybin, a flavonolignan with high hepatoprotective, anticancer and antioxidant activities was used to produce the Flavonolignan silybin-phytosome with trade name Siliphos™. The availability of this structure in human hepatoma cells was confirmed by Fluorescence spectrometry. The study demonstrated the potential of the phytosome to pass through the cell membrane and deliver the phytoconstituent to

target cells, for the treatment of acute or chronic liver diseases.^[242] The phytosome enhances the permeability of the hepatoprotective agent to the liver cells, allowing reducing the oxidation caused by free radicals, as well.^[243] A hepatoprotective synergistic effect of curcumin and silybin loaded on phytosome was also proved for the treatment of liver cirrhosis as well as hepatoprotective synergistic effect of phytosome (section 2.7.).^[244] Furthermore, a silybin loaded on phytosome, in another investigation, revealed the proportion of 1:5 silybin: phospholipid provides good stability after freeze-drying, with an optimized size (133.534 ± 8.76 nm), loading capacity ($12.18 \pm 0.30\%$) and entrapped efficiency ($97.169 \pm 2.412\%$).^[245]

1.3.4.12. Antioxidant Delivery

Antioxidant compounds are phytoconstituents linked to a vital role in reduction risk of oxidative stress and cancer. Nowadays, the target delivery of phytoconstituents with antioxidant activity is challenging and liposome structures can contribute to that (section 2.7.). In this field, a good example is pomegranate extract (natural strong antioxidant)^[246] loaded on liposome obtained by freeze-dried technique, that revealed to be a right candidate for the delivery and protection of antioxidants.^[247]

Grape pomace contains a wide range of polyphenolic compounds that provides antioxidant and antimicrobial properties, allowing its application in nutraceutical, pharmaceutical and food industries. A liposome structure modified with sodium alginate and gum arabic loaded with grape pomace showed protection on Caco-2 cells against oxidative stress. This behavior was due to the high-entrapped efficiency and a considerable protection of the polyphenolic compounds. Furthermore, the size of this structure, grape pomace-liposome conjugated sodium alginate and gum arabic (300 nm) protects the polyphenolic compounds against decomposition in gastric media and improves its functionality.^[248]

Resveratrol is another putative antioxidant and health-protective agent with low bioavailability, limited solubility, and fast metabolism *in vivo*. Liposome encapsulated resveratrol, in different studies, proved to be more effective to reduce the amount of intracellular ROS accumulation and provide a better antioxidant functionality.^[249,250]

1.3.4.13. Anti-inflammatory Delivery

Salvia triloba and *Rosmarinus officinalis* essential oils (EOs) have antibacterial, antioxidant, and anticancer activities. These EOs were incorporated on a liposome vehicle to improve stability, UV protection and prevent oil evaporation. EO-liposome have

a particle size around 200 nm, zeta potential between -20 mV and -35 mV, encapsulation efficacy between 57- 65% and stability over one month at 4 °C. EO-liposome revealed a considerable reduction on effective dose, as well as antioxidant, anti-inflammatory, and antibacterial activities against *Klebsiella pneumonia*, compared to unprotect EOs.^[251]

Anthocyanin-rich hibiscus extract exhibited high anti-hypertensive, apoptosis, antioxidant, antimicrobial,^[252] anti-obesity,^[253] anti-diabetes,^[254] and hepatoprotective activities.^[255] A minimum amount of *H. sabdariffa* was loaded on three-layer liposome conjugated by chitosan and pectin. The liposome showed high stability for multilayer liposome (30 days), small particle size (less than 200 nm) with high homogeneity. Interestingly, the extract with the lowest concentration of *H. sabdariffa* has the highest stability and high concentration of anthocyanidins, as well.^[256]

Centella asiatica is a medicinal plant with anti-inflammatory action (skin and mucosal relaxation) on rheumatism and mental illness as well as wound healing.^[257,258] A phytosome loaded with *C. asiatica*, formulated by anti-solvent precipitation and co-solvent lyophilization techniques, showed to be effective in both skin and allergic responses. *C. asiatica*-phytosome inhibited the infiltration of the inflammatory cells and inhibited the expression of iNOS and COX-2, the activity of NF- κ B, and the release of TNF- α , IL-1 β , and IgE, as well. Indeed, *C. asiatica*-phytosome also revealed effectiveness on the inflammatory response by macrophages in an atopic dermatitis (AD) mouse model.^[259] A quercetin-phytosome proved to be another effective candidate for healing itching, injury, or inflammation skin signs.^[260]

A leukocyte-mimetic (LM) structure was recently designed to improve delivery of drugs into target cells. The researchers used an endothelial barrier coupled to the liposome surface to obtain an accurate selectivity on inflammatory receptors of the target cells. Moreover, LM-liposome was a suitable carrier to transfer an anti-inflammatory agent through a cell barrier and directly to the inflammation site. This liposome modified by leukocytes facilitate the drug transfer through the inflamed endothelium via membrane protein to decrease or stop the inflammation focus. The results demonstrated that LM-liposome considerably increased the vascular endothelial-cadherin displacement.^[261]

1.3.4.14. Gastrointestinal Disorders

Although the protection of bioactive compounds against the gastrointestinal media

is a big challenge, select a suitable carrier to improve absorption through gastrointestinal cells or treat this organ is also becoming a global concern. L/P structures can be a good candidate once they considerably enhance the absorption on intestinal lumen barriers compared to other delivery carriers (section 2.7.1. point 1, 3, and 5, and 2.7.2. point 1, and 11). A Eudragit-modified liposome loaded with quercetin was formulated to prevent structural decomposition in the acidic digestion system. This liposome could protect a human intestinal cell against oxidative agents and reduce ROS production.^[262]

1.3.4.15. Artificial Joints

Bio-lubricants are oil or a combination of oils extracted from seeds (canola, rapeseed, sunflower or soybean oils) or fruits (palm or coconut oils).^[263,264] They can be coupled to the liposome surface through several carbon chains like an unilamellar vesicle for artificial joints application. They could be used as synovial joints (such as the elbow, hip, knee, and in mammals through interacting between bones covered by a thin layer of cartilage tissue, and the synovial fluid (SF) between them), dental and heart valve prosthesis. Negative-charged polymer conjugated liposome through structural integrity of small unilamellar lipid layer of Si_3N_4 surface, increase the carbon chain length and cause considerable lubricant functionalities. Meanwhile, the specific interaction between negative charge polymer and lipid layer of liposome provides a high-density boundary-lubricating layer coupled to polymeric liposome which significantly enhances the lubrication functionalities.^[265] Bio-lubrication liposome has been formulated by Goldberg, and Klein, aiming a strong correlation with a carbon bond in artificial joints, as well.^[266]

1.3.4.16. Gene Therapy

By improving numerous prototypes, DNA-based biopharmaceuticals are a novel established pathway to control disease progression by induction or inhibition of genes. L/P are suitable candidates for this purpose since the related structures such as enzymosome, virosome, aptamosome, and protein corona-Liposome were specialized for gene delivery. In spite of the fact that DNA is generally used for gene therapy, its poor stability limits the functionality. Taking into account all above, Table 4 presents several examples of used liposomes in gene therapy in what concerns structure formation, type of incorporated gene for conjugation (DNA, RNA, siRNA, Antibody) and the more important experimental data observed. It has to be referenced that only some gene delivery-based liposomal structures passed already the approval from regulatory agencies and several candidates are in advanced stages of human clinical trials.^[267]

Table 4. Conjugation of Liposome with Protein, Peptide, DNA, SiRNA, and Enzyme for Gene Therapy

Incorporator	Type	Method	Result	Ref
siRNA	Liposome 2018	Film hydration technique	- siRNA delivery could be a specialized treatment of Acanthamoeba keratitis disease (reversed 60% of keratitis <i>in vivo</i>). - PEGylated cationic liposomes provide a unique technique for the protection of siRNA delivery. - Combination of liposome with chlorhexidine reported as the only solution for the treatment of severe lesions associated with keratitis. - Significant efficiency of siRNA-Liposome compared with conventional drugs. <i>In vitro</i> and <i>in vivo</i> studies revealed the positive effect of glycogen phosphorylase and phosphatase proteins coupled on PEGylated cationic liposomes for treatment of Acanthamoeba trophozoites.	[268]
Acanthamoeba keratitis is an eye corona disease caused by amoebae found in soil and water. Acanthamoeba keratitis occurs in a lack of personal hygiene. Serine phosphatase (SP) and glycogen phosphorylase (GP) proteins have SiRNA sequences that decrease the cytopathic effect of amoebae.				
Adamantyltripeptides	Liposome 2003	Dry lipid hydration	- Liposome has a negatively charged multilamellar interaction with adamantyltripeptides. - Electron spin resonance spectroscopy proved the un-direct correlation between lipid surface and peptidoglycan monomer (PGM).	[75]
Transferrin (Tf)	Liposome 2018	Thin-lipid film hydration method	- Gene therapy is a useful technique for the treatment of neurodegenerative disease. - Liposome act as a dual-function carrier to deliver genes to neuronal cells and provide a triple co-culture blood-brain barrier. - Enhance the permeation of genes transport across BBB and neuronal cells by combining liposome with transferrin (Tf) receptor. - Multifunctional liposomes proved as a unique platform on gene delivery as a function of neurodegenerative diseases.	[269]
Dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), Dioleoyl-3-tri-methylammonium-propane chloride (DOTAP).				
Anti-ovalbumin antibodies	Liposome 2007	Dry lipid hydration	- OVA (Ovalbumin) encapsulated in liposome structure to enhance the efficiency of immuno-competent cells. - OVA was used as an antigen model in a various investigation for immune reaction. - Electron spin resonance (ESR) spectroscopy confirmed spin-labelled OVA enable the detection of interactions between the lipids in liposomal bilayers.	[76]
siRNA	Exosome 2018	Thin-film hydration	- Exosome as an efficient cargo delivery confirmed as anti-serum aggregation effect with a long-term storage ability, and three folded safer than cationic liposomes, in addition of inherent properties such as low immunogenicity, and possibly intrinsic homing capacity. - Exosome passes through A549 cells by micropinocytosis, caveolae-mediated endocytosis, and membrane fusion, considering the type of cell, the mechanism of cell passing change appropriately.	[270]
The exosome is a subgroup of liposome used to encapsulate macromolecular nucleic acids and proteins. Exosome has a potent ability to transferring the macromolecule for long distances with high storage stability and long release time as well as sustained release mechanism.				

Table 4. Continued 1. Conjugation of Liposome with Protein, Peptide, DNA, SiRNA, and Enzyme for Gene Therapy.

Incorporator	Type	Method	Result	Ref
siRNA	Liposome 2019	Thin-film hydration method	• Cationic liposomes/siRNA complexes modified by neutral helper (cholesterol). • <i>In vivo</i> studies revealed the efficiency of cationic liposomes/siRNA complexes modified by cholesterol complexes, after the intravenous injection of siRNA lipoplexes. • The siRNAs are degraded by endogenous nucleases in the blood and are rapidly eliminated from the circulation when injected intravenously. • <i>In-vivo</i> efficiency on the intravenous injection of cationic liposomes/siRNA was greatly influenced by an aggregate of a cationic lipid, neutral helper lipid (cholesterol), and siRNA in cationic liposomes. • This finding is another proof for gene delivery of liposome.	[273]
LDL-derived peptide antigen	Liposome 2018	Thin-film dehydration-rehydration method	• DSPG(1,2-distearoyl-sn-glycero-3-phosphoglycerol)-liposome loaded with LDL-derived peptide antigen significantly decrease the concentration of cholesterol on arteries. • DSPG-liposome loaded with LDL-derived peptide antigen was efficient in the prevention and treatment of Atherosclerosis. • DSPG-liposome loaded with LDL-derived peptide antigen eliminated the amount of plaque formation up to 50% and stabilized the plaques on arteries. • DSPG-liposomes proved to be a potent option for vaccination against atherosclerosis. • DSPG-liposome (anionic) loaded with LDL-derived peptide antigen could be a good option for the curing of atherosclerosis and other autoimmune diseases, as well.	[274]
Atherosclerosis is a type of cardiovascular disease in which arteries become narrow and hardened due to a buildup of plaque around the artery wall. Atherosclerosis slowly blocks arteries and stop passing blood is an important worldwide death reason.				[275–277]
Enzyme	Liposome 2019	-	• Using a horseradish peroxidase (HRP)-loaded on liposome provide amplification potentiometric immunosensing platform based on enzymatic biocatalytic design for detection of thyroid-stimulating hormone (TSH). • Liposome loaded by HRP provoke a sensitive detection of thyroid-stimulating hormone on immunosensing platform within the linear range of 0.01–30 mIU/mL at a detection limit of 0.0067 mIU/mL • Liposome loaded with enzyme have good reproducibility, long-time stability and high efficiency were achieved for potentiometric immunosensor for detection of thyroid-stimulating hormone (TSH), embedded on commercial human TSH ELISA kit, as well.	[278]

Table 4. Continued 2. Conjugation of Liposome with Protein, Peptide, DNA, SiRNA, and Enzyme for Gene Therapy.

Incorporator	Type	Method	Result	Ref
CB5005 peptide/ NF-κB inhibitor	Liposome 2018	Thin-film hydration and extrusion method	- Dual-function PEGylated liposome with doxorubicin conjugated by fluorescent dye synthesized to enhance a cell membrane penetration and NF- κB inhibition for treatment of glioma. - CB5005-LS/dye liposome successfully enhanced the penetration of liposome into a tumor cell. - CB5005-LS/dye revealed that modification improve the concentration of DOX into glioma cells through the nucleus. - The cytotoxicity data evaluated the effect of surface modification of liposome loaded with doxorubicin and CB5005 peptide, which was verified a fivefold increase on killing glioma cells. - In-vivo assay proved the penetration of CB5005-modified liposomes loaded by DOX through brain barrier and through a tumor cell. - CB5005 peptide loaded on PEGylated liposome might have a synergist effect on chemotherapy for other human tumors, as well.	[279]
CB5005; designed for improve cell-penetrating peptides through the cell barrier and into a nucleus as well as tumor growth inhibitor (blocking intracellular NF-κB activation)				[280]
Dual antibody	Liposome 2019	Post-insertion method	- CD123/CD33-LP-DNR loaded on liposome could be a practical structure to improve a potent targeting ability against myeloid leukemia. - The cytotoxicity and cellular uptake were tested on Dual antibodies (THP-1 (CD123brightCD33bright) and HL-60 (CD123dimCD33bright) cells) showed THP-1 and HL-60 liposome are higher cellular uptake 1.8 and 1.6-times, respectively. In contrast, the cytotoxicity, and cellular uptake 1.5-times only in single-antibody modified liposome was observed. - MTT assay proved dual-antibody modified liposomes (CD123/CD33) could be an efficient structure to eliminate the antigen-negative escape.	[281]
Myeloid leukemia (AML) is an abnormal and rapid growth of myeloid line of blood cells that build up in the bone marrow which cause deformation on healthy blood cells. CD123 or CD33 is effective either induvial or using the dual-targeting agents for treatment of AML.				

1.3.4.17. COVID-19 (SARS-CoV-2) Therapy

SARS-CoV-2 (COVID-19) is a severe respiratory syndrome, induced by a coronavirus that prompt unprecedented worldwide pandemic in last eighty years. Due to the thousands of deaths daily and the increasing number of cases, find accurate urgent treatments in a short time became a challenge. Liposome technology seems to be a promising technique, not only for target delivery of anti-virus drug, but also for an efficient vaccination. Several researchers focused on suitable treatments for this pandemic, taking liposome technology in mind and the effects of L/P discussed on section 4.2.

Meanwhile, lectin is a natural based carbohydrate-binding protein able to conjugate to viruses, bacteria, fungi and parasites, leading to agglutination and neutralization of these pathogens. The already referred inherent ability of lectin to bind carbohydrate components (especially natural-based one) of human pathogens made it a critical agent for surface modification of liposome. Liposome, as a functionalized carrier could be modified by lectin for target delivery of antiviral agents or vaccines across the cell membranes and provides an inhibition against coronaviruses. Nevertheless, the use of lipids with immuno-stimulatory effects is another factor to improve the efficiency of target delivery. By the way, using the liposomal carriers functionalized by lectin should be a promising strategy to target delivery of anti-Covid-19 agents for further investigation.^[282]

Hydroxychloroquine is a potent antimalarial and anti-inflammatory drug that showed *in vitro* inhibition of SARS-CoV-2 infection. This drug shows a considerable anti-infective activity against SARS-CoV-2, but also has a systemic toxicity (cardiotoxicity) in high dosage. Therefore, hydroxychloroquine-liposome formulated to overcome the toxicity and high dosage efficiency of this drug is a way. This structure shows thirty-fold increase on lung exposure and decrease blood and heart exposure, comparatively to unprotected hydroxychloroquine. Finally, the Hydroxychloroquine-liposome proved to be a promising candidate with high efficiency and minimum side effects for treatment of respiratory syndrome COVID-19 (SARS-CoV-2).^[283]

1.3.4.18. Immunological Vaccination

Nanocarriers play a vital role in immunological vaccination. Considering the conventional bases of the vaccines, the combination of lipid nanocarriers with antigen and adjuvant can be the solution for specific target-delivery of vaccines (section 3.13., 3.15., 3.20., and 3.21). Concluding, nanocarriers might be modified by all mentioned techniques, designed to improve the permeability and labelled with a specific antigen/adjuvant to improve a target-specific delivery. Different lipid-based carriers were

developed to improve the efficiency of vaccination *in vivo/in vitro* such as, emulsome,^[94] virosome,^[185] corona protein,^[156] which was already discussed in the current review. Different factors play a vital role on the development and efficiency of vaccines. This is the case of appropriate carriers to protect the RNAi, nAbs, antigens, and proteins from degradation named *loss of function* in intracellular systemic delivery. Other factor is the elimination of high dosage toxicity of based-drugs, especially peptide and protein by dose reduction. Also, the modification of the pharmacokinetic-pharmacodynamics of the vaccine, enhances high loading capacity to reduce the side effects and finally improve the half-life of based-drugs, and cellular uptake.^[284]

Accordingly, liposome-vaccine based (lipid-based nanocarriers in vaccine approach) is proved since 1999. For instance, the combination of phospholipids, cholesterol and a lipid emulsifier were used to produce a vaccine with immunogenic properties, in a perfect distribution media designed by O/W emulsome, prepared by stepwise addition process, following the extrusion technique. The O/W emulsome contained a prostate-specific antigen that exhibited antigen-specific immunostimulant effect in mice. The O/W emulsome improved the oil concentration from 2.5 to 42.5 % from a liquid to viscus form. This technique highlighted liposome *as the best carriers for cell-mediated immune responses* and allows developing an excellent carrier for antigen on oil-in-water vaccines media.^[285]

Moreover, leishmania antigen-Liposome formulated for *in vivo* was investigated on the susceptible BALB/c mice for vaccination against leishmaniosis. Leishmania antigen-Liposome proved to improve protection of the antigen enhancing it targeted delivery and decreasing the concentration of parasite in spleen (almost for 14 weeks). Vaccinated mice showed strong immunity due to high levels of SLA IgG antibodies with a predominant IgG2a titer. Therefore, *in vivo* investigation confirms the potential credit of liposome structure to be used in vaccines.^[286]

Diphtheria toxoid is the drug of the diphtheria vaccine, purified from inactivated diphtheria toxin, being a pharmaceutical product used against *Corynebacterium diphtheria*. Diphtheria toxoid with combination of poly (I:C), loaded on cationic liposome, showed high efficiency and significantly improves the immunogenicity after intradermal vaccination in mice. Diphtheria toxin-liposomal-based vaccine have a similar effect on the antibody response (IgG and IgG1). These findings could be another vigorous prove for the potential of intradermal liposomal vaccine delivery.^[287] The surface of a cationic lipid-conjugated DNA vaccine was modified by Influenza nucleoprotein encoding plasmid and designed for influenza vaccination complex. Cationic virosome-plasmid complex

cause immunity against intra-subtyped on adapted H1N1 PR8 virus. However, the vaccination complex without nucleoprotein encoding plasmid was not efficient on immunity. It could be concluded that influenza cationic virosome should be modified with a nucleoprotein encoding plasmid to provoke anti-influenza cytotoxic immune responses and enhance the vaccine coverage against antigenic drift.^[288] Nevertheless, the significant effect of using virosome in immunological vaccination was discussed in detailed on chapter "3.20. Virosome" in current manuscript.

In conclusion, the significant similarity among SARS-CoV-2 with two other coronaviruses, as well as a considerable similarity between influenza viruses, facilitates the vaccine development toward a promising vaccine and nanotechnology. Eventually, considering the necessity of immunological vaccination against the Covid-19 pandemic, it is essential developing appropriate nanocarriers with a minimum of targeting delivery risk, high immunological efficiency, and minimum side effects. Emulsome, virosome, corona protein or a liposome-based carrier are suggested as decisive candidates in immunological vaccination of SARS-CoV-2 (Covid-19) or any similar condition for further *in vitro* and *in vivo* investigation.

1.3.5. Function of Liposome/Phytosome on Skin

Liposome/Phytosome delivery carriers can help to overcome the skin barrier and enhance drugs permeation, regarding desirable similarity of L/P to human cell membranes (explained in section 2.1.) and high transdermal permeation ability (section 2.7). Therefore, there are different L/P structures that could be promising carriers for delivery of phytoconstituents/drugs to specific cells, taking into consideration the best choice of lipid layer and modification process mostly targeted to the desire site.

1.3.5.1. Skin Disorders

Sinigrin is a natural compound from the glucosinolates group produced by *brassicaceae* family. This compound has anticancer, antibacterial, antifungal, antioxidant and anti-inflammatory activities especially on skin.^{[289] [290]} Taking this in account, a Sinigrin-phytosome, prepared by a thin-film hydration method with 153nm in size, zeta potential of 10.09 mV and encapsulation efficiency of 69.5% was produced aiming wound healing and cytotoxicity on A-375 and HaCaT cells. A functional and practical wound healing effect was observed after 42 hours comparatively to pure Sinigrin. A minimum toxicity towards HaCaT cells, at high concentrations, was observed.^[291]

In another investigation, a Sinigrin-phytosome proved to enhance the permeation of this phytoconstituent into the *stratum corneum* - *epidermis* on human abdominal skin compared to phytosome free of sinigrin, as well. In conclusion, sinigrin-phytosome could be introduced as a promising agent for skin wound treatment or permeation through *stratum corneum–epidermis*. Phytosome structure could be an excellent candidate to overcome the permeation of different type of drugs through the skin. Moreover, cationic curcumin-liposome, among anionic, and neutral one observed putative high penetration properties and no toxicity on *ex vivo* penetration of curcumin through the full thickness human skin.^[292]

1.3.5.2. Skin-Care Products

Skin-care products include various types of formulations, from cosmetics to pharmaceuticals. For instances, Clove (*Allium sativum*) EO have a wide range of bioactive compounds with skincare properties like anti-inflammatory, antiacaricidal, antiviral, anticarcinogenic, antioxidant and neuroprotective^[293–296] when formulated on liposome carrier. Different liposome structures were used to protect the EO with optimized stability.

Clove EO cover by cyclodextrin-liposome showed to be a suitable carrier due to small particle size, high loading efficiency and storage stability (around one month) for functionality in cosmetic, pharmaceuticals and food industry. Ethanol injection method and membrane contactor were also introduced as suitable techniques for production of clove EO-liposomes either in laboratory and at industrial scale.^[297] The particle stability was enhanced to 2 months of storage at 4°C, in another investigation, maintaining a good functionality in pharmaceutical, food and cosmetic industry, with special attention on skin.^[298]

Polygonum aviculare L. (knotgrass) loaded on cell-penetrating peptide (CPP) conjugated liposome (cationic arginine-rich peptide), due to its high antioxidant and cellular membrane protective activities was produced to improve the transdermal delivery in terms of depigmentation and anti-wrinkle studies. Knotgrass-liposome-cationic arginine-rich peptide was effective due to the high permeation of CPP-conjugated liposomes, verified by Franz diffusion cell experiments, and confocal laser scanning microscopy. It showed more effectiveness on depigmentation and anti-wrinkle studies *in vivo*. It also revealed to be effective on skin penetration and could have an effective functionality on transdermal delivery to prevent skin ROS destruction (skin-care) as well as skin-therapy.^[299]

A resveratrol-liposome conjugated by chitosan enhances the skin permeability from 30.84 % (free resveratrol) to 40.42 %. This structure is another powerful transdermal delivery candidate for anti-ageing skin cream formulation.^[300]

1.3.6. Liposome/Phytosome Functionality in Food Products and Dietary Supplements

Flavors are main ingredients of a food product, playing an essential role in food product attraction by the consumer. Natural flavors besides give a good aroma/smell to the product can also have nutritional or medicinal properties.^[300,301] Suitable lipid carriers are needed to overcome the low bioavailability and stability of natural-based products, prevent their decomposition during storage or through the digestive system and improve desirable features and cover undesirable properties.^[302]

In case of anethole, a natural flavoring loaded liposome and a complex of cyclodextrin/liposome using single and double loading by extrusion technique. The complex of cyclodextrin/liposome showed better encapsulation and photo protection of anethole compared to liposome, as well as the improvement of antitumor and anti-inflammatory activities with a considerable impact on stroke prevention and heart disease risks.^[303]

Black carrot is a rich source of anthocyanin.^[304,305] Its encapsulation on cholesterol-free liposome provides a significant increase on the bioavailability and stability of anthocyanin color during storage. Moreover, the efficiency of anthocyanin-cholesterol-free liposome improved up to 50% by increasing lecithin concentrations. Liposome is an effective carrier to eliminate the effect of ascorbic acid on the decomposition of anthocyanin in aqueous solution.^[306] A liposome loaded by a mixture of thirteen spices, similar to high amount of natural antioxidants, improves the quality of cooked pork. It prevents changes on red meat color after storage, as well as kept the pork shapes and taste fresh during storage time.^[307]

Lemongrass oil is a source of antifungal, antioxidant, and anti-inflammatory compounds, traditionally used to alleviate pain, digestion disorders, fever and infections.^[308] This oil was coupled to a liposome structure, aiming to prevent the infectious disease caused by *Listeria monocytogenes* in contaminated cheeses. Lemongrass oil-liposome showed considerable antibiotic activity against *L. monocytogenes* and enhanced the shelf life of the bioactive compounds. Therefore, liposome proved to be a suitable antibiotic carrier for food application against some pathogens and for prevention of food contamination.^[309]

Garlic-phytosome was applied as a natural preservative on a yogurt model. This structure was stable for a long time compared to normal yogurt, cover a bad smell and taste, prevent decomposition and oxidation of garlic, as well as enhanced the quality of the yogurt during storage time.^[310]

At commercial level, some food supplements use this technology. This is the case of *Thorne*, a mixture of Quercetin/sunflower phosphatidylcholine in capsule, functionalized for health-care practitioner. *Leucoselect* is a grape seed polyphenol functionalized as a natural antioxidant, being available more examples on Table 5. Furthermore, table 5 describes commercial products in terms of active ingredients, type of carriers, functionality, and brand. The acceptance of L/P structures in pharmaceuticals, food products and cosmetics is proved, wisely confirming the positive impact of such structures to answer all the issues in drugs/phytoconstituents delivery.

Table 5. Commercial Product Available in Market.

Trade name	Type	Component	Brand	Properties
Siliphos	Phytosome	Silybin from milk thistle seed	Indena (Powder)	Hepatocyte protection
Vitamin C	Liposome	Vitamin C	Dr. Mercola (Capsule)	Help collagen synthesis
Glutathione	Liposome	Sterial Glutathione and phospholipid, Beeswax	(Capsule)	-
Jamieson Green Tea	phytosome	Green tea extract	(Capsule)	antioxidant
PA2 Phytosome	phytosome	Proanthocyanidin A2 from horse chestnut bark	Indena (powder)	Anti-wrinkles, UV protectant
Hemp	Liposome	Cannabidiol	CBD Essential (Capsule)	-
Glutathione	Liposome	Glutathione	(Capsule)	skin whitening action
Glutathione	Liposome	Glutathione	Bio max (Capsule)	-
Meriva	phytosome	Curcuminoids from turmeric rhizome	Jarrow Formulas (Capsule)	-
Meriva Turmeric	Phytosome	Curcumin and phosphatidylcholine	Piping Rock (Capsule)	Healthy inflammation
Green tea CR	phytosome	Green tea extract with Resveratrol and phosphatidylcholine	Purity product (Capsule)	Brain function, cardio health and antioxidant, immune defense
Boswellia	phytosome	Boswellia Extract	Thorne Research	Vitamins & Supplement
Virtiva	phytosome	Ginkgo flavone glucosides, ginkgolides bilobalide from Ginkgo biloba leaf	(Capsule)	Vasokinetic
Artery & Vein	phytosome	Hawthorn Berry & Meriva Phytosome	Emerald (Capsule)	cardiovascular health
Inflamma-X		Turmeric, boswellin, bromelain, tart cherry, protease enzymes	Life seasons (Capsule)	Inflammation support
Ginkgo biloba Terpenes Phytosome	phytosome	Ginkgolides and bilobalide from Ginkgo biloba leaf	(Capsule)	Soothing
Bio-Quercetin	phytosome	Quercetin	Life Extension (Capsule)	Immune and cardiovascular support
Curcumin	Liposome	Turmeric Curcumin (95% curcuminoids), ginger root extract, and shiitake mushroom powder	Zenwise (Capsule)	Healthy immune response, provides cardiovascular support, supports digestive function
Curcumin	Liposome	Ginger Root Extract, Quinquin (bark extract), Vitamin B1, B6, Soybean oil	Copmed (Capsule)	-
UltraThistle	phytosome	Silybin	Natural wellness (Capsule)	-
Melatonin	Liposome	Melatonin	Oncotonin (Capsule)	Cardiovascular research

Table 5. Continued. Commercial Product Available in Market.

Trade name	Type	Component	Brand	Properties
Curosome	Liposome	curcumin	Cure support	-
Curcuma Sorb	Phytosome	Curcumin (Meriva)	Pure encapsulation (Capsule)	-
Centella Phytosome	Phytosome	Triterpenes from <i>Centella asiatica</i> leaf	(Capsule)	Cicatrizing, trophodermic
T-Laen Extreme	Phytosome	Green tea and forsalean	(Capsule)	promote a healthy metabolic rate and support weight management
Thorne	Phytosome	Quercetin/sunflower phosphatidylcholine	(Capsule)	Health-care practitioner.
Hawthorn Phytosome	Phytosome	flavonoids	Enzymatic (Capsule)	Healthy Cardiovascular function
Silymarin Phytosome	Phytosome	Silymarin from milk thistle seed	(Capsule)	Anti-hepatotoxic
Ginkgo biloba Dimeric Flavonoids Phytosome	Phytosome	Dimeric flavonoids from Ginkgo biloba leaf	(Capsule)	Lipolytic, vasokinetic
18 β -glycyrrhetic acid Phytosome	Phytosome	18 β -glycyrrhetic acid from licorice rhizome	Indena (powder)	Soothing
Milk Thistle	Phytosome	Silybum marianum	SOLARAY	
<i>Crataegus</i> Phytosome	Phytosome	Vitexin-2"-O-rhamnoside from Hawthorn flower	Hawthorn (Capsule)	Antioxidant
Ginkgo select	Phytosome	Ginkgo flavone glucosides, Ginkgolides, bilobalide from Ginkgo biloba lea	Indena (powder)	Vasokinetic
Giloba	Phytosome	Ginkgo biloba	Mega We Care (Capsule)	Improves circulation of blood
Visnadex	Phytosome	Visnadin from <i>Amni visnaga</i> umbel	-	anticellulite
Sericoside Phytosome	Phytosome	Sericoside from <i>Terminalia sericea</i> bark root	Indena (powder)	Anti-wrinkles
Ginselect	Phytosome	Ginsenosides from <i>Panax ginseng</i> rhizome	Indena (powder)	Skin elasticity improver, adaptogenic
Ginkgo Biloba (iHerb)	Phytosome	Ginkgo biloba	Herbal factors (Capsule)	
Meriva-SF	Phytosome	Curcumin	Thorne (Capsule)	healthy inflammatory response
Leucoselect	Phytosome	Polyphenols from grape seed	Indena (powder)	Antioxidant, capillarotropic
Escin β -sitosterol Phytosome	Phytosome	Escin β -sitosterol from horse chestnut fruit	Indena (powder)	Anti-edema

1.3.7. Clinically Approved Active Ingredients

FDA approved bioactive compounds are not directly used as medicines but as food and dietary supplement assign to different functions at nutritional point of view, skincare products and cancer preventing, among others. Curcumin, estragole, silymarin, resveratrol, quercetin, hypericin, apigenin and xanthan are bioactive compounds used in the referred areas with FDA approve. Table 6 compiles and briefly explains the application of these compounds as liposome and related subgroups for various therapeutic activities.

Table 6. Clinically Approved Active Ingredients.

Compounds	Type	year	Method	Application	Ref
Curcumin /Rhamnolipids	Liposome	2019	Ethanol Injection Method followed by High-Pressure Micro fluidization	Functional foods, dietary supplements, and pharmaceutical industries	[211]
Rhamnolipids are a natural glycolipid surfactant extracted from <i>Pseudomonas aeruginosa</i> bacteria					[311,312]
Curcumin	Liposome	2019	Thin film hydration method	Antioxidant	[313]
Curcumin/gemcitabine	Phytosome	2018	-	Pancreatic cancer	[223]
Glycyrrhetic acid, Silibinin	Liposome/p hytosome	2015	Thin-film hydration followed by sonication	Liver cancer (Hepatocellular Carcinoma Cells)	[314]
Silybin (Silymarin)	Phytosome	2016	Thin layer-hydration method	Oral delivery	[245]
Silybin, Curcumin, α -R-Lipoic Acid	Phytosome	2016	-	Hepatoprotective (Chronic Hepatitis)	[315]
Estragole	Liposome	2019	Ethanol-injection method	Antibacterial (Food Preservative)	[316]
4-methoxyallylbenzene called estragole is a phenylpropene; with a potent antimicrobial, antioxidant, antifungal and cardiovascular activities					[317,318]
Resveratrol	Liposome	2018	Film dispersion followed by a membrane extrusion method	Anti-obesity/obesity treatment	[319]
Resveratrol, Quercetin	Liposome	2020	Thin-film hydration follow by sonication	Skin inflammation, stress, and cancer	[320]
Usnic acid	Liposome	2019	Combination of both passive and active method	Bacterial Infection	[321]
Quercetin	Phytosome	2018	-	Hormone therapy food product, and supplement	[322]
	Phytosome	2016	-	Skin disorder	[260]
Apigenin	Phytosome	2017	-	Antioxidant	[323]
Hypericin	Liposome	2019	Thin-film hydration method	Antimicrobial	[324]
Hypericin is a natural anthraquinone derivative from <i>Hypericum</i> (Saint John's worth). Hypericin is a famous photosensitizer as well as present antibiotic and antiviral activities.					[325,326]

1.3.8. Encapsulated Drugs in Liposome/Phytosome

Nowadays L/P are practical techniques to increase the highlighted features of active ingredients. Consequently, there are a significant number of drugs encapsulated by these techniques. Several scientists improved probable weak points of L/P techniques and showed their interest to drug encapsulation via L/P for the global market. Table 7 reports drugs clinically used to treat several diseases using L/P techniques.

1.3.9. Patents

Hundreds of papers, patents or marketing products are approved every year regarding the high demand of L/P. Table 8 compiles thirty years of patents in this area, where is explained the phytoconstituent, the technique, the region and the Patent reference. The variety on patent region strongly proved the acceptance of such structure on worldwide market. Furthermore, considering the therapy area various patent reported its use on cancer, brain and hepatoprotective therapy. The phytoconstituent variety and preparation method show the wide application of L/P.

1.3.10. Scale-up

A structure with high-entrapped efficiency, high stability, high targeting potential, as well as a programmable time-release is the initial purpose for L/P as carriers. Over and above that, find a suitable scale-up method, with acceptable yields is another challenging concern in this field.^[301] For instance, microfluidic techniques via extrusion in scale up was reported to be more efficient in all steps. It was proved that the aqueous phase concentration does not interfere on the particle size distribution and the efficiency, while the ratio between aqueous and organic phases is a critical parameter to control the liposome size due to Ostwald ripening. According to it, the higher aqueous volumes originate shaping smaller lipid vesicles based on fast mixing and reduction of organic solvent. In addition, the optimized concentration of lipid is around 3 mg/mL to obtain particles around 100 nm for most of the lipids and a molar ratio higher than thirty times provide more stable lipid particles. It was eventually concluded that both microfluidic and extrusion techniques for large scale preparation of liposomes have identical *in vitro* and *in vivo* properties.^[327]

Table 7. Drug loaded on Liposome/ Phytosome Technology

Drugs	Type	Method	Result	Ref
Acetylcholinesterase reactivator, 2-PAM	Liposome 2018	Thin-layer hydration	- Cationic-liposome improves efficiency of drug permeation by improving the features of a cationic liposome, enhance solubility, and enhance efficient colloidal stability, high encapsulation efficiency (90%), optimized particle size (100nm), and a significant increase in bioavailability of cholinesterase reactivator, 2-PAM. - Cationic-liposomes revealed hemolytic and antimicrobial activities against human red blood cells and gram-positive bacteria, respectively. - Cationic liposome loaded by 2-PAM proved to be effective against paraoxon-induced acetylcholinesterase inhibition in the brain.	[328]
Cholinesterase reactivator can directly link to alkyl phosphorylated enzymes to reverse the inactivation of cholinesterase activates by phosphate or sulfate derivation. The recent investigation recommends the intranasal delivery method of a cationic liposomes carrier to transfer the active ingredient.				
Camptothecin (CPT) Camptothecin glycerol-phosphorylcholine (di-CPT-GPC) (Synthesized drug)	Liposome 2018	Thin-film hydration	- High loading capacity (62% of CPT-loaded on liposome), optimized particle size approximately 165 nm, and multilamellar structural with 4nm bilayer. - CPT-liposome has dynamic stability in physiological conditions. - CPT-liposome release occurs in poor acidic conditions. - Di-CPT-GPC-liposome proved a considerable cytotoxicity against MCF-7, HeLa, and HepG-2 Cells <i>in vitro</i> and inhibited tumor growth <i>in vivo</i>.	[329]
Gemcitabine	Liposome 2019	Thin-film hydration	- Poor pharmacokinetics and hypo-vascularization restricted the delivery of Gemcitabine to the pancreatic ductal adenocarcinoma - Gemcitabin-activated liposome with a high loading capacity improve the serum stability, Gemcitabin circulation, and target delivery to diffuse within the tumor. - Gemcitabin-activated liposome has optimized release time in 5 minutes. - Have a potent <i>in vitro</i> activity against murine breast cancer and pancreatic cancer with limited toxicity - Gemcitabin combined with hyperthermia-liposome provokes apoptosis and necrosis in cancer cell death, in more aggressive tumor model of murine pancreatic cancer.	[330]
Gemcitabine (Gemzar) is a nucleoside analog used to treat ovary, lung (big cancer cell), bladder, pancreas, and breast cancers.				[331]

Table 7. Continued 1. Drug loaded on Liposome/ Phytosome Technology.

Drugs	Type	Method	Result	Ref
Colistin and ciprofloxacin	Liposome 2019	Lipid-film hydration	• A considerable in-vitro antimicrobial activity showed by the liposome loaded with combination of colistin and ciprofloxacin observed against multidrug-resistant pseudomonas aeruginosa. Liposome loaded by combination of colistin and ciprofloxacin used against lung epithelial model which was cultured under the air-interfaced condition (Calu-3 cell monolayer), <i>in vitro</i>. • liposome loaded by combination of colistin and ciprofloxacin exhibited no drug toxicity at the concentration ≤ 500 $\mu\text{g/mL}$ • <i>In vitro</i> activities of liposome loaded with colistin and ciprofloxacin observed against the human lung epithelial cell model. • Colistin (an antibiotic commercially known as polymyxin E) has less absorption in the mucus or attach to cell membrane as well as less transported across the cell monolayer of apical cell membrane compared to ciprofloxacin. • No significant drug-drug interaction was observed through the permeation. Although liposome loaded by the combination of colistin and ciprofloxacin, decrease conveyor capacity of both drugs over the lung epithelial cell monolayer, and enhance the retention of drugs during the time on the lung epithelial surfaces.	[332]
Doxorubicin	Liposome 2019	pH-gradient remote	• Doxorubicin-PEGylated-liposome fabricated in small particle size (100 nm), high loading capacity approximately 80%, Low polydispersity, by pH-gradient remote loading method.	[334]
Doxorubicin	Liposome 2018	Mathematical report: Mass conservation equation	• Numerical simulator equation used to clarify the effect of doxorubicin in concentration on target cell and permeation in cancer therapy (brain tumor therapy). • Doxorubicin loaded in liposome could enhances the cellular uptake and the transference of a drug to a brain cell tumor. • The numerical simulator equation optimized the sufficient amount of doxorubicin as an effective dosage of killing cancer cells. • The liposome efficiency could be improved by enhancing the liposome accumulation solution and infusion rate, normalized microvasculature density by administrating liposomes in the tumor cells and enhancing the drug penetration that provides sufficient free doxorubicin for cell killing might be precious.	[335]
Insulin	Liposome	Reverse phase evaporation	• Insulin loaded in lectin-modified liposomes certified the oral absorption of liposome due to specific-site combination on GI cell membrane. • No significant hypoglycemic effects observed from wheat germ agglutinin, tomato lectin, and ulex europaeus agglutinin liposome modifier compared to conventional insulin-liposome.	[336]
Hemoglobin (Artificial Blood Substitute)	Liposome 2005	Dry-lipid hydration extrusion	• Nanoscale hydrogel-based oxygen carriers synthesized for the delivery of oxygen in the blood media. • Lipogel and Nanoscale hydrogel-based liposome exhibit a new generation blood substitute with desirable hemoglobin-based oxygen carriers through the polymer matrix, which limits both dissociations of hemoglobin and <i>in vivo</i> exposure of acellular hemoglobin.	[337]

Table 7. Continued 2. Drug loaded on Liposome/ Phytosome Technology

Drugs	Type	Method	Result	Ref
VISTA	Liposome 2019	Thin-film hydration followed by Extrusion method	• VISTA-conjugated liposome by biotin-streptavidin is a right candidate for treatment of allograft rejection of islet transplantation • VISTA-conjugated liposome by biotin-streptavidin improve the circulating half-life of immunosuppressive effect on allograft survival to 60 hours. • VISTA-conjugated liposome by biotin-streptavidin can control the blood glucose level during the treatment phase. • VISTA-conjugated liposome by biotin-streptavidin showed a considerable anti-rejection effect, <i>in vivo</i>. • VISTA-conjugated liposome by biotin-streptavidin was observed to inhibit SYK-VAV pathway by activation of SHP1, <i>in vivo</i>.	[338]
V-domain Ig-containing suppressor of T-cell activation (VISTA). VISTA is a negative bio-modulator used for various pharmaceutical activities. A combination of VISTA with another checkpoint receptors sufficiently might be an excellent solution to stop a tumor tolerance.				[339,340]
Double-branched biotin	Liposome 2019	Thin-film hydration	• Paclitaxel liposome modified with biotin and double-branched biotin might be suitable candidate to treat a breast cancer. • Modifying the surface of liposome enhance the anti-tumor activity of paclitaxel combined with biotin in liposome structure.	[341]
Biotin one of the essential B-complex vitamins; is a potent factor to obtain energy from the foods which have a potent anticancer targeting activity.				[342,343]
Physostigmine	Liposome	Thin-film hydration	• PEGylated liposomes prepared by thin-film hydration followed by the remote-loading method based on pH-gradient. • Physostigmine-PEGylated liposomes has prophylaxis activity against nerve poisoning and decreases cholinergic side effects. • Physostigmine- PEGylated liposomes size around 130 μm improve considerable release time. • Physostigmine-liposome improves the activity of Physostigmine during the cholinesterase-protection, <i>in vitro</i>. • Physostigmine-PEGylated liposomes exhibited a high plasma physostigmine concentration as well as cholinesterase inhibition ratio compared to liposome and free liposome and free physostigmine. • PEGylated liposome increases the duration cholinesterase-protecting effect of physostigmine.	[344]
Physostigmine is a hydrophilic toxic Para-sympathomimetic alkaloid from Calabar bean and the manchineel tree, generally used for a variety of cholinesterase inhibitors.				

Table 7. Continued 3. Drug loaded on Liposome/ Phytosome Technology

Drugs	Type	Method	Result	Ref
Methotrexate	Liposome	Fusion method	• MTX-liposome improves transferring of MTX on target cells to treat psoriasis. • MTX-liposome provide a high-entrapped efficiency of up to 85%, with less toxicity. • Low skin permeation of MTX was intimated due to Franz cell permeability assay through the healthy BALB/c mice. By the way, permeation of MTX through an inflammatory skin significantly enhance around 50%, which was induced by imiquimod. • Skin pathological assay, as well as inflammatory investigation, was proved the advantages of MTX-Liposome treatment without no toxicity the mice organs. • Entirely, due to various skin assay, it was proved that MTX-liposomes could be an interesting for the treatment of psoriasis with less toxicity and deserve further investigations.	[345]
Methotrexate (MTX), commercially known as amethopterin, is a potent suppressant of immune system. Generally used for treatment of lung, breast, and leukemia cancers, basal skin cancers, autoimmune diseases, lymphoma, osteosarcoma, resorption, hypotrophy, and abortion as well as cardiovascular disease, infection, and rheumatoid arthritis.				
Temozolomide	Liposome	Mathematical model	• The mathematical model investigates the ability of temozolomide for the treatment of a brain tumor. • Delivery capacity of temozolomide-liposome directly depends on liposome features such as enhance the transvascular permeability or improve extracellular release rates, while it is independent to an intravenous infusion duration. • Temozolomide-liposome improve the drug concentration ratio in brain tumor cells, and reducing the drug accumulation in blood, as well.	[349]
Temozolomide, with a commercial name of Temodal, is a triazine derivative; alkylating anticancer agent in preclinical trials.				
Docetaxel	Liposome 2018	Thin-film hydration	• Docetaxel conjugated anacardic acid functionalized by PEG-stealth-Liposome considerably reduces the tumor cell and decrease the toxicity compared to Taxotere® (marketing product). • Quantitative cell uptake assay revealed 2.64 and 2.88-fold on docetaxel conjugated anacardic acid by PEG-Liposome uptake, compared to free docetaxel. • Docetaxel conjugated anacardic acid functionalized by PEG-Liposome enhances the cell inhibition and apoptosis in MCF-7 cells compared to free docetaxel and a significant increase (around 3.7 and 4.5-fold) on AUC and t1/2 value synthesized liposome compared to Taxotere® as a control.	[350]
Docetaxel (DTX) is a clinically effective anti-tumor agent used to treat a variety of cancers like breast, lung, ovarian, prostate, and head.				[351]

Table 7. Continued 4. Drug loaded on Liposome/ Phytosome Technology

Drugs	Type	Method	Result	Ref
Bufalin	Liposome 2019	Modified ethanol injection method	- High encapsulation efficiency >85 was observed from different liposome structure such as Bufalin-liposome, bufalin-PEGylated liposome, Bufalin PEGylated- RGD targeted- liposome. - Higher inhibition of A549 cells' increase was observed from bufalin PEGylated- RGD targeted-liposome in a cytotoxicity assay compared to un-targeted liposome sample. Bufalin PEGylated-RGD targeted- liposome showed more effective cancer cells than non-targeted bufalin liposomes, well.	[352]
Bufalin, a potent antitumor agent is a cardiotonic steroid isolated from Chansu (toad venom).				[353,354]
Ciprofloxacin	Liposome 2019	-	- Ciprofloxacin loaded in porphyrin-liposome (photoactivatable liposomes) could increase the penetration of antibiotics. - Porphyrin-phospholipid was modifying the liposomes structures by 2% mole cause active loading of ciprofloxacin, which provides significant growth inhibition of <i>Bacillus subtilis</i> in liquid media, accelerated light-triggered drug release, and 90% antibiotic release (> 90%) at less than 30s.	[355]
Porphyrin–phospholipids a biodegradable phospholipid-based nano-carriers used as photo-activated agents to formulate a photoactivatable liposome				[356–358]
Keratinocyte	Liposome 2019	Reverse-phase evaporation method	- Keratinocyte encapsulated by neutrophil membrane vesicle modified liposome with 1:50 ratio enhances the stability of Keratinocyte and Keratinocyte liposome and provide high-entrapped efficiency (98.3%). - Keratinocyte encapsulated by neutrophil membrane vesicle modified liposome provides an inflammatory HUVEC and cause recovery of the morphology and function of the bowel - Keratinocyte encapsulated by neutrophil membrane vesicle modified liposome follows a suitable nanopatform delivery pattern to the delivery of KGF.	[359]

Table 8. Thirty years of Liposome/phytosome Patents in the World.

Phytoconstituents	Method	Region	Patent number	Ref
Resveratrol	Phytosome	China	CN101579291A	[360]
Aescin- β -sitosterol	Phytosome	United States, Indena	US5118671	[361]
Cylated peptides	Phytosome	Jan marine skincare	US9808654B2	[362]
Polyphenolic compounds	Liposome	Mashhad, Iran	US20170172921A1	[363]
<i>Ginkgo biloba</i>	Phytosome	Europe	EP1813280A1	[364]
Breviscopine	Phytosome	China	CN103110578B	[365]
Silybin	Phytosome	Europe	WO2014147119A1	[366]
Curcumin	Phytosome	Europe	EP2228062A1	[367]
Curcumin	Phytosome	Europe	EP1837030A1	[368]
Method for Pharmaceutical composition	Liposome	Europe	WO2001082897A2	[369]
PEGylated liposome (liposome coated with polymer)	Liposome	Australia	AU2002305094C1	[370]
Flavonoids	Phytosome	China	CN103120798A	[371]
Polynucleotide	Liposome	United States	US6120798A	[372]
Glycosylated protein-liposome	Liposome	United States	US6027726A	[373]
Procyanidins (<i>Vitis vinifera</i>)	Phytosome	United States, Indena	US6297218B1	[374]
Fusogenic	Liposome	United States	US5891468A	[375]
Therapeutic liposome composition	Liposome	United States	US6936272B2	[376]
Amphiphilic compounds	Liposome	United States	US6284267B1	[377]
Fusogenic liposome	Liposome	Japan	JP4128617B2	[378]
Gene-liposome	Liposome	United state	US5552157A	[379]
Fused liposome and acid-induced method	Liposome	United States	US4789633A	[380]
Apparatus for liposome production	Liposome	Europe	EP1146959B1	[381]
Drug loading method	Liposome	Japan	JP4874548B2	[382]
Quinolone	Liposome	United States	US5972379A	[383]
High drug-loaded method	Liposome	United States	US5616341A	[384]
Liposome method	Liposome	China	CN1980637B	[385]
Adriamycin	Liposome	United states	US4873088A	[386]
Liposomes useful for drug delivery to the brain	Liposome	United states	US20070110798A1	[387]
Lipid vehicles	Liposome	United states	US20070003610A1	[388]
Curcumin for cancer therapy	Liposome	United states	US9283185B2	[389]
Phytosome nanoscale method	Phytosome	Russia	RU2680809C2	[390]

1.3.11. Future Perspectives

Current paper aimed to emphasize the importance of L/P in transferring phytoconstituents/drugs via target cells as well as various L/P surface modifications to enhance the physiochemical properties of these promising lipid-based carriers. By this purpose, different modifications of liposome were discussed, while only few researches done regarding phytosome surface modifications. For future studies, it will be advisable to occupy the free space in the phytosome surface modification such as PEGylated phytosome, temperature responsive and thermo-sensitive phytosome, pseudo-ceramide phytosome, biopolymer coating phytosome, marking phytosome (virus, bacteria, RNA, protein, antibody, conjugated the phytosome layer) and aptamer phytosome. Furthermore, a new investigation on drug incompatibilities could be a good challenge in the field of L/P to apply in intensive care units or in clinical trials. Taking into account the limited scientific investigation in this sensible area, the effect of lipid-based nanoparticles especially L/P on drug incompatibilities, at clinical trial on intensive care units, could be a good concept for new challenging developments.

1.4. Conclusions

L/P is a novel advanced carrier for drugs/bioactive compounds with a modifiable surface layer. L/P structures had high oral and transdermal permeation and high injectable levels according to specific modifiable phospholipid surface around phytomedicine or gene carried. Although phytosome is a phytoconstituent format of liposome, both have considerable similarities and differences, being the choice influenced by the intended application. Different modifications on liposome surface allow more flexible and prevailing structures comparatively to other delivery functions. Concluding, considering the research topics discussed in this review, in different areas, L/P technology is a promising flexible technique, providing transformative structures for target delivery of drugs/bioactive compounds, as well as gene in various applications from pharmaceutic, and food to a cosmetic product.

Declaration of competing interest

The authors declare no conflict of interest.

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1-5-References

- [1] C. Ye, H. Chi, *Mater. Sci. Eng ., C.* **2018**, 83, 233.
- [2] D. Sharma, A. A. E. Ali, L. R. Trivedi, *PharmaTutor.* **2018**, 6, 50.
- [3] F. Casanova, L. Santos, *J. Microencapsul.* **2016**, 33, 1.
- [4] S. M. Jafari, *Lipid-Based Nanostructures for Food Encapsulation Purposes*, Elsevier, Amsterdam, Netherlands **2019**.
- [5] V. Nedovic, A. Kalusevic, V. Manojlovic, S. Levic, B. Bugarski, *Procedia Food Sci.* **2011**, 1, 1806.
- [6] P. Vejan, T. Khadiran, R. Abdullah, S. Ismail, A. Dadrasnia, *J. Plant Nutr.* **2019**, 42, 2600.
- [7] V. Suganya, V. Anuradha, *Int. J. Pharm. Clin. Res.* **2017**, 9, 233.
- [8] M. R. I. Shishir, L. Xie, C. Sun, X. Zheng, W. Chen, *Trends Food Sci. Technol.* **2018**, 78, 34.
- [9] M. Fathi, M. R. Mozafari, M. Mohebbi, *Trends Food Sci. Technol.* **2012**, 23, 13.
- [10] M. Sun, X. Su, B. Ding, X. He, X. Liu, A. Yu, H. Lou, G. Zhai, *Nanomedicine* **2012**, 7, 1085.
- [11] J. L. Zaro, *AAPS J.* **2015**, 17, 83.
- [12] G. B. Singhal, R. P. Patel, B. G. Prajapati, N. A. Patel, *Int. Res. J. Pharm.* **2011**, 2, 40.
- [13] C. Jaafar-Maalej, A. Elaissari, H. Fessi, *Expert Opin. Drug Deliv.* **2012**, 9, 1111.

- [14] S. B. Lim, A. Banerjee, H. Önyüksel, *J. Control. Release* **2012**, 163, 34.
- [15] A. E. Oprea, A. M. Grumezescu, *Nanotechnology Applications in Food*, Elsevier, Amsterdam, Netherlands **2017**.
- [16] F. Tamjidi, M. Shahedi, J. Varshosaz, A. Nasirpour, *Innov. Food Sci. Emerg. Technol.* **2013**, 19, 29.
- [17] L. Feng, R. J. Mumper, *Cancer lett.* **2013**, 334, 157.
- [18] S. Szunerits, R. Boukherroub, *Front. Bioeng. Biotechnol.* **2018**, 6, 15.
- [19] G. Bozzuto, A. Molinari, *Int. J. Nanomedicine.* **2015**, 10, 975.
- [20] A. L. Petersen, A. E. Hansen, A. Gabizon, T. L. Andresen, *Adv. Drug Deliv. Rev.* **2012**, 64, 1417.
- [21] B. Ghanbarzadeh, A. Babazadeh, H. Hamishehkar, *Food Biosci.* **2016**, 15, 126.
- [22] T. Amin, S. V. Bhat, *Int. J. Adv. Res. Technol.* **2012**, 1, 43.
- [23] R. Awasthi, G. T. Kulkarni, V. K. Pawar, *Int. J. Pharm. Pharm. Sci.* **2011**, 3, 1.
- [24] D. Sharma, A. A. Bhujbale, *Pharmatutor* **2018**, 6, 23.
- [25] T. Li, D. Cipolla, T. Rades, B. J. Boyd, *J. Control. Release* **2018**, 288, 96.
- [26] H. Anwekar, S. Patel, A. Singhai, *Int. J. Pharm. Life Sci.* **2011**, 2, 945.
- [27] J. Khan, A. Alexander, Ajazuddin, S. Saraf, S. Saraf, *J. Control. Release* **2013**, 168, 50.
- [28] M. Marsanasco, A. L. Márquez, J. R. Wagner, S. del V. Alonso, N. S. Chiaramoni, *Food Res. Int.* **2011**, 44, 3039.
- [29] T. B. Dudala, P. R. Yalavarthi, H. C. Vadlamudi, J. Thanniru, G. Yaga, N. L. Mudumala, V. K. Pasupati, *Int J Pharm Investig.* **2014**, 4, 149.
- [30] S. Kaddah, N. Khreich, F. Kaddah, C. Charcosset, H. G. -Gerges, *Food Chem. Toxicol.* **2018**, 113, 40.
- [31] P. P. Constantinides, K. M. Wasan, *J. Pharm. Sci.* **2007**, 96, 235.
- [32] W.-C. Huang, B. Deng, C. Lin, K. A. Carter, J. Geng, A. Razi, X. He, U. Chitgupi, J. Federizon, B. Sun, C. A. Long, J. Ortega, S. Dutta, C. R. King, K. Miura, S.-M. Lee, J. F. Lovell, *Nat. Nanotechnol.* **2018**, 13, 1174.
- [33] A. Jesorka, O. Orwar, *Annu. Rev. Anal. Chem.* **2008**, 1, 801.

- [34] A. Guliani, R. Singla, A. Kumari, S. K. Yadav, *Nanoscale Materials in Targeted Drug Delivery, Theragnosis and Tissue Regeneration*, Springer, Singapore, **2016**.
- [35] J. A. S. Suryawanshi, *J. Med. Genet. Genomics* **2011**, 3, 109.
- [36] A. Singh, V. A. Saharan, M. Singh, A. Bhandari, *Iran. J. Pharm. Sci.* **2011**, 7, 209.
- [37] S. Z. Vahed, R. Salehi, S. Davaran, S. Sharifi, *Mater. Sci. Eng. C* **2017**, 71, 1327.
- [38] N. Wang, M. Chen, T. Wang, , *J. Control. Release* **2019**, 303, 130.
- [39] A. K. Verma, A. K. Sachan, S. Kumar, N. Singh, A. K. Rai, *Int. Res J Pharm. App Sci.* **2014**, 4, 74.
- [40] H. E. Swaisgood, *Chemistry of the Caseins*, Springer, Boston, MA, **2003**.
- [41] F. Ansari, A. Sobhani, M. Salavati-Niasari, *J. Colloid Interface Sci.* **2018**, 514, 723.
- [42] S. Panja, D. K. Khatua, M. Halder, *Colloids Surfaces B.* **2019**, 180, 298.
- [43] T. Garg, A. K Goyal, *Drug deliv. Lett.* **2014**, 4, 62.
- [44] A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifehpour, M. Samiei, M. Kouhi, K. Nejati-Koshki, *Nanoscale Res. Lett.* **2013**, 8, 1.
- [45] M. J. Glade, K. Smith, *Nutrition* **2015**, 31, 781.
- [46] N. Jain, B. P. Gupta, N. Thakur, R. Jain, J. Banweer, D. K. Jain, S. Jain, *Int. j. pharm.* **2010**, 2, 224.
- [47] J. Patel, R. Patel, K. Khambholja, N. Patel, *Asian J. Pharm. Sci.* **2009**, 4, 363.
- [48] P. M. Kidd, *Altern. Med. Rev.* **2009**, 14, 226.
- [49] J. A. Kareparamban, P. H. Nikam, A. P. Jadhav, V. J. Kadam, *Int. J. Res. Pharm. Chem.* **2012**, 2, 299.
- [50] Z. Huang, X. Li, T. Zhang, Y. Song, Z. She, J. Li, Y. Deng, *Asian J. Pharm. Sci.* **2014**, 9, 176.
- [51] K. Kataria, A. Sharma, T. Garg, A. K Goyal, G. Rath, *Drug Deliv. Lett.* **2014**, 4, 79.
- [52] W. Zhang, G. Wang, J. R. Falconer, B. C. Baguley, J. P. Shaw, J. Liu, H. Xu, E. See, J. Sun, J. Aa, Z. Wu., *Pharm. Res.* **2015**, 32, 1451.
- [53] Z. Wen, B. Liu, Z. Zheng, X. You, Y. Pu, Q. Li, *Chem. Eng. Res. Des.* **2010**, 88, 1102.
- [54] C.-Q. Li, L.-C. He, H.-Y. Dong, J.-Q. Jin, *J. Ethnopharmacol.* **2007**, 114, 212.

- [55] S. Kumar, A. Baldi, D. K. Sharma, *J. Dev. Drugs* **2020**, 9, 1.
- [56] Y. P. Patil, S. Jadhav, *Chem. Phys. Lipids* **2014**, 177, 8.
- [57] S. Rasaei, S. Ghanbarzadeh, M. Mohammadi, H. Hamishehkar, *Pharm. Sci.* **2014**, 20, 96.
- [58] A. Wagner, K. Vorauer-Uhl, *J. Drug Deliv.* **2011**, 2011, 1.
- [59] H. Mirzaei, A. Shakeri, B. Rashidi, A. Jalili, Z. Banikazemi, A. Sahebkar, *Biomed. Pharmacother.* **2017**, 85, 102.
- [60] D. R. Telange, A. T. Patil, A. Tatode, B. Bhoyar, *Pharm. Methods* **2014**, 5, 34.
- [61] R. Singh, S. Parpani, R. Narke, R. Chavan, *Asian J. Pharm. Res. Dev.* **2014**, 2, 15.
- [62] N. Dewan, D. Dasgupta, S. Pandit, P. Ahmed, *J. Pharmacogn. Phytochem.* **2016**, 5, 104.
- [63] A. Semalty, M. Semalty, D. Singh, M. S. M. Rawat, *J. Incl. Phenom. Macrocycl. Chem.* **2010**, 67, 253.
- [64] M. S. Freag, Y. S. R. Elnaggar, O. Y. Abdallah, *Int J Nanomedicine.* **2013**, 8, 2385.
- [65] S. Tripathy, D. K. Patel, L. Barob, S. K. Naira, *J. Drug Deliv. Ther.* **2013**, 3, 147.
- [66] P.-F. Yue, W.-J. Zhang, H.-L. Yuan, M. Yang, W.-F. Zhu, P.-L. Cai, X.-H. Xiao, *AAPS PharmSciTech.* **2008**, 9, 322.
- [67] N. K. Gupta, V. K. Dixit, *Arch. Dermatol. Res.* **2011**, 303, 89.
- [68] A. Semalty, M. Semalty, M. S. M. Rawat, F. Franceschi, *Fitoterapia* **2010**, 81, 306.
- [69] M. Kumar, M. Ahuja, S. K. Sharma, *Sci. Pharm.* **2008**, 76, 761.
- [70] F. Franceschi, A. Giori, *US Patent 20100068316*, **2010**.
- [71] P. Amit, T. Y. S., S. Rakesh, P. Poojan, *J Pharm Sci Biosci Res* **2013**, 3, 51.
- [72] D. Guimarães, J. Noro, A. Loureiro, A. Cavaco-Paulo, E. Nogueira, *Colloid Surface B* **2019**, 179, 414.
- [73] H. Qu, C. Hao, Z. Nan, X. Zhang, R. Sun, *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2020**, 224, 117459.
- [74] C. K. Hindarto, S. Surini, F. C. Saputri, C. Irawan, *Pharma Innov. J.* **2017**, 6, 347.
- [75] R. Frkanec, V. Noethig-Laslo, B. Vranešić, K. Miroslavljević, J. Tomašić, *Biochim. Biophys. Acta - Biomembr.* **2003**, 1611, 187.

- [76] M. Brgles, K. Miroslavljević, V. Noethig-Laslo, R. Frkanec, J. Tomašić, *Int. J. Biol. Macromol.* **2007**, *40*, 312.
- [77] K. Togami, T. Daisho, Y. Yumita, A. Kitayama, H. Tada, S. Chono, *Int. J. Pharm.* **2019**, *562*, 218.
- [78] S. Simões, A. Filipe, H. Faneca, M. Mano, N. Penacho, N. Düzgünes, M. Pedroso de Lima, *Expert Opin. Drug Deliv.* **2005**, *2*, 237.
- [79] M. Kornek, V. Lukacs-Kornek, A. Limmer, E. Raskopf, U. Becker, M. Klöckner, T. Sauerbruch, V. Schmitz, *Mol. Med.* **2008**, *14*, 365.
- [80] T. N. Pashirova, N. A. Zhukova, S. S. Lukashenko, F. G. Valeeva, E. A. Burilova, A. S. Sapunova, A. D. Voloshina, A. B. Mirgorodskaya, L. Y. Zakharova, O. G. Sinyashin, *Colloid Surface B* **2019**, *178*, 317.
- [81] K. S. Pfaendler, K. S. Tewari, *Am. J. Obstet. Gynecol.* **2016**, *214*, 22.
- [82] W. Zhong, L. Jiang, X. Wang, A. Huang, L. Li, L. Ma, *Colloid Surface B* **2018**, *167*, 509.
- [83] G. A. Joanitti, R. S. Sawant, V. P. Torchilin, S. M. de Freitas, R. B. Azevedo, *Colloid Surface B* **2018**, *167*, 474.
- [84] G. Lou, G. Anderluzzi, S. Woods, C. W. Roberts, Y. Perrie, *Eur. J. Pharm. Biopharm.* **2019**, *143*, 51.
- [85] K. Tahara, M. Kobayashi, S. Yoshida, R. Onodera, N. Inoue, H. Takeuchi, *Int. J. Pharm.* **2018**, *543*, 311.
- [86] D. Li, J. Zhuang, Y. Yang, D. Wang, J. Yang, H. He, W. Fan, A. Banerjee, Y. Lu, W. Wu, L. Gan, J. Qi, *Colloid Surface B* **2018**, *171*, 224.
- [87] L. F. Neves, J. Duan, A. Voelker, A. Khanal, L. McNally, J. Steinbach-Rankins, B. P. Ceresa, *J. Microencapsul.* **2016**, *33*, 391.
- [88] D. A. Balazs, W. Godbey, *J. Drug Deliv.* **2011**, *2011*, 1.
- [89] Z. Zhong, J. Han, Y. Wan, Z. Zhang, X. Sun, *Mol. Pharm.* **2011**, *8*, 673.
- [90] S. D. Patil, D. G. Rhodes, D. J. Burgess, *AAPS J.* **2004**, *6*, 13.
- [91] S. Khadke, C. B. Roces, A. Cameron, A. Devitt, Y. Perrie, *J. Control. Release* **2019**, *307*, 211.
- [92] P. Trucillo, R. Campardelli, E. Reverchon, *J. Supercrit. Fluids* **2019**, *154*, 104626.
- [93] B. Gill, J. Singh, V. Sharma, S. L. H. Kumar, *Asian J. Pharm.* **2012**, *6*, 87.

- [94] M. H. Ucisik, U. B. Sleytr, B. Schuster, *Curr. Pharm. Biotechnol.* **2015**, 16, 392.
- [95] C.-F. Hung, J.-K. Chen, M.-H. Liao, H.-M. Lo, J.-Y. Fang, *J. Nanosci. Nanotechnol.* **2006**, 6, 2950.
- [96] X. Zhou, Z. Chen, *Arch. Pharm. Res.* **2015**, 38, 2193.
- [97] K. Pal, I. Banerjee, P. Sarkar, D. Kim, W. P. Deng, N. K. Dubey, K. Majumder, *Biopolymer-Based Formulations: Biomedical and Food Applications*, Elsevier, Amsterdam, Netherlands **2020**.
- [98] A. T. Yousefi, S. Ikeda, M. R. Mahmood, J. Rouhi, H. T. Yousefi, *World Applied Sciences Journal* **2012**, 17, 524.
- [99] J. Pushpamalar, A. K. Veeramachineni, C. Owh, X. J. Loh, *ChemPlusChem* **2016**, 81, 504.
- [100] J. Sun, R. Zhao, J. Zeng, G. Li, X. Li, *Molecules* **2010**, 15, 5162.
- [101] I. Mourtzinou, N. Kalogeropoulos, S. E. Papadakis, K. Konstantinou, V. T. Karathanos, *J. Food Sci.* **2008**, 73, 89.
- [102] N. Devi, M. Sarmah, B. Khatun, T. K. Maji, *Adv. Colloid Interface Sci.* **2017**, 239, 136.
- [103] L. Liao, Y. Luo, M. Zhao, Q. Wang, *Colloid Surface B* **2012**, 92, 305.
- [104] R. J. Moore, K. G. Jackson, A. M. Minihane, *Br. J. Nutr.* **2009**, 102, 1790.
- [105] N. Li, L. S. Taylor, L. J. Mauer, *J. Agric. Food Chem.* **2011**, 59, 6082.
- [106] D. Dag, S. Guner, M. H. Oztop, *Int. J. Biol. Macromol.* **2019**, 138, 473.
- [107] E.-H. Lee, S.-J. Lim, M.-K. Lee, *Carbohydr. Polym.* **2019**, 224, 115143.
- [108] J. Zhang, Q. Tang, X. Xu, N. Li, *Int. J. Pharm.* **2013**, 448, 168.
- [109] J. Azzi, A. Jraij, L. Auezova, S. Fourmentin, H. Greige-Gerges, *Food Hydrocoll.* **2018**, 81, 328.
- [110] Y. Li, H. Liu, Q. Han, B. Kong, Q. Liu, *Food Chem.* **2017**, 222, 74.
- [111] A. J. Bradley, D. V. Devine, S. M. Ansell, J. Janzen, D. E. Brooks, *Arch. Biochem. Biophys.* **1998**, 357, 185.
- [112] R. Mare, D. Paolino, C. Celia, R. Molinaro, M. Fresta, D. Cosco, *Int. J. Pharm.* **2018**, 552, 414.
- [113] X. Wang, H. Wang, K. Jiang, Y. Zhang, C. Zhan, M. Ying, M. Zhang, L. Lu, R. Wang, S. Wang, D. J. Burgess, H. Wang, W. Lu, *J. Control. Release* **2019**, 293, 201.

- [114] Y. Xiao, Q. Liu, A. J. Clulow, T. Li, M. Manohar, E. P. Gilbert, L. de Campo, A. Hawley, B. J. Boyd, *Colloid Surface B* **2019**, 182, 110362.
- [115] T. Lajunen, R. Nurmi, D. Wilbie, T. Ruoslahti, N. G. Johansson, O. Korhonen, T. Rog, A. Bunker, M. Ruponen, A. Urtti, *J. Control. Release* **2018**, 284, 213.
- [116] T. Lajunen, L.-S. Kontturi, L. Viitala, M. Manna, O. Cramariuc, T. Róg, A. Bunker, T. Laaksonen, T. Viitala, L. Murtomäki, A. Urtti, *Mol. Pharm.* **2016**, 13, 2095.
- [117] Y. Cheng, T. Zou, M. Dai, X.-Y. He, N. Peng, K. Wu, X.-Q. Wang, C.-Y. Liao, Y. Liu, *Colloid Surface B* **2019**, 178, 263.
- [118] M. Wang, J.-C. Kim, *Int. J. Pharm.* **2014**, 468, 243.
- [119] L.-A. Tai, P.-J. Tsai, Y.-C. Wang, Y.-J. Wang, L.-W. Lo, C.-S. Yang, *Nanotechnology* **2009**, 20, 135101.
- [120] R. Nemoto, K. Fujieda, Y. Hiruta, M. Hishida, E. Ayano, Y. Maitani, K. Nagase, H. Kanazawa, *Colloid Surface B* **2019**, 176, 309.
- [121] Y. Chang, K. Yang, P. Wei, S. Huang, Y. Pei, W. Zhao, Z. Pei, *Angew. Chem. Int. Engl.* **2014**, 53, 13126.
- [122] J. Bae, A. Maurya, Z. Shariat-Madar, S. N. Murthy, S. Jo, *AAPS J.* **2015**, 17, 1357.
- [123] R. L. McCarley, *Annu. Rev. Anal. Chem.* **2012**, 5, 391.
- [124] E. Heidarli, S. Dadashzadeh, A. Haeri, *Iran. J. Pharm. Res.* **2017**, 16, 1273.
- [125] Y.-C. Wang, F. Wang, T.-M. Sun, J. Wang, , *Bioconjug. Chem.* **2011**, 22, 1939.
- [126] A. K. Singla, A. Garg, D. Aggarwal, *Int. J. Pharm.* **2002**, 235, 179.
- [127] Z. Wang, L. Ling, Y. Du, C. Yao, X. Li, *Int. J. Pharm.* **2019**, 564, 244.
- [128] Y. Fan, C. Chen, Y. Huang, F. Zhang, G. Lin, *Colloid Surfaces B* **2017**, 151, 19.
- [129] J. J. Sudimack, W. Guo, W. Tjarks, R. J. Lee, *Biochim. Biophys. Acta - Biomembr.* **2002**, 1564, 31.
- [130] J. Liu, Y. Huang, A. Kumar, A. Tan, S. Jin, A. Mozhi, X.-J. Liang, *Biotechnol. Adv.* **2014**, 32, 693.
- [131] M. Tang, D. Svirskis, E. Leung, M. Kanamala, H. Wang, Z. Wu, *J. Control. Release* **2019**, 305, 89.
- [132] E. Fattal, P. Couvreur, C. Dubernet, *Adv. Drug Deliv. Rev.* **2004**, 56, 931.

- [133] J. de Oliveira Silva, R. S. Fernandes, C. M. Ramos Oda, T. H. Ferreira, A. F. M. Botelho, M. M. Melo, M. C. de Miranda, D. A. Gomes, G. D. Cassali, D. M. Townsend, *Biomed . Pharmacother.* **2019**, *118*, 109323.
- [134] S. H. Abd El-Alim, A. A. Kassem, M. Basha, A. Salama, , *Int. J. Pharm.* **2019**, *563*, 293.
- [135] Y.-P. Fang, Y.-B. Huang, P.-C. Wu, Y.-H. Tsai, *Eur. J. Pharm. Biopharm.* **2009**, *73*, 391.
- [136] E. Touitou, B. Godin, *J. Drug Deliv. Sci. Technol.* **2007**, *17*, 303.
- [137] E. Touitou, B. Godin, M. Shumilov, N. Bishouty, D. Ainbinder, R. Shouval, A. Ingber, V. Leibovici, *J. Eur. Acad. Dermatol. Venereol.* **2008**, *22*, 629.
- [138] V. Wilson, K. Siram, S. Rajendran, V. Sankar, *Artif. Cell. Nanomed. B* **2018**, *46*, 1892.
- [139] S. Rai, V. Pandey, G. Rai, *Nano Rev. Exp.* **2017**, *8*, 1325708.
- [140] H. A. Benson, *Expert Opin. Drug Deliv.* **2006**, *3*, 727.
- [141] S. Jain, P. Jain, R. Umamaheshwari, N. Jain, *Drug Dev. Ind. Pharm.* **2003**, *29*, 1013.
- [142] S. Duangjit, P. Opanasopit, T. Rojanarata, T. Ngawhirunpat, *J. Drug Deliv.* **2011**, *2011*, 1.
- [143] S. Duangjit, P. Opanasopit, T. Rojanarata, T. Ngawhirunpat, *AAPS PharmSciTech* **2013**, *14*, 133.
- [144] X. Ge, M. Wei, S. He, W.-E. Yuan, *Pharmaceutics* **2019**, *11*, 55.
- [145] P. Bhardwaj, P. Tripathi, R. Gupta, S. Pandey, *J. Drug Deliv. Sci. Technol.* **2020**, *56*, 101581.
- [146] R. Bartelds, M. H. Nematollahi, T. Pols, M. C. Stuart, A. Pardakhty, G. Asadikaram, B. Poolman, *PLoS One* **2018**, *13*, 0194179.
- [147] M. Gharbavi, J. Amani, H. Kheiri-Manjili, H. Danafar, A. Sharafi, *Adv. Pharmacol. Sci.* **2018**, *2018*, 1.
- [148] R. Muzzalupo, E. Mazzotta, *Expert Opin. Drug Deliv.* **2019**, *16*, 1145.
- [149] H. B. Mohamed, S. M. El-Shanawany, M. A. Hamad, M. Elsabahy, *Sci. Rep.* **2017**, *7*, 1.
- [150] M. A. Obeid, I. Khadra, A. Albaloushi, M. Mullin, H. Alyamani, V. A. Ferro, *Beilstein J. Nanotechnol.* **2019**, *10*, 1826.

- [151] M. S. Webb, M. B. Bally, L. D. Mayer, J. J. Miller, P. G. Tardi (Arbutus Biopharma Corp), *US5741516A*, **1996**.
- [152] S. Saraf, D. Gupta, C. D. Kaur, S. Saraf, *Int J Cur Sci Res.* **2011**, *1*, 63.
- [153] B. Sarmento, J. d. Neves, *Biomedical applications of functionalized nanomaterials: concepts, development and clinical translation*, Elsevier, Amsterdam, Netherland **2018**.
- [154] B.-J. Lee, V. H. Nguyen, B.-J. Lee, *Int. J. Nanomedicine*, **2017**, *12*, 3137.
- [155] A. Kumar, A. Dhawan, *Nanoparticle–Protein Corona: Biophysics to Biology*, The Royal Society of Chemistry London, United Kingdom **2019**.
- [156] M. Bros, L. Nuhn, J. Simon, L. Moll, V. Mailänder, K. Landfester, S. Grabbe, *Front. Immunol.* **2018**, *9*, 1760.
- [157] C. Corbo, R. Molinaro, F. Taraballi, N. E. T. Furman, M. B. Sherman, A. Parodi, F. Salvatore, E. Tasciotti, *Int. J. Nanomedicine* **2016**, *11*, 3049.
- [158] V. Colapicchioni, M. Tilio, L. Digiacomo, V. Gambini, S. Palchetti, C. Marchini, D. Pozzi, S. Occhipinti, A. Amici, G. Caracciolo, *Int. J. Biochem. Cell Biol.* **2016**, *75*, 180.
- [159] K. Kristensen, T. B. Engel, A. Stensballe, J. B. Simonsen, T. L. Andresen, *J. Control. Release* **2019**, *307*, 1.
- [160] S. Palchetti, V. Colapicchioni, L. Digiacomo, G. Caracciolo, D. Pozzi, A. L. Capriotti, G. La Barbera, A. Laganà, *Biochim Biophys Acta Biomembr.* **2016**, 1858, 189.
- [161] Y. Takagi, X. Ning, A. Takahashi, C. Ishizaki, T. Nishizaka, K. Kaizu, M. Miyaki, H. Yamamuro, M. Kawashima, *Clin. Cosmet. Investig. Dermatol.* **2018**, *11*, 141.
- [162] M. K. Kim, E. S. Jeong, K. N. Kim, S. H. Park, J. W. Kim, *Colloids Surfaces B* **2014**, *116*, 597.
- [163] D.-H. Kim, S.-G. Oh, Y.-J. Lee, Y.-J. Kim, H.-K. Kim, H.-H. Kang, *Colloids Surfaces A* **2002**, *207*, 177.
- [164] D.-H. Kim, E. J. An, J. Kim, S.-H. Han, J.-W. Kim, S.-G. Oh, K.-D. Suh, E. C. Cho, *Colloids Surfaces B* **2009**, *73*, 207.
- [165] D.-H. Kim, W. R. Park, J. H. Kim, E. C. Cho, E. J. An, J.-W. Kim, S.-G. Oh, *Colloids Surfaces* **2012**, *94*, 236.
- [166] A. R. Kim, N. H. Lee, Y. M. Park, S. N. Park, *J. Drug Deliv. Sci. Technol.* **2019**, *52*, 150.
- [167] Y. Zhang, B. S. Lai, M. Juhas, *Molecules* **2019**, *24*, 941.

- [168] M. H. Ali, M. E. Elsherbiny, M. Emara, *Int. J. Mol. Sci.* **2019**, *20*, 2511.
- [169] C. L. Esposito, S. Catuogno, G. Condorelli, P. Ungaro, V. De Franciscis, *Genes* **2018**, *9*, 529.
- [170] S. A. Moosavian, A. Sahebkar, *Cancer Lett.* **2019**, *448*, 144.
- [171] K. Plourde, R. M. Derbali, A. Desrosiers, C. Dubath, A. Vallée-Bélisle, J. Leblond, *J. Control. Release* **2017**, *251*, 82.
- [172] X.-q. Dou, H. Wang, J. Zhang, F. Wang, G.-I. Xu, C.-c. Xu, H.-h. Xu, S.-s. Xiang, J. Fu, H.-f. Song, *Int. J. Nanomedicine* **2018**, *13*, 763.
- [173] M. N. Ara, T. Matsuda, M. Hyodo, Y. Sakurai, N. Ohga, K. Hida, H. Harashima, *Biol. Pharm. Bull.* **2014**, *37*, 1742.
- [174] S. Hameed, P. Bhattarai, Z. Dai, *Front. Chem.* **2018**, *6*, 127.
- [175] A. George, *Advances in biomimetics*, InTech, Rijeka, Croatia **2011**.
- [176] M. Hashizume, H. Inoue, K. Katagiri, A. Ikeda, J.-I. Kikuchi, , *J. Sol-Gel Sci. Technol.* **2004**, *31*, 99.
- [177] G. Zhou, L. Li, J. Xing, S. Jalde, Y. Li, J. Cai, J. Chen, P. Liu, N. Gu, M. Ji, *Colloids Surfaces B* **2016**, *148*, 518.
- [178] Y. Fan, Y. Fang, L. Ma, *Colloids Surfaces B* **2014**, *123*, 8.
- [179] D. Patel, R. Jani, C. Patel, *Sys Rev Pharm.* **2011**, *2*, 72.
- [180] M. Kamalesh, B. Diraj, B. Kiran, W. Kalpesh, , *Indo AM. J. Pharm. Res.* **2014**, *4*, 1363.
- [181] P.-F. Yue, Q. Zheng, B. Wu, M. Yang, M.-S. Wang, H.-Y. Zhang, P.-Y. Hu, Z.-F. Wu, *Pharm. Dev. Technol.* **2012**, *17*, 94.
- [182] A. Pandita, P. Sharma, *Int. Sch. Res. Notices* **2013**, *2013*, 1.
- [183] A. Huckriede, L. Bungener, T. Stegmann, T. Daemen, J. Medema, A. M. Palache, J. Wilschut, *Vaccine* **2005**, *23*, S26.
- [184] J. Mestecky, M. W. Russell, H. Cheroutre, W. Strober, B. L. Kelsall, B. N. Lambrecht, *Mucosal Immunology*, Elsevier, Amsterdam, Netherlands **2015**.
- [185] P. Rathore, G. Swami, *Int. J. Pharm. Sci. Res.* **2012**, *3*, 3591.
- [186] Y. R Hundekar, B. K Nanjwade, A. S Mohamied, N. F Idris, T. Srichana, A. S. Md Shafioul, J. Nanomed. Nanotechnol. **2015**, *2*.
- [187] S. Shefrin, C. Sreelaxmi, V. Vijayan, S. C. Nair, *Int. J. Appl. Pharm.* **2017**, *1*.

- [188] M. M. Gaspar, O. C. Boerman, P. Laverman, M. L. Corvo, G. Storm, M. E. M. Cruz, *J. Control. Release* **2007**, *117*, 186.
- [189] P. Chandaroy, A. Sen, P. Alexandridis, S. W. Hui, *Biochim Biophys Acta Biomembr.* **2002**, *1559*, 32.
- [190] M. U. Aiswarya, K. Raju, R. B. Menon, V. S. Lakshmi, S. C. NAIR, *Int. J. App. Pharm.* **2019**, *11*, 7.
- [191] A. D. Dinsmore, M. F. Hsu, M. Nikolaides, M. Marquez, A. R. Bausch, D. A. Weitz, *Science* **2002**, *298*, 1006.
- [192] K. L. Thompson, M. Williams, S. P. Armes, *J. Colloid Interface Sci.* **2015**, *447*, 217.
- [193] L. Decome, M. De Méo, A. Geffard, O. Doucet, G. Duménil, A. Botta, *J. Photochem. Photobiol. B Biol.* **2005**, *79*, 101.
- [194] X. Zhang, W. Zong, J. Wang, M. Dong, W. Cheng, T. Sun, X. Han, *New J. Chem.* **2019**, *43*, 4895.
- [195] G. Kaur, T. Garg, G. Rath, A. K. Goyal, *Drug Deliv.* **2016**, *23*, 2497.
- [196] T. Benvegnu, L. Lemiègre, S. Cammas-Marion, *Recent Pat. Drug Deliv. Formul.* **2009**, *3*, 206.
- [197] G. Garg, S. Saraf, S. Saraf, *Biol. Pharm. Bull.* **2007**, *30*, 350.
- [198] Z. Karami, M. Hamidi, *Drug Discov. Today* **2016**, *21*, 789.
- [199] S. Massadeh, *Nanotechnology in Drug Delivery*, One Central Press, Manchester, United Kingdom **2016**.
- [200] F. A. Plamper, W. Richtering, *Acc. Chem. Res.* **2017**, *50*, 131.
- [201] E. An, C. B. Jeong, C. Cha, D. H. Kim, H. Lee, H. Kong, J. Kim, J. W. Kim, *Langmuir* **2012**, *28*, 4095.
- [202] B. C. Borro, M. S. Toussaint, S. Bucciarelli, M. Malmsten, *Biochim. Biophys. Acta - Gen. Subj* **2019**, *1865*, 129485.
- [203] O. V. Ivashkov, T. M. Yakimova, E. G. Evtushenko, A. P. Gelissen, F. A. Plamper, W. Richtering, A. A. Yaroslavov, *Colloids Surfaces A* **2019**, *570*, 396.
- [204] E. S. Jeong, H. Am Son, M. K. Kim, K.-H. Park, S. Kay, P. S. Chae, J. W. Kim, *Colloids and Surfaces B: Biointerfaces* **2014**, *123*, 339.
- [205] M. W. Jøraholmen, P. Basnet, M. J. Tostrup, S. Moueffaq, N. Škalko-Basnet, *Pharmaceutics* **2019**, *11*, 53.

- [206] Y.-H. Ma, J. Yang, B. Li, Y.-W. Jiang, X. Lu, Z. Chen, *Polym. Chem.* **2016**, 7, 2037.
- [207] H. Abdelkader, M. R. Longman, R. G. Alany, B. Pierscionek, *Int. J. Nanomedicine* **2016**, 11, 2815.
- [208] S. F. El-Menshawe, A. A. Ali, M. A. Rabeh, N. M. Khalil, *Int. J. Nanomedicine* **2018**, 13, 307.
- [209] R. M. Hathout, H. A. Gad, S. M. Abdel-Hafez, N. Nasser, N. Khalil, T. Ateyya, A. Amr, N. Yasser, S. Nasr, A. A. Metwally, *Int. J. Pharm.* **2019**, 556, 192.
- [210] A. Chaudhury, S. Das, R. F. Lee, K.-B. Tan, W.-K. Ng, R. B. Tan, G. N. Chiu, *Int. J. Pharm.* **2012**, 430, 167.
- [211] C. Cheng, Z. Wu, D. J. McClements, L. Zou, S. Peng, W. Zhou, W. Liu, *Colloids surfaces B* **2019**, 183, 110460.
- [212] M. Okumura, H. Ichihara, Y. Matsumoto, *Drug Deliv.* **2018**, 25, 1192.
- [213] J.-H. Lee, Y. Shin, W. Lee, K. Whang, D. Kim, L. P. Lee, J.-W. Choi, T. Kang, *Sci. Adv.* **2016**, 2, e1601838.
- [214] M. Gómez-Ballesteros, J. J. López-Cano, I. Bravo-Osuna, R. Herrero-Vanrell, I. T. Molina-Martínez, *Polymers* **2019**, 11, 929.
- [215] H. Ichihara, S. Nakagawa, Y. Matsuoka, K. Yoshida, Y. Matsumoto, R. Ueoka, *Anticancer Res.* **2014**, 34, 4701.
- [216] K. Sen, S. Banerjee, M. Mandal, *Colloids Surfaces B* **2019**, 180, 9.
- [217] L. Giansanti, M. Condello, B. Altieri, L. Galantini, S. Meschini, G. Mancini, *Chem. Phys. Lipids* **2019**, 223, 104781.
- [218] Y. Pu, H. Zhang, Y. Peng, Q. Fu, Q. Yue, Y. Zhao, L. Guo, Y. Wu, *Eur. J. Med. Chem.* **2019**, 183, 111720.
- [219] A. Salminen, M. Lehtonen, T. Paimela, K. Kaarniranta, *Biochem. Biophys. Res. Commun* **2010**, 394, 439.
- [220] J. Liu, J. Lee, M. A. S. Hernandez, R. Mazitschek, U. Ozcan, *Cell* **2015**, 161, 999.
- [221] J. Wolfram, K. Suri, Y. Huang, R. Molinaro, C. Borsoi, B. Scott, K. Boom, D. Paolino, M. Fresta, J. Wang, *Journal of microencapsulation* **2014**, 31, 501.
- [222] M. S. Freag, W. M. Saleh, O. Y. Abdallah, *Int. J. Pharm.* **2018**, 535, 18.
- [223] D. Pastorelli, A. S. Fabricio, P. Giovanis, S. D'Ippolito, P. Fiduccia, C. Soldà, A. Buda, C. Sperti, R. Bardini, G. Da Dalt, *Pharmacological research* **2018**, 132, 72.

- [224] S. Mukherjee, A. Fried, R. Hussaini, R. White, J. Baidoo, S. Yalamanchi, P. Banerjee, *J. Exp. Clin. Cancer Res.* **2018**, 37, 1.
- [225] C. F. de Freitas, M. C. Montanha, D. S. Pelloso, E. Kimura, W. Caetano, N. Hioka, *Mater. Sci. Eng. C* **2019**, 104, 109923.
- [226] T. Ferrara, G. De Vincentiis, F. Di Pierro, *Eur. Rev. Med. Pharmacol. Sci.* **2015**, 19, 3757.
- [227] E.-C. Kim, J.-K. Min, T.-Y. Kim, S.-J. Lee, H.-O. Yang, S. Han, Y.-M. Kim, Y.-G. Kwon, *Biochem. Biophys. Res. Commun.* **2005**, 335, 300.
- [228] W. Kukula-Koch, L. Czernicka, *Handbook of Dietary Phytochemicals* **2019**, 1.
- [229] R. P. Singh, H. V. Gangadharappa, K. Mruthunjaya, *EUR. J. Pharm. Sci.* **2018**, 122, 214.
- [230] J. N. Reginald-Opara, D. Svirskis, S. J. O'Carroll, S. Sreebhavan, J. M. Dean, Z. Wu, *Int. J. Pharm.* **2019**, 567, 118451.
- [231] Y. Zhao, G. Tang, Q. Tang, J. Zhang, Y. Hou, E. Cai, S. Liu, D. Lei, L. Zhang, S. Wang, *Eur. J. Drug Metab. Pharmacokinet.* **2016**, 41, 605.
- [232] Z.-L. Chen, M. Huang, X.-R. Wang, J. Fu, M. Han, Y.-Q. Shen, Z. Xia, J.-Q. Gao, *Nanomedicine: NBM* **2016**, 12, 421.
- [233] F. Yu, Y. Li, Q. Chen, Y. He, H. Wang, L. Yang, S. Guo, Z. Meng, J. Cui, M. Xue, *Eur. J. Pharm. Biopharm.* **2016**, 103, 136.
- [234] L. Gilardini, L. Pasqualinotto, F. Di Pierro, P. Risso, C. Invitti, *BMC Complement. Altern. Med.* **2016**, 16, 3.
- [235] R. Toita, T. Kawano, M. Murata, J.-H. Kang, *Biomaterials* **2016**, 110, 81.
- [236] S. A. da Silveira, A. Perez, *Clin. Pulm. Med.* **2018**, 25, 84.
- [237] S. A. da Silveira, A. Perez, *Expert Rev. Anti. Infect. Ther.* **2015**, 13, 531.
- [238] V. S. Panda, S. R. Naik, *Altern. Med. Rev.* **2009**, 14, 161.
- [239] S. Gajalakshmi, S. Vijayalakshmi, R. V. Devi, *Int. J. Pharm. Pharm. Sci.* **2012**, 4, 3.
- [240] S. Mancini, L. Nardo, M. Gregori, I. Ribeiro, F. Mantegazza, C. Delerue-Matos, M. Masserini, C. Grosso, *Phytomedicine* **2018**, 42, 233.
- [241] Z. hui-hui, L. qiu-hua, Y. zhi-jun, P. wei-san, N. shu-fang, *Drug Deliv.* **2011**, 18, 502.
- [242] R. Angelico, A. Ceglie, P. Sacco, G. Colafemmina, M. Ripoli, A. Mangia, *Int. J. Pharm.* **2014**, 471, 173.

- [243] S. R. Naik, V. S. Panda, *Fitoterapia* **2008**, 79, 439.
- [244] S. O. Ali, H. A. E.-m. Darwish, N. A. E.-f. Ismail, *Chem.-Biol. Interact.* **2014**, 216, 26.
- [245] W. Maryana, H. Rachmawati, D. Mudhakhir, *Mater. Today* **2016**, 3, 855.
- [246] S. R. Kanatt, R. Chander, A. Sharma, *Iny. J. Food. Sci. Tech.* **2010**, 45, 216.
- [247] D. Marín, A. Alemán, P. Montero, M. Gómez-Guillén, *J. Food Eng.* **2018**, 223, 132.
- [248] M. Manconi, F. Marongiu, I. Castangia, M. L. Manca, C. Caddeo, C. I. G. Tuberoso, G. Bacchetta, A. M. Fadda, *Colloids surfaces B* **2016**, 146, 910.
- [249] C. Bonechi, S. Martini, L. Ciani, S. Lamponi, H. Rebmann, C. Rossi, S. Ristori, *PLoS One* **2012**, 7, 41438
- [250] J. Kristl, K. Teskač, C. Caddeo, Z. Abramović, M. Šentjunc, *Eur. J. Pharm. Biopharm* **2009**, 73, 253.
- [251] L. Risaliti, A. Kehagia, E. Daoultzi, D. Lazari, M. C. Bergonzi, S. Vergkizi-Nikolakaki, D. Hadjipavlou-Litina, A. R. Bilia, *J. Drug Deliv. Sci. Technol.* **2019**, 51, 493.
- [252] K. R. Christian, J. C. Jackson, *J. Food Compos. Anal.* **2009**, 22, 663.
- [253] O. V. Ojulari, S. G. Lee, J.-O. Nam, *Molecules* **2019**, 24, 210.
- [254] M. H. M. Idris, S. B. Budin, M. Osman, J. Mohamed, *EXCLI J.* **2012**, 11, 659.
- [255] V. H. Shruthi, C. T. Ramachandra, U. Nidoni, S. Hiregoudar, N. Naik, A. R. Kurubar, *Plant Archives* **2016**, 16, 515.
- [256] M. Gibis, B. Zeeb, J. Weiss, *Food Hydrocoll.* **2014**, 38, 28.
- [257] A. Rahmawati, B. Fachri, S. Oktavia, F. Abrori, *IOP Conf. Ser.: Mater. Sci. Closely. (discontin.)* **2021**, 1053, 1.
- [258] V. Prakash, N. Jaiswal, M. Srivastava, *Asian J. Pharm. Clin. Res.* **2017**, 10, 69.
- [259] P. J. Ho, J. J. Sung, K. K. Cheon, H. J. Tae, *Phytomedicine* **2018**, 43, 110.
- [260] G. Maramaldi, S. Togni, I. Pagnin, L. Giacomelli, R. Cattaneo, R. Eggenhöfner, S. E. Burastero, *Clin. Cosmet. Investig. Dermatol.* **2016**, 26, 55.
- [261] X. Chen, R. Wong, I. Khalidov, A. Y. Wang, J. Leelawattanachai, Y. Wang, M. M. Jin, *Biomaterials* **2011**, 32, 7651.
- [262] C. Caddeo, M. Gabriele, X. Fernández-Busquets, D. Valenti, A. M. Fadda, L. Pucci, M. Manconi, *Int. J. Pharm.* **2019**, 565, 64.

- [263] T. Rao, A. M. A. Rani, M. Awang, M. Baharom, Y. Uemura, P. D. R. Iskandar, *J. Tribol.* **2018**, 18, 40.
- [264] T. M. Panchal, A. Patel, D. Chauhan, M. Thomas, J. V. Patel, *Renew. Sustain. Energy Rev.* **2017**, 70, 65.
- [265] Z. Wang, J. Li, X. Ge, Y. Liu, J. Luo, D. G. Chetwynd, K. Mao, *Colloids Surfaces B* **2019**, 178, 469.
- [266] R. Goldberg, J. Klein, *Chem. Phys. Lipids* **2012**, 165, 374.
- [267] S. D. Patil, D. G. Rhodes, D. J. Burgess, *AAPS J.* **2005**, 7, E61.
- [268] G. K. Zorzi, R. S. Schuh, V. J. Maschio, N. T. Brazil, M. B. Rott, H. F. Teixeira, *Colloids Surfaces B* **2019**, 173, 725.
- [269] B. dos Santos Rodrigues, H. Oue, A. Banerjee, T. Kanekiyo, J. Singh, *J. Control. Release* **2018**, 286, 264.
- [270] M. Lu, X. Zhao, H. Xing, Z. Xun, S. Zhu, L. Lang, T. Yang, C. Cai, D. Wang, P. Ding, *Int. J. Pharm.* **2018**, 550, 100.
- [271] S. Antimisiaris, S. Mourtas, K. Papadia, *Int. J. Pharm.* **2017**, 525, 293.
- [272] H. Valadi, K. Ekström, A. Bossios, M. Sjöstrand, J. J. Lee, J. O. Lötvall, *Nat. Cell Biol.* **2007**, 9, 654.
- [273] Y. Hattori, K. Tamaki, K.-i. Ozaki, K. Kawano, H. Onishi, *J. Drug Deliv. Sci. Technol.* **2019**, 52, 1042.
- [274] N. Benne, J. van Duijn, F. L. Vigario, R. J. Lebourg, P. van Veelen, J. Kuiper, W. Jiskoot, B. Slütter, *J. Control. Release* **2018**, 291, 135.
- [275] S. J. Lewis, *Am. J. Med.* **2009**, 122, S38.
- [276] S. Bergheanu, M. Bodde, J. Jukema, , *Netherlands Hear. J.* **2017**, 25, 231.
- [277] R. Mota, J. W. Homeister, M. S. Willis, E. M. Bahnson, *Issues Sci. Technol. Librariansh.* **2017**, 1.
- [278] Y. Cao, M. Zheng, W. Cai, Z. Wang, *Chinese Chem. Lett.* **2020**, 31, 463.
- [279] Y. Zhang, L. Zhang, Y. Hu, K. Jiang, Z. Li, Y.-Z. Lin, G. Wei, W. Lu, *J. Control. Release* **2018**, 289, 102.
- [280] L. Zhang, Y. Zhang, L. Tai, K. Jiang, C. Xie, Z. Li, Y.-Z. Lin, G. Wei, W. Lu, W. Pan, *Acta Biomater.* **2016**, 42, 90.
- [281] S. Sun, H. Zou, L. Li, Q. Liu, N. Ding, L. Zeng, H. Li, S. Mao, *Int. J. Pharm.* **2019**, 568,

118518.

- [282] M. Alavi, K. Asare-Addo, A. Nokhodchi, *Biomedicines* **2020**, *8*, 580.
- [283] T. T. Tai, T. J. Wu, H. D. Wu, Y. C. Tsai, H. T. Wang, A. M. Wang, S. F. Shih, Y. C. Chen, *Clin. Transl. Sci.* **2021**, *14*, 132.
- [284] G. Chauhan, M. J. Madou, S. Kalra, V. Chopra, D. Ghosh, S. O. Martinez-Chapa, *ACS nano* **2020**, *14*, 7760.
- [285] J. M. Muderhwa, G. R. Matyas, L. E. Spitler, C. R. Alving, *J. Pharm. Sci.* **1999**, *88*, 1332.
- [286] A. Badiie, M. R. Jaafari, A. Khamesipour, *Exp. Parasitol.* **2007**, *115*, 127.
- [287] G. Du, M. Leone, S. Romeijn, G. Kersten, W. Jiskoot, J. A. Bouwstra, *Int. J. Pharm.* **2018**, *547*, 250.
- [288] M. T. Kheiri, A. Jamali, M. Shenagari, H. Hashemi, F. Sabahi, F. Atyabi, R. Saghiri, *Antiviral Res.* **2012**, *95*, 229.
- [289] C. Krul, C. Humblot, C. Philippe, M. Vermeulen, M. van Nuenen, R. Havenaar, S. Rabot, *Carcinogenesis* **2002**, *23*, 1009.
- [290] B. Shofran, S. Purrington, F. Breidt, H. Fleming, *J. Food Sci.* **1998**, *63*, 621.
- [291] A. Mazumder, A. Dwivedi, J. L. Du Preez, J. Du Plessis, *Int. J. Pharm.* **2016**, *498*, 283.
- [292] S. Ternullo, E. Gagnat, K. Julin, M. Johannessen, P. Basnet, Ž. Vanić, N. Škalko-Basnet, *Eur. J. Pharm. Biopharm.* **2019**, *144*, 154.
- [293] B. K. Jadhav, K. R. Khandelwal, A. R. Ketkar, S. S. Pisal, *Drug Dev. Ind. Pharm.* **2004**, *30*, 195.
- [294] M. Müller, H.-C. Pape, E.-J. Speckmann, A. Gorji, *Neuroscience* **2006**, *140*, 743.
- [295] T. F. Bachiega, J. P. B. de Sousa, J. K. Bastos, J. M. Sforcin, *J. Pharm. Pharmacol.* **2012**, *64*, 610.
- [296] E. Pinto, L. Vale-Silva, C. Cavaleiro, L. Salgueiro, *J. Med. Microbiol.* **2009**, *58*, 1454.
- [297] C. Sebaaly, C. Charcosset, S. Stainmesse, H. Fessi, H. Greige-Gerges, *Carbohydr. Polym.* **2016**, *138*, 75.
- [298] C. Sebaaly, A. Jraij, H. Fessi, C. Charcosset, H. Greige-Gerges, *Food Chem.* **2015**, *178*, 52.
- [299] S. S. Kwon, S. Y. Kim, B. J. Kong, K. J. Kim, G. Y. Noh, N. R. Im, J. W. Lim, J. H. Ha, J. Kim, S. N. Park, *Int. J. Pharm.* **2015**, *483*, 26.

- [300] S. N. Park, N. R. Jo, S. H. Jeon, *J. Ind. Eng. Chem.* **2014**, 20, 1481.
- [301] K. Kaur, R. Sharma, S. Singh, *Pigment. Resin Technol.* **2020**, 49, 110.
- [302] K. K. Ajeeshkumar, P. A. Aneesh, N. Raju, M. Suseela, C. N. Ravishankar, S. Benjakul, *Compr. Rev. Food Sci. F.* **2021**, 20, 1280.
- [303] R. Gharib, L. Auezova, C. Charcosset, H. Greige-Gerges, *Food Chem.* **2017**, 218, 365.
- [304] M. Schwarz, V. Wray, P. Winterhalter, *J. Agric. Food Chem.* **2004**, 52, 5095.
- [305] M. Netzel, G. Netzel, D. R. Kammerer, A. Schieber, R. Carle, L. Simons, I. Bitsch, R. Bitsch, I. Konczak, *Innov. Food Sci. Emerg. Technol.* **2007**, 8, 365.
- [306] B. Guldiken, M. Gibis, D. Boyacioglu, E. Capanoglu, J. Weiss, *Food Funct.* **2017**, 8, 1085.
- [307] B. Kong, H. Zhang, Y. L. Xiong, *Meat Sci.* **2010**, 85, 772.
- [308] A. N. M. A. Haque, R. Remadevi, M. Naebe, *Cellulose* **2018**, 25, 5455.
- [309] H. Cui, J. Wu, L. Lin, *J. Dairy Sci.* **2016**, 99, 6097.
- [310] M. Nazari, B. Ghanbarzadeh, H. S. Kafil, M. Zeinali, H. Hamishehkar, *Colloid Interface Sci. Commun.* **2019**, 30, 100176.
- [311] M. M. Müller, J. H. Kügler, M. Henkel, M. Gerlitzki, B. Hörmann, M. Pöhnlein, C. Syltatk, R. Hausmann, *J. Biotechnol.* **2012**, 162, 366.
- [312] G. Soberón-Chávez, M. Aguirre-Ramírez, R. Sánchez, *J. Ind. Microbiol. Biotechnol.* **2005**, 32, 675.
- [313] C. Pu, W. Tang, X. Li, M. Li, Q. Sun, *Food Hydrocoll.* **2019**, 87, 29.
- [314] M. M. Ochi, G. Amoabediny, S. M. Rezayat, A. Akbarzadeh, B. Ebrahimi, *Cell J.* **2016**, 18, 135.
- [315] S. O. Ali, H. A. Darwish, N. A. Ismail, *Basic Clin. Pharmacol. Toxicol.* **2016**, 118, 369.
- [316] R. Gharib, S. Haydar, C. Charcosset, S. Fourmentin, H. Greige-Gerges, *J. Drug Deliv. Sci. Technol.* **2019**, 52, 794.
- [317] R. J. B. de Siqueira, P. J. C. Magalhães, J. H. Leal-Cardoso, G. P. Duarte, S. Lahlou, *Life Sci.* **2006**, 78, 2365.
- [318] S. Hemaiswarya, M. Doble, *Phytomedicine* **2009**, 16, 997.
- [319] Y. Zu, H. Overby, G. Ren, Z. Fan, L. Zhao, S. Wang, *Colloids and Surfaces B* **2018**, 164, 414.

- [320] C. Caddeo, A. Nacher, A. Vassallo, M. F. Armentano, R. Pons, X. Fernández-Busquets, C. Carbone, D. Valenti, A. M. Fadda, M. Manconi, *Int. J. Pharm.* **2016**, 513, 153.
- [321] I. Francolini, L. Giansanti, A. Piozzi, B. Altieri, A. Mauceri, G. Mancini, *Colloids Surfaces B* **2019**, 181, 632.
- [322] A. I. Abd El-Fattah, M. M. Fathy, Z. Y. Ali, A. E.-R. A. El-Garawany, E. K. Mohamed, *Chem. Biol. Interact.* **2017**, 271, 30.
- [323] D. R. Telange, A. T. Patil, A. M. Pethe, H. Fegade, S. Anand, V. S. Dave, *Eur. J. Pharm. Sci.* **2017**, 108, 36.
- [324] N. Plenagl, B. S. Seitz, L. Duse, S. R. Pinnapireddy, J. Jedelska, J. Brüllner, U. Bakowsky, *Int. J. Pharm.* **2019**, 570, 118666.
- [325] Y. Zhang, K. Shang, X. Wu, S. Song, Z. Li, Z. Pei, Y. Pei, *RSC Adv.* **2018**, 8, 21786.
- [326] Z. Jendželovská, R. Jendželovský, B. Kuchárová, P. Fedoročko, *Front. Plant Sci.* **2016**, 7, 560.
- [327] V. M. Shah, D. X. Nguyen, P. Patel, B. Cote, A. Al-Fatease, Y. Pham, M. G. Huynh, Y. Woo, A. W. Alani, *Nanomedicine: NBM* **2019**, 18, 146.
- [328] T. N. Pashirova, I. V. Zueva, K. A. Petrov, S. S. Lukashenko, I. R. Nizameev, N. V. Kulik, A. D. Voloshina, L. Almasy, M. K. Kadirov, P. Masson, *Colloids Surfaces B* **2018**, 171, 358.
- [329] S. Fang, Y. Hou, L. Ling, D. Wang, M. Ismail, Y. Du, Y. Zhang, C. Yao, X. Li, *Colloids Surfaces B* **2018**, 166, 235.
- [330] S. T. Tucci, A. Kheirloom, E. S. Ingham, L. M. Mahakian, S. M. Tam, J. Foiret, N. E. Hubbard, A. D. Borowsky, M. Baikoghli, R. H. Cheng, *J. Control. Release* **2019**, 309, 277.
- [331] L. de S. Cavalcante, G. Monteiro, *Eur. J. Pharmacol.* **2014**, 741, 8.
- [332] G. Chai, H. Park, S. Yu, F. Zhou, J. Li, Q. Xu, Q. T. Zhou, , *Int. J. Pharm.* **2019**, 569, 118616.
- [333] D. Sharma, R. P. Patel, S. T. R. Zaidi, M. Sarker, M. Rahman, Q. Y. Lean, L. C. Ming, *Front. Pharmacol.* **2017**, 8, 546.
- [334] C. C. Cheung, W. T. Al-Jamal, *Int. J. Pharm.* **2019**, 566, 687.
- [335] W. Zhan, C.-H. Wang, *Journal of Controlled Release* **2018**, 285, 212.
- [336] N. Zhang, Q. N. Ping, G. H. Huang, W. F. Xu, *Int. J. Pharm.* **2005**, 294, 247.

- [337] J. N. Patton, A. F. Palmer, *Biomacromolecules* **2005**, 6, 414.
- [338] M. Guo, S. Han, Y. Liu, W. Guo, Y. Zhao, F. Liu, X. Shi, G. Ding, Q. Wang, *Biochem. Biophys. Res. Commun.* **2019**, 516, 914.
- [339] L. Wang, R. Rubinstein, J. L. Lines, A. Wasiuk, C. Ahonen, Y. Guo, L.-F. Lu, D. Gondek, Y. Wang, R. A. Fava, A. Fiser, S. Almo, R. J. Noelle, *J. Exp. Med.* **2011**, 208, 577.
- [340] J. L. Lines, E. Pantazi, J. Mak, L. F. Sempere, L. Wang, S. O'Connell, S. Ceeraz, A. A. Suriawinata, S. Yan, M. S. Ernstoff, *Cancer Res.* **2014**, 74, 1924.
- [341] R. Lu, L. Zhou, Q. Yue, Q. Liu, X. Cai, W. Xiao, L. Hai, L. Guo, Y. Wu, *Bioorg. Med. Chem.* **2019**, 27, 3115.
- [342] J. Zemleni, S. S. Wijeratne, Y. I. Hassan, *Biofactors* **2009**, 35, 36.
- [343] N. Muhammad, N. Sadia, C. Zhu, C. Luo, Z. Guo, X. Wang, *Chem. Commun.* **2017**, 53, 9971.
- [344] J.-H. Park, J.-Y. Lee, K.-T. Kim, H.-E. Joe, H.-J. Cho, Y.-K. Shin, D.-D. Kim, *Int. J. Pharm.* **2018**, 553, 467.
- [345] M. Bahramizadeh, M. Bahramizadeh, B. Kiafar, A. H. Jafarian, A. R. Nikpoor, M. Hatamipour, H. Esmaily, Z. Rezaeemehr, S. Golmohammadzadeh, S. A. Moosavian, *International journal of pharmaceutics Int. J. Pharm.* **2019**, 569, 118623.
- [346] P. Taylor, A. Balsa Criado, A.-B. Mongey, J. Avouac, H. Marotte, R. B. Mueller, *J. Clin. Med.* **2019**, 8, 515.
- [347] J. Braun, *Clin Exp Rheumatol* **2010**, 28, S46.
- [348] J. M. Kremer, *Arthritis Rheumatol.* **2004**, 50, 1370.
- [349] W. Zhan, *Int. J. Pharm.* **2019**, 557, 280.
- [350] V. Kushwah, D. K. Jain, A. K. Agrawal, S. Jain, *Colloids and Surfaces B* **2018**, 172, 213.
- [351] K. A. Lyseng-Williamson, C. Fenton, *Drugs* **2005**, 65, 2513.
- [352] Y. Zhang, Z. Tian, X. Zhao, N. Li, V. M. Garamus, P. Yin, A. Zou, *Colloids Surf, A Physicochem Eng Asp* **2019**, 571, 72.
- [353] Y. L. Lan, J. C. Lou, X. W. Jiang, X. Wang, J. S. Xing, S. Li, B. Zhang, *Oncol. Lett.* **2019**, 17, 3635.
- [354] Q. Miao, L.-L. Bi, X. Li, S. Miao, J. Zhang, S. Zhang, Q. Yang, Y.-H. Xie, J. Zhang, S.-W. Wang, *Int. J. Mol. Sci.* **2013**, 14, 1370.

- [355] S. Ghosh, R. Qi, K. A. Carter, G. Zhang, B. A. Pfeifer, J. F. Lovell, *Biochem. Eng. J.* **2019**, *141*, 43.
- [356] D. Luo, J. Geng, N. Li, K. A. Carter, S. Shao, G. E. Atilla-Gokcumen, J. F. Lovell, *Molecular cancer therapeutics* **2017**, *16*, 2452. *Mol. Cancer Ther.* **2017**, *16*, 2452.
- [357] K. A. Carter, D. Luo, A. Razi, J. Geng, S. Shao, J. Ortega, J. F. Lovell, *Theranostics* **2016**, *6*, 2329.
- [358] S. Shao, T. N. Do, A. Razi, U. Chitgupi, J. Geng, R. J. Alsop, B. G. Dzikovski, M. C. Rheinstädter, J. Ortega, M. Karttunen, J. A. Spornyak, J. F. Lovell, *Small* **2017**, *13*, 1602505.
- [359] Y.-Z. Zhao, D.-L. ZhuGe, M.-Q. Tong, M.-T. Lin, Y.-W. Zheng, X. Jiang, W.-G. Yang, Q. Yao, Q. Xiang, X.-K. Li, H.-L. Xu, , *J. Control. Release* **2019**, 299, 90.
- [360] D. Mingzing, J. Huafeng (Tsinghua University, Jiangsu Longliqi Biotechnology Co., Ltd.), *CN101579291A*, **2009**.
- [361] E. Bombardelli, G. Patri, R. Pozzi (Indena S.p.A), *US5118671A*, **1992**.
- [362] J. Marini, S. J. Saxena (Jan Marini Skin Research), *US9808654B2*, **2017**.
- [363] M. R. Jaafari, F. Gheybi, S. M. R. Sorkhabadi (M. R. Jaafari, F. Gheybi, S. M. R. Sorkhabadi), *US20170172921A1*, **2017**.
- [364] F. D. Pierro (Pharmextracta S.r.l), *EP1813280A1*, **2007**.
- [365] C. Xiaoxin, L. Chaofeng, X. Chengshi, W. Xiong, L. Xiaoying, Z. Xiaoli, O. Su (Guangdong Huanan Pharmaceutical Group Co., Ltd.), *CN103110578B*, China Patent, **2013**.
- [366] C. Loguercio, A. Federico, P. Stiuso (Istituto Biochimico Italiano Giovanni Lorenzini S.P.A.), *WO2014147119A1*, **2014**.
- [367] F. D. Pierro (Velleja Research S.r.l), *EP2228062A1*, **2010**.
- [368] A. Giori, F. Franceschi (Indena S.p.A), *EP1837030A1*, **2007**.
- [369] M. B. Y. Betageri (WIPO), *WO2001082897A2*, **2001**.
- [370] T. M. Allen, S. K. Huang, S. Zalipsky (Alza Corp.), *AU2002305094C1*, **2002**.
- [371] S. Dongyun, L. Shanlin, X. Feijoa (Nanjing), *CN103120798A*, **2013**.
- [372] T. M. Allen, D. D. Stuart (Alza Corp.), *US6120798A*, **2000**.
- [373] S. M. Ansell (University of British Columbia), *US6027726A*, **2000**.

- [374] P. Morazzoni, E. Bombardelli (Indena S.p.A), *US6297218B1*, **2001**.
- [375] F. J. Martin, S. Zalipsky (Alza Corp), *US5891468A*, **1999**.
- [376] F. J. Martin, S. Zalipsky (Alza Corp.), *US6936272B2*, **2005**.
- [377] R. Aneja (Nutrimed Biotech), *US6284267B1*, **2001**.
- [378] A. Karisu, P. Horando, J. Maden, T. A. Karisu (The University of British Columbia), *JP4128617B2*, **2008**.
- [379] K. Yagi, H. Noda, N. Ohishi, Ma. Kurono (Vitamin Kenkyuso K.K.), *US5552157A*, **1996**.
- [380] L. Huang, J. Connor (Biowhittaker Inc. A. Corp. OF Delaware Lipogen Inc A. Corp. of De. University of Tennessee), *US4789633A*, **1988**.
- [381] M. T. Baker, W. A. Heriot (William A. Heriot), *EP1146959B1*, **2010**.
- [382] G. M. Jensen, M. Srivan, N. Fu, S. Yang (Gilead Sciences Inc.), *JP4874548B2*, **2010**.
- [383] L. S. S. Guo, J. Gittelman, S. Zalipsky, F. J. Martin (Alza Corp), *US5972379A*, **1999**.
- [384] L. D. Mayer, M. B. Bally, P. R. Cullis, R. S. Ginsberg, G. N. Mitlenes (Cephalon Ltd., Elan Pharmaceuticals LLC.), *US5616341A*, **1997**.
- [385] K. Hong, D. C. Drummond, D. B. Kilpotin (Ipsen Biopharm Ltd), *CN1980637B*, **2014**.
- [386] E. Mayhew, M. J. Ehrke, K. Mace, F. Szoka, F. C. Olson (Liposome Technology Inc.), *US4873088A*, **1989**.
- [387] D. Drummond, D. Kirpotin (Ipsen Biopharm Ltd.), *US20070110798A1*, **2007**.
- [388] M. Chancellor, M. Fraser, Y.-C. Chuang, W. D. Groat, L. Huang, N. Yoshimura (University of Pittsburgh), *US20070003610A1*, **2007**.
- [389] R. Kurzrock, L. Li, K. Mehta, B. B. Aggarwal (University of Texas System), *US9283185B2*, **2016**.
- [390] A. I. Marakhova, P. Kezimana, Y. M. Stanishevsky, B. S. Shvitko (Federal State Autonomous Educational Institution of Higher Education "Peoples' Friendship University of Russia" (PFUR)), *RU2680809C2*, **2019**.

Chapter 2

Emerging on Recent Drying technique on Food Safety and Quality:
A Review

Emerging on Recent Drying technique on Food Safety and Quality: A Review

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Reviews and Trends

2.1. Abstract

The new trends in drying technology seek a promising alternative to synthetic preservatives to improve the shelf-life and storage stability of food products. On the other hand, the drying process can result in deformation and degradation of phytoconstituents due to their thermal sensitivity. The main purpose of this review is to give a general overview of common drying techniques with special attention to food industrial applications, focusing on recent advances to maintain the features of the active phytoconstituents and nutrients, and improve their release, and storage stability. Furthermore, a drying technique that extends the shelf-life of the food products by reducing trapped water, will negatively affects the spoilage of microorganisms and enzymes that are responsible for undesired chemical composition changes, but can protect beneficial microorganisms like probiotics. This paper

also explores recent efficient improvements in drying technologies that produce high-quality and, low-cost final products compared to conventional methods. However, despite the recent advances in drying technologies, hybrid drying (a combination of different drying techniques) and spray drying (drying with the help of encapsulation methods) are still promising techniques in food industries. In conclusion, spray-drying encapsulation can improve the morphology and texture of dry materials, preserve natural components for a long time, and increase storage times (shelf-life). Optimizing a drying technique and using a suitable drying agent should also be a promising solution to preserve probiotic bacteria and antimicrobial compounds.

Keywords: Drying techniques, Food quality, Storage stability, Drying agents.

2.2. Main text

2.2.1. Introduction

Fruits, vegetables, and especially protein-rich food products possess high water contents (80–90%), allowing food spoilage and the consequent degradation/deformation of phytoconstituents. Drying technologies are physical preservation/stabilization techniques used from ancient times for food preservation (Land, 2011; Mujumdar, 2006). Given that consumers have definite expectations for product quality, a proper drying technique is needed to eliminate undesirable water and ensure that the perishable, shrinkable, and porous food components maintain the nutritional value for a long shelf-life (Mahiuddin et al., 2018). For this purpose, it is vital to find appropriate drying techniques that provide new products with high porous dried textures, high retention of the active ingredients, high drying yield, and high preservation of phytoconstituents as well as higher energy efficiency, low cost, and environmentally safe operations. Here, the common drying techniques, with a special focus on industrial food applications, are grouped and discussed comprehensively in terms of drying operation description, drying theory (heat and mass transfer), common mathematical models (if applicable), and finally some related recent examples. Therefore, a comprehensive overview at the end of the paper is available and the readers can easily choose the better technique for their related application in the food area.

2.2.2. Drying Techniques

Drying occurs when the solvent (liquid) is vaporized by the heat supplier, the removal occurring from both the bound and unbound liquid phases. There are two types of food

moisture: bound moisture which corresponds to the water trapped in the solid microstructure or present as liquid solution within the solid phase, and unbound moisture that is the free water content. Evaporation and vaporization are two techniques used to remove unbound moisture. When the vapor pressure on the solid surface is equal to the atmospheric pressure, evaporation occurs. On the other hand, when hot air passes through forced convection over the solid, moisture is drawn in and vaporization occurs. In this case, the saturation vapor pressure is lower than atmospheric pressure and moisture removal is complete (Mujumdar, 2006). Conduction, convection, and radiation are three forms of heat transfer that involve mass transfer and dry the component (Farid, 2019). However, heat and mass transfer are critical processes that take place at the same time in the drying step. Equation (1) allows the calculation of the moisture content (g) (M_c), where W_t and W_g are the residual mass and the dried powder mass (g), respectively.

$$M_c = \frac{W_t - W_g}{W_g} \quad \text{Eq.1}$$

Hence, the mass transfer occurs in two steps; one is the transfer of internal moisture to the surface of the solid and from the surface of the material to the surrounding environment. At the same time, heat transfer occurs from the surrounding environment to the material. The drying process can also have two steps. In the first, the particles are heated up to wet-bulb temperature. The extra heat evaporates free moisture from the surface of particles. Then the temperature decreases to normalize the process, maintaining an almost constant drying rate. Due to the drying process, particles shrinkage, swelling, or crushing occurs. The evaporation of trapped liquids in the surface is faster and needs less energy. In the second step, a dried thick layer at the surface surrounds the wet core. Although subjected to the heat transfer, the mass transfer rate decreases due to the formed porous solid layer. Therefore, the water trapped in the inner layer needs to be transferred to the outer layer to be eliminated, requiring more energy. The drying rate decreases due to an enhanced resistance of mass transfer. When mass and heat transfer are suspended, it becomes impossible to dry the material and the drying process stops in this stage (Figure 1). To clarify the presented theory, Equation (2) allows the calculation of the drying rate (g/(g·s)) in which M_t is the dried moisture content (g%), Δt in this case is the interval time (s) (Du et al., 2020; Mujumdar, 2006).

$$R_D = \frac{M_t - M_{t+\Delta t}}{\Delta t} \quad \text{Eq. 2}$$

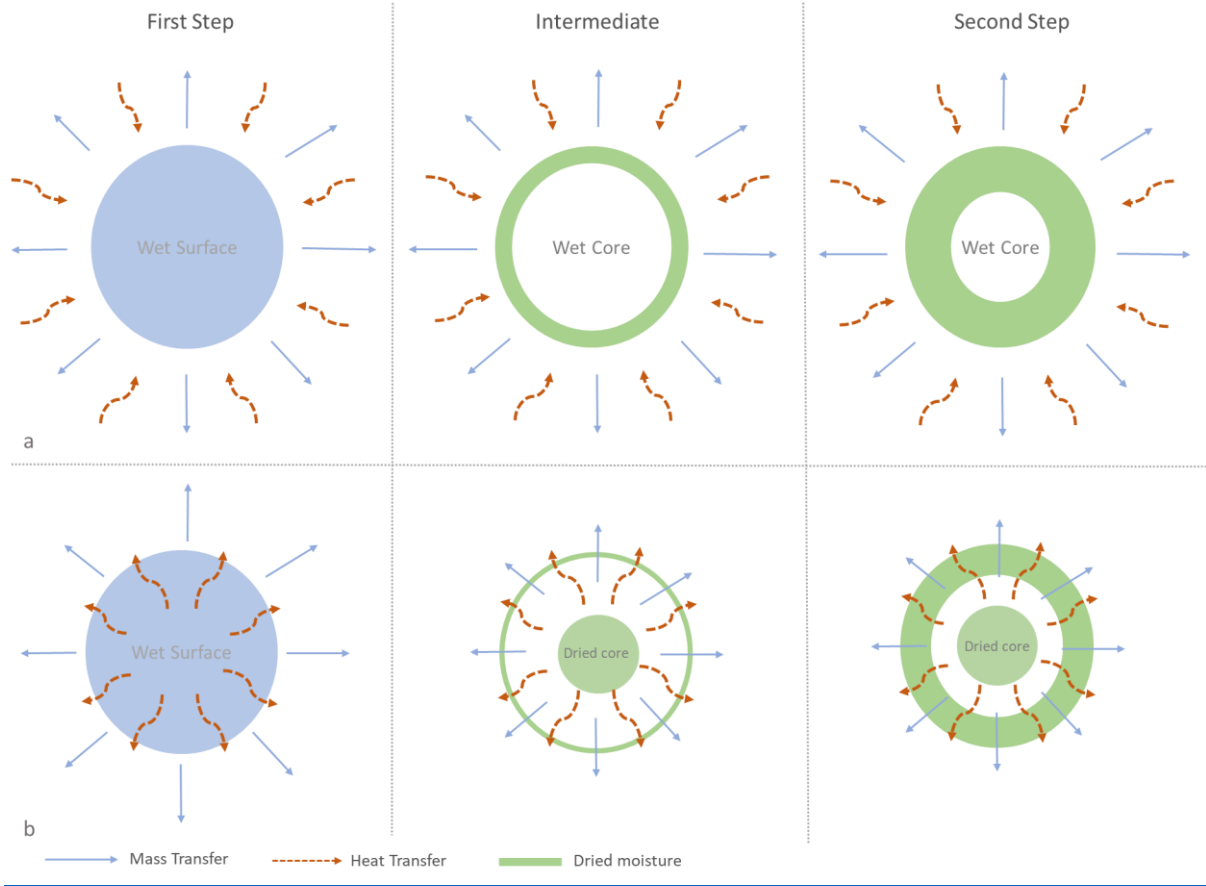


Figure 1- The heat transfer was subjected from the surrounding environment to the wet solid by convection, conduction, radiation or combination of some. The heat was transfer to the external layer and then internal layer in case of convection, and conduction (a), and transfer from internal to external in case of dielectric and radio frequency (b). First drying step directly related on external temperature condition, environmental humidity, surfaced air exposed and drying pressure. Second step was directly related to drying temperature and moisture content (Mujumdar, 2006).

2.2.3. Mass Transfer

There are two types of mass transfer in drying processes, convective and diffusive. Mathematical models and practical equations were developed over the years to describe the drying process. However, dryer's design is mainly done by fusing theory with practical knowledge. The drying state of solids is determined by the moisture content (M_c) in g, (Equation 3), expressed by the ratio between the m_l : mass of liquid and the m_{dm} : mass of dried material in (g) (Mujumdar, 2006; Sinnott, 2014) by:

$$M_c = m_l / m_{dm} \quad \text{Eq.3}$$

Meanwhile, convection depends on the fluid dynamic but diffusive depends on different factors like molecular diffusion, capillary flow, Knudsen flow, hydrodynamic flow, or surface diffusion (Mujumdar, 2006). The moisture transfer mechanism (drying process) is generally influenced by different parameters such as moisture content, capillary forces, and gas phase vapor pressure gradients. The moisture ratio (MR) is calculated using Equation (4) in which M_e is the dried moisture at equilibrium and M_0 is the initial moisture content (%). Since M_e can be ignored, the equation can be simplified (Du et al. 2020):

$$M_R = \frac{M_{ct} - M_e}{M_0 - M_e} \quad \text{Eq.4}$$

There are several mathematical models to describe the convection drying kinetics in terms of experimental data, such as Page (Equation 5) and modified Page (Equation 6) (Pattanayak et al., 2019), two-term exponential (Equation 7), diffusion approach (Equation 8) (Gao et al., 2021), Henderson and Pabis (Equation 9), modified Handerson and Pabis (Equation 10) (Ivanova et al., 2020), logarithmic (Equation 11), simplified Fick's diffusion equation (Equation 12) (Du et al., 2020), and others. K_1 K_0 , and K_1 are drying coefficients specific to related equations; t is time (k); L_t is thickness (m); a , b , c , and e are constant coefficients specific to related equation and n is the drying exponent.

$$M_R = e^{-kt^n} \quad \text{(Eq.5)}$$

$$M_R = e^{-(kt)^n} \quad \text{(Eq.6)}$$

$$M_R = ae^{-kt} + (1 - a)e^{-kat} \quad \text{(Eq.7)}$$

$$M_R = ae^{-kt} + (1 - a)e^{-kbt} \quad \text{(Eq.8)}$$

$$M_R = ae^{-kt} \quad \text{(Eq.9)}$$

$$M_R = ae^{-kt} + be^{-gt} + ce^{-ht} \quad \text{(Eq.10)}$$

$$M_R = ae^{-kt} + c \quad \text{(Eq.11)}$$

$$M_R = ae^{-c(t/L_t^2)} \quad \text{(Eq.12)}$$

However, diffusion occurs by molecular motion and can be described by Fick's law (Equation 13); J is the rate of solvent loss; D the diffusion coefficient; A the heat transfer area; R the universal gas constant; T the temperature; and ∇P the pressure gradient

between the vapor pressure of water at the surface and the partial pressure in the air (Mujumdar, 2006).

$$J = \frac{DA}{RT} \nabla P \quad (\text{Eq.13})$$

The Knudsen diffusion is another theory to define the mass diffusion transfer in which the free mean path of the particles has the same magnitude order of the container (pore) diameter. In this case, the molecules interact very often with the walls of the container. The Knudsen diffusion (D_K) is expressed as (Eq.14) in which d is the mean free path. R is the gas constant, T is temperature (in this case assumed equal to 293 K); and M the molecular mass (Maalal et al., 2021).

$$D_K = \frac{d_{fp}}{3} \sqrt{\frac{8RT}{\pi M}} \quad (\text{Eq.14})$$

In another approach, Caccavale et al. (2016) calculated the mass transfer for food drying industries in bulk. The mass transfer (moisture content, M_c) is an average bulk value for the water diffusivity, since no mass transfer in the liquid or vapor is expected (kg/kg wb). Therefore, to obtain the total mass transfer, no output of liquid water is present and the system is entirely closed. In this case, the mass transfer is evaluated by Equation (15); M_{tv} is the mass transfer of vapor; v the water vapor of working air, and ρ_{av} is the density of vapor of working air (kg/m³) (Caccavale et al., 2016).

$$\frac{\partial M_{tv}}{\partial t} + V \cdot \nabla M_{tv} = \nabla \cdot (\rho_{av} \nabla M_{tv}) \quad (\text{Eq.15})$$

2.2.4. Heat Transfer

Heat transfer can occur by conduction, convention, or radiation (Farid, 2019). In conduction, the energy is transferred to the wet material through a hot surface, while convention takes place when hot gas flows through the solid. Considering direct and indirect drying mechanisms, the heating method requires a preliminary characterization of the material: the features of the solid, the moisture equilibrium, and material temperature sensitivity. For this purpose, conduction is more appropriate for wet solids and thin products. The heat provided by the hot surface

causes moisture to evaporate, which is removed by vacuum or carrier gas. The energy is efficiently trapped in the system, and just a small loss occurs.

Fourier's law (Equation 16) calculates the rate of heat transfer by conduction (H_{tcond}); U is the transfer coefficient by diffusion (m^2/s); A the heat transfer surface area (m^2) and T_i is the inlet temperature ($^{\circ}C/k$) (Mujumdar, 2006).

$$H_{tcond} = UA(T - T_i) \quad (Eq.16)$$

Furthermore, convective drying is one of the most common heat resources, supplying a gas flow to the exposed surface of the material and carrying away the moisture. The convective drying is calculated by Equation (17); H_{tconv} is the convective heat transfer coefficient if $H > 0$, T_s is the temperature of the surface and T_f is the temperature of the bulk fluid, $^{\circ}C/k$, (Halonen, 2020; Mujumdar, 2006).

$$Q = H_{tconv} (T_s - T_f) \quad (Eq.17)$$

Also, Caccavale et al. (2016) calculated the heat transfer for food-drying industries in bulk. The evaporation heat transfer (H_{EV}) in closed systems is calculated by Equation (18); K is the rate of evaporation defined by first-order Arrhenius kinetics; Δh_{EV} the latent heat of evaporation (J/kg); M_i the initial water content in the system; and M the molecular mass.

$$H_{EV} = M \Delta h_{EV} K M_i \quad (Eq.18)$$

In terms of circulated full energy toward the component and considering K as the rate of evaporation, the heat transfer is calculated in the working air by Equation (19); ρ_a is the density (kg/m^3); C_{Pa} the constant specific heat pressure, (J/kgK); $\partial T/\partial t$ the rate of heat ($^{\circ}C/s$); λ_a the thermal conductivity, (W/mK), (Caccavale et al., 2016).

$$\rho C_{Pa} \frac{\partial T}{\partial t} + \rho C_{Pa} V \cdot \nabla T = \nabla \cdot (\lambda_a \nabla T) \quad (Eq.19)$$

Time and energy directly depend on several conditions like external air or gas temperature, flow rate, humidity, pressure, and exposed surface. A carrier gas like air or

neutral nitrogen can provide convection drying, while conduction occurs by indirect heat, and the carrier gas helps to transfer the steam (Land, 2011). Extending the shelf-life of the final food product, by reducing trapped water and consequently deactivating the spoilage microorganisms and enzymes, depends on several parameters aiming to produce a final product with minimal structural changes. It could be more evident if the drying techniques were categorized as high-temperature, low-temperature, and dielectric drying (Chart 1). Industrial drying systems differ in the heat transfer model, principle, and type and should be categorized in three different subgroups as follows.

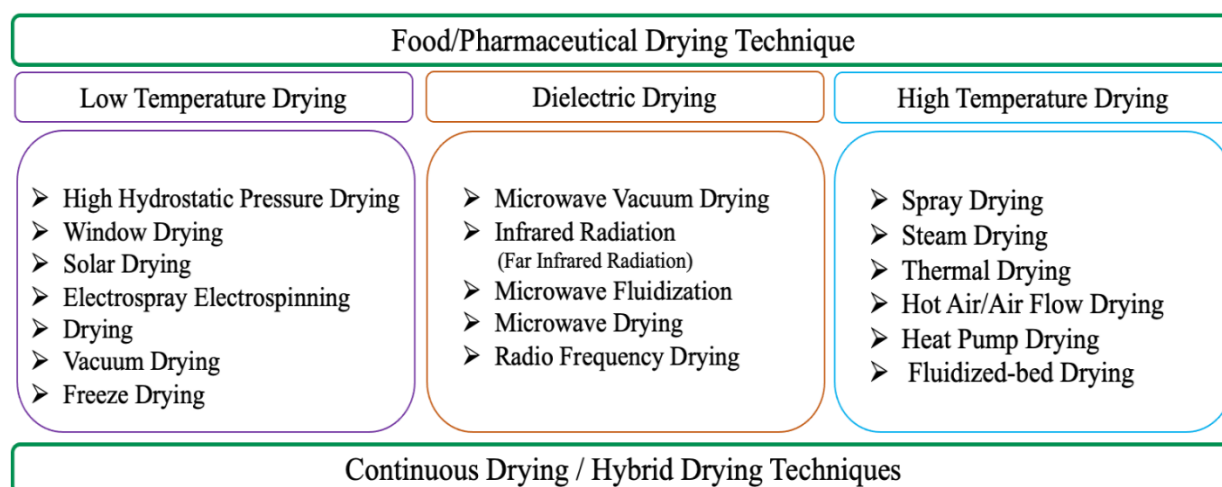


Chart 1- Drying technique categorization regarding a type of heating system; low temperature, high temperature and Dielectric-based drying mechanism.

2.2.5. High-Temperature-based Drying Techniques

2.2.5.1. Heat/Thermal Drying

Heat drying occurs by direct or indirect heat transfer from a hot plate (heat transfer surface) to a conductor, under controlled conditions, to remove water by direct evaporation from the surface or the inner layer and produce a dried porous product. Heat drying has different advantages such as less air volume, fast moisture equilibrium, and high thermal efficiency (Land, 2011). Meanwhile, the total amount of active phytoconstituents and nutrients such as antioxidants decrease considerably (Hung & Duy, 2012; Neikov, 2009). For instance, the impact of thermal drying in food samples was analyzed regarding the content of polyphenolic compounds, with special attention to tannins and stilbenes in the bark of Norway spruce (*Picea abies* L.). A significant decrease of tannins was observed (from 35–36 to 25–31 mg/g). It was also verified that the stilbenes content decreased (36–43%) after 10 h, with losses up to 60% recorded through the drying process. Although thermal drying at 50°C was considered as a moderate and optimized drying temperature,

the authors concluded that it is not an efficient industrial process due to significant loss of chemical components (Jylhä et al., 2021). It must be noted that the heat and mass transfer can be evaluated by equation presented in Sections 2.1 and 2.2.

2.2.5.2. Hot Air/Airflow Drying

Hot airflow drying is another traditional drying method in the food industry. The hot air/air flow-drying technique uses direct or indirect heat transfer from hot air (50–90°C) to the drying material, causing rapid water evaporation. Hot airflow is the main drying process that minimizes packaging, reduces transport cost, and enhances storage stability with acceptable retention of nutrients and phytoconstituents. Due to the high temperatures applied to the material, some decomposition, deformation, and/or degradation of the natural products might occur. However, hot air drying is a fast drying technique (Zhang et al., 2017). For instance, a persimmon sample was subjected to hot air drying (45–65°C) to evaluate the drying efficiency by convective dryer (FCV/E6L3, Zanussi, Pordenone, Italy). The results showed the effect of temperature on drying time as well as shrinkage, and color of end product. The drying process decreased by increasing the drying temperature; better resistances for color and optimum shrinkage were observed at 65°C. Mathematical models such as Enderson and Pabis, Page, Logarithmic, and Two term were proved in this research (Senadeera et al., 2020).

The corn kernel was used for a case study to evaluate the theory of hot air drying as a dimension of temperature and moisture ratio (Equation 4). Furthermore, general heat transfer (H_t ; Equation 23) and convective heat transfer (H_{tc} ; Equation 24) were evaluated by Fourier's law of heat conduction, considering the Reynold (R ; Equation 20), Nusselt (Nu ; Equation 21), and Prandtl number (Pr , Equation 22) (Wei et al., 2019) as follows:

$$h = \frac{Nu \ k}{D_h} \quad (\text{Eq.20})$$

in which k is the thermal conductivity (W/m-K), and D_h is the hydraulic diameter (m). Also, the mass transfer was calculated considering the Sherwood number (Sh) in which D is the diffusion coefficient (m²/s) by:

$$M_t = \frac{Sh \cdot D}{D_h} \quad (\text{Eq.21})$$

$$Pr = \frac{C_p \mu}{k_\infty} \quad (\text{Eq.22})$$

$$\rho C_p \frac{\partial T}{\partial t} = k \left(\frac{\partial^2 T}{\partial x^2} + \frac{\partial^2 T}{\partial y^2} + \frac{\partial^2 T}{\partial z^2} \right) + h_g \rho \frac{\partial X}{\partial t} \quad (\text{Eq.23})$$

$$H_t = k_a \frac{2 + \left(0.4 \sqrt{\frac{v_{dried\ medium} d_s}{\mu_v}} + 0.06 \sqrt[3]{\frac{v_{dried\ medium} d_s}{\mu_v}} \right) \left(\frac{\mu_v C_p}{k_a} \right)^{0.4}}{d} \quad (\text{Eq.24})$$

in which k_∞ is the thermal conductivity in W(mK)⁻¹, ρ the density (in this case corn kernel, kg/m³), C_p the specific heat of food sample (J/kg K). T and t are the temperature and time presented in (K) and (s) in Equations (20) and (21). k_a is the thermal conductivity of drying medium in W/mK. μ is the Viscosity (Pa), μ_v is the dynamic viscosity in (Pa.s), v is the velocity (m/s), d is the equivalent diameter of corn kernel in (m). The convective mass transfer (moisture) of the corn kernel was evaluated by assuming a spheroid analogy, Sherwood, and Schmidt number of the external flow (Wei et al., 2019) as follows:

$$M_t = \frac{D_a \left(2 + 0.552 \sqrt{\frac{v_{dried\ medium} d_s}{\mu_v}} \sqrt[3]{\frac{\mu_v}{\rho D_a}} \right)}{d} \quad (\text{Eq.25})$$

The theoretical mathematical equation revealed that the inner layer play a vital role in drying process and the results were quite different in different corn kernels (Wei et al., 2019).

2.2.5.3. Steam Drying

Steam drying is a convective drying method in which the drying occurs by direct contact of superheated steam produced via a heat exchanger through the sample. The water content is evaporated by superheated steam and the steam cools down after the sample contact. Then the superheated steam becomes saturated, and is recycled and reheated in a closed loop while the amount of steam raised by water evaporation is blended into the loop. This process occurs simultaneously until the sample moisture vanishes. Spray dryer, fluid-bed dryer, and flash dryer can be considered as a subgroup or improvements of steam drying. Safe operation (water steam), lack of fires and dust explosions, environmentally acceptable, and preserving product quality are some advantages of steam drying. The large heat exchange can be considered as a significant disadvantage (Land, 2011). Different rice species were submitted to superheated-steam drying to evaluate the nutrient value and

chalkiness properties. The drying process at 120, 140, and 160°C showed higher milling quality in heated rice samples. The superheated-steam drying can successfully decrease the chalky rice kernels. In terms of rice color, this drying process provides a darker color compared to the conventional parboiling process. The sample dried at 120°C provides an initial moisture content of paddy of 32% w.b., high nutritional value, as well as a less intense color and low chalkiness degree. As a result, superheated steam drying was introduced as a promising technique for the rice-milling industry, with low energy consumption compared to the conventional system (Jittanit & Angkaew, 2020). Furthermore, the heat and mass transfer were calculated in real samples (in this case, potato) for superheated steam drying and evaluated based on comparison between the theoretical and experimental results. The heat transfers in the following case were considered as convective and radiative heat transfer (W/m^2K) as follows:

$$H_t = H_{tconv} + H_{trad} \quad (\text{Eq.26})$$

in which the H_{tconv} is constant at drying conditions and could be calculated based on Nusselt, Reynold, and Prandtl numbers:

$$H_{tconv} = \frac{\lambda_v \left(2 + 0.664 \sqrt{\frac{v_{steam} d_s}{\nu_v}} \sqrt{\frac{\nu \cdot c_p \mu_v}{\lambda_v}} \right)}{d_s} \quad (\text{Eq.27})$$

where λ_v is the thermal conductivity of the specific heat capacity ($W/m/K$), ν , v_{steam} , ν_v is the general, steam, and vapor velocity (m/s), respectively. d_s is the characteristic diameter (m), c_p is the specific heat capacity at constant pressure in ($J/kg/K$), μ_v is the dynamic viscosity ($Pa \cdot s$). Moreover, the radiative heat transfer (H_{trad} ; temperature dependent) can be evaluated based on Stefan–Boltzmann constant ($\sigma_{rad} = 5.67 \times 10^{-8} W/W/m^2K^4$) and following Equation (28):

$$H_{trad} = \sigma_{rad} \delta_t \frac{(T_s + T_{st})^3}{2} \quad (\text{Eq.28})$$

where δ_t is the thermal emissivity, T in this case presented in kelvin (K) in which T_s and T_{st} are the surface and steam temperatures, respectively. It should be taken into account that the open surface mass transfer is very large based on the existence of gas content in the gaseous medium. Therefore, gas pressure equal to steam pressure was generated during the drying process. An acceptable coherence between experimental

investigation and numerical calculation defined by the theoretical process is reported. The water conductivity of the extracellular layer greatly affected the drying characteristic (uniformity and porosity) due to the diffusion of intracellular moisture toward extracellular moisture (Le et al., 2018).

2.2.5.4. Heat pump drying

The heat pump drying consists of two leading groups: a heat pump (an evaporator, a condenser, a compressor, and an expansion valve) and a drying chamber. The last oneThe drying chamber might operate as a rotary evaporator, fluid bed, tray, conveyer, or vacuum spray. While different natural sources supply the heat, the hot air passes through the container (drying chamber) with the material to be dried. The hot air is then removed from the drying chamber and returns to the heat cycle. The energy consumption of the heat pump dryer is less than the one for traditional heat dryers (Colak & Hepbasli, 2009). Banana slices were subjected to heat pump drying system at 37, 40, and 43°C in closed loop systems. The results revealed a significant increase in drying time and temperature with increases in thickness. The greatest moisture extraction and energy activation were observed at high drying temperatures. Drying time was directly affected by drying temperature (increasing drying temperatures result in shorter drying times), and color value was improved by improving drying temperature (Tunckal & Doymaz, 2020). Table 1 shows some application examples of different drying techniques, hybrid drying techniques, and pretreatment mechanisms.

The heat transfer by convection in contact with the airflow was evaluated in real samples, in this case mint leaves, considering the Reynold and Nusselt numbers. Moisture content and moisture ratio were calculated by Equations (29) and (3, 4).

$$M_c = \frac{M_i - M_d}{M_d} \quad (\text{Eq.29})$$

where M_c , M_i , M_d , M_e , M_o are the general moisture content (water/dried content), basic wet content, and final dry content, all expressed in kg, or g depended to process condition, respectively (Le et al., 2018). It must be noted that the heat and mass transfer can also be evaluated by equations presented in Sections 2.1 and 2.2. Also, moisture content could be evaluated in g or kg throughout the paper in a related equation depended to process condition.

Table 1-Different drying technique, Hybrid drying (combination of some techniques), and pretreatment mechanism.

Initial feed	Drying techniques	Note	References
Potato slices	Microwave/ Air drying, Conventional fluidized bed drying, Fluidized bed drying/ microwaves	• Drying technique and temperature directly influenced drying rate, porosity, and color of dried potato slices. • Penetration force and rehydration capacity not influenced by drying technique and temperature. • Both statistical and experimental results show microwaves assisted by fluidized bed have shorter drying time compared to other techniques.	(Reyes et al., 2007)
Vegetables	Hot-air/ heat pump drying	• Low moisture content in final product. • Low drying temperature 40–80 °C. • High retention of heat-sensitive phytoconstituent/nutrient on vegetables.	(Zhang et al., 2017)
Shiitake mushroom	Mid-infrared drying/ freeze-drying, Freeze-drying/ Mid-infrared drying	• Freeze-drying:Mid-infrared drying for 4h saves 48% time with a retention of product quality in same level compared to freeze drying. • Mid-infrared drying/ freeze-drying observed to have a lower quality compared to freeze-drying – Mid-infrared drying. • Freeze-drying – Mid-infrared drying not only affected on retention of aromatic compound but also have a considerable retention of sulfur compounds such as dimethyl, trisulfide and 1,2,4-trithiolane.	(Wang et al., 2015)
Vegetables	Ultrasound/ freeze drying	• Using lower temperature and less drying time due to water removal by ultrasonic waves. • Induce cavitation by high-intensity ultrasonic energy and increase water removal from the inner layer. • Improve freeze drying efficiency and significantly decreased energy consumption.	(Zhang et al., 2017)
Vegetables	Microwave /freeze drying	• Microwave considerably increase the drying rate of freeze drying. • Enhance drying rate, reduced drying time and cost was estimated by experiments and numerical predictions of microwave heating • microwave energy directly absorbed by the water content within molecules (cause fast sublimation). • 55% of total water content was sublimated during the Microwave /freeze drying process, then 60% of the total water was sublimated during the last Freeze-drying process.	(Zhang et al., 2017)
Garlic powder	Microwave/ vacuum, vacuum drying, drying by microencapsulation	• Brighter colour (red-blue), high retention of volatile oils at Microwave energy of 720W, 376.1W, then 282.1W for 3min assisted by 188.0W for 9min, and finally for 3min under vacuum.	(Li et al., 2007)

Table 1-Continued- Different drying technique, Hybrid drying (combination of some techniques), and pretreatment mechanism.

Initial feed	Drying techniques	Note	References
Grape pomace	Heat Pump Drying	- Changing the air velocity from 1.5 to 2.5 m/s, the drying yield increased from 50 to 69 %, and the energy consumption and drying time was decrease significantly. - Changing in air velocities increase yield and provide a cost-effective technique while cause a degradation of trans-resveratrol, catechin and epicatechin.	(Taşeri et al., 2018).
Potato starch	Dielectric drying	- Amylose and amylopectin, the main components of starch potato, were used as standards. - Considerable enhance was observed on crystallization rate, which was revealed by degree of crystallization, surface morphology, and XRD diagrams - SEM images show a significant decrease on amylose and amylopectin concentration while the gelatinization properties were maintained.	(Jiang et al., 2016).
Diced Carrot	Microwave/ fluidized Bed Drying	- Higher removal of moisture content compared to conventional mechanisms. - Good retention of the physiochemical and biological properties compared to fluidized bed dryer. - Keep initial color, flavor, and uniform quality.	(Stanisławski, 2005).
Soybeans	Fluidized bed drying	- Keeping protein level at drying temperature around 110 - 140°C. - Soybean should not be dried lower than 23.5% to preserve the active phytoconstituent. - Cost effective, low energy consumption and high drying capacity.	(Soponronnarit et al., 2001).
<i>Abelmoschus esculentus</i> L. Moench (Okra pods)	Vacuum pulsation/ vacuum drying	- Considerable increase on hardness and brightness was proved. - Flavonoids (Polyphenolic compound) content, and antioxidant capacity were improved. - vacuum pulsation accepted as a suitable pretreatment of vacuum drying to improving the final product quality.	(Xu et al., 2020).
<i>Myrciaria jaboticaba</i> (Jaboticaba, a Brazilian berry).	Freeze, oven, and HHP drying	- The HHP show a similar impact on the total content of polyphenolic compounds but with different profile compared to freeze-drying, and oven drying at 75 °C. - Freeze-drying is more adjusted for natural food colorant. - Oven drying at 75 °C is the more adjusted for sample with high ellagitannins.	(Inada et al., 2020).
oleogels from K-carrageenan	Supercritical CO ₂ drying and Freeze-drying	- The supercritical CO₂ drying preserve the oil content up to 94% and provide a strength and stability on oleogels. - Freeze-drying preserve the oil content up to 97%. - Both techniques provide a porous morphology of components.	(Plazzotta et al., 2019).

2.2.5.5. Spray Drying

Spray drying is the most popular drying system in the food, pharmaceutical, chemical, and biochemical industries. It converts droplets into low bulk density dried particles/capsules/powders by spraying a liquid phase into a hot drying chamber. Here, the conduction heat supplier (typically air) causes rapid evaporation and solidification. Although spray drying is considered a one-step process, it can be divided into four phases: (i) atomization of input feed; (ii) droplets mix (spraying gas, air/neutral nitrogen) and free/coated moisture; (iii) spraying the mixture, air, and droplet to nozzle; and (iv) product recovery (Figure 2) (Broadhead et al., 1992; Land, 2011). The initial step of this process (a feature of input feed) plays an essential role in the efficiency and quality of the output powder, so it is still challenging to distribute the natural or sensitive bioactive components, in the form of an emulsion or solution, before the feed to spray dryer (Reineccius, 2019). The final product remains with 2– 6% moisture (Zhang et al., 2017). It must be taken into account that by decreasing the water content in the final product and increasing water in the initial feed, the degradation of natural products can be prevented significantly. In fact, the use of drying agents under optimized feeding conditions based on the end use of the product promotes its value (Mezhericher et al., 2008). Several parameters affect the drying process and product quality, such as dryer geometry (continuous phase flow hydrodynamics), which directly affect cost and energy. Therefore, a theoretical calculation such as computational fluid dynamics (CFD) is an effective tool for optimizing the drying process and provides a real-object simulation. For this purpose, the particles density (ρ) can be obtained by Equation (30) in which the density could be constant or an average of the volume density; m is the mass of particle or the average of initial component mass and ρ_i is the density of sample i in kg.m^{-3} .

$$\rho = \left(\sum \frac{m_i}{m \rho_i} \right)^{-1} \quad (\text{Eq.30})$$

Heat balance between the particles and the drying chamber was calculated by Equation (31); T is particle temperature (include the radiation, convective heat transfer, and vaporization condition in which radiation could be equal to zero, $\text{W/m}^2\text{K}$); m_i is the mass of the particles (just affected by vaporization); and M_w is the molecular weight; A_p is the internal cross field of vial section (cm^2); and σ_{rad} is the Stefan-Boltzmann constant.

$$m_i C_p = \left(\frac{dT}{dt} \right) = A_p \varepsilon_p \sigma (\theta_R^4 - T_p^4) + h A_p (T_\infty - T_p) + \sum \frac{dm_i}{dt} (h_{i,p} - h_{i,g}) \quad (\text{Eq.31})$$

Moreover the mass transfer were obtained by Eq.32 in which the U is diffusion coefficient factor of cumulate vapor presented in m^2/s .

$$Sh_{AB} = \frac{M_c U}{U} = 2.0 + 0.6 Re^{1/2} Sc^{1/3} \quad (\text{Eq.32})$$

The heat transfer coefficient (H_i) is obtained by the Ranz and Marshall equation (33); d_p the particle diameter (m), K_i is the thermal conductivity (W/mK); Re is the Reynolds number (related to particle diameter and relative viscosity); and Pr is the Prandtl number related to continuous phase ($C_p \mu/k_\infty$).

$$Nu = \frac{H_t d_p}{k_\infty} = 2.0 + 0.6 Re_d^{1/2} Pr^{1/3} \quad (\text{Eq.33})$$

The heat transfer efficiency of the drying chamber is obtained by the average heat transfer coefficient (K_{Ht} , Equation 34) allowing also evaluating the dryer volume.

$$k_{Ht} = \frac{H_t}{V \Delta t} \quad (\text{Eq.31})$$

H_t is the total energy that circulates inside the chamber (J/kg, kg/kg air, and cal/g), coming from the particles and the continuous phase; Δt is the mean temperature of the gas and particles chamber (Khanh & Hang, 2021). Table 2 shows examples of application of the spray drying technique on food samples.

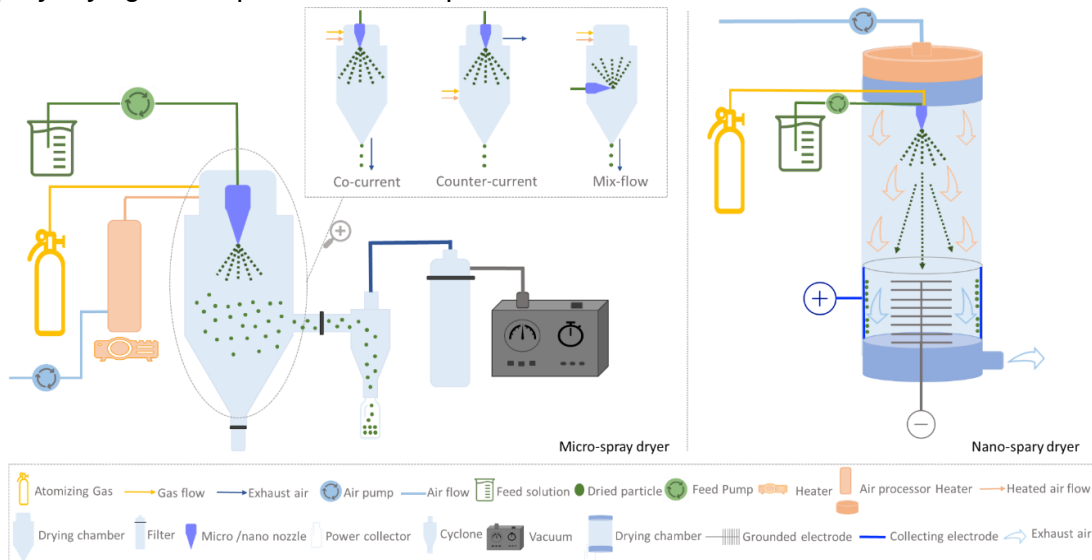


Figure 2- Schematic of Nano/micro spray dryer.

Table 2-Example of freeze and spray drying in which used drying agent as adjunctive

Drying Technique	Drying agent	Effectiveness	References
Spray drying	Chickpea	• Spray drying was used to preserve polyphenol-protein of Blueberry as a function of food. • Spray-dried capsules presenting a higher content of phytochemicals and biological value compared to lyophilized particles. • Spray drying proved to be a more convenient, fast, simple, and cost-effective technique compared to freeze drying.	(Hoskin et al., 2019)
Spray drying	Maltodextrin	• Avocado is a rich tropical and Mediterranean fruit with a high nutritional value with and high content of oil and water. • Spray drying authorize a long retention of the chemical composition with high bioactivities. • Dried avocado revealed a high content of protein, ascorbic acid, phenolic compounds, high-quality nutritional product without harmful synthesis preservatives function on food and juice product.	(Dantas et al., 2018)
Freeze and spray drying	Whey protein, maltodextrin and gum arabic with different ratio	• Krill oil in form of nanoemulsion was used as a feed solution submitted to freeze and spray dryer. • 8% (w/v) is an optimized concentration of krill oil on dried powder. • Sustainable release time was occurred during the digestion period (two hours). • Both spray and freeze dry sample was observed the same oxidative stability within fifteen days. • Spray dryer observed to be a more encapsulation efficiency and better in vitro bio accessibility.	(El-Messery et al., 2020)
Freeze drying	Alginate gel	• Nanosized alginate fluid gel particles release was not significantly change by the drying system; in contrast, the micro sized particles were considerably affected. • Freeze-drying could extend shelf life without compromising the material properties.	(Smaniotto et al., 2020).
Vacuum, spray, and freeze-drying methods	Insulin and maltodextrin	• Chemical composition and biological properties of Japanese quince (<i>Chaenomeles japonica</i>) was investigated. • high protection of polyphenolic compound, antioxidant activity, and undesirable hydroxymethylfurfural were observed by maltodextrin dried by freeze drying. • High porosity, more favorable color parameters, high retention of polyphenolic was observed from freeze drying.	(Turkiewicz et al., 2020).

2.2.5.6. Fluidized Bed Drying

The fluid-bed dryer depends on sample fluidization. It runs by continuous feeding of wet particulate contacting with a warm surface or hot air blown through to maintain the material in a fluidized state. Fluidized bed drying is used in the food, pharmaceutical, and chemical industries for drying humid powders and solid capsules/particles (Figure 3). The fluid-bed dryer provides efficient heat and mass transfer, short drying time, high drying rate, high efficiency, and uniform condensation. Therefore, the operating units require high thermal power, high fluidizing gas flow rate, and high solids transport rate to and from the dryer (Sivakumar et al., 2016; Land, 2011). Overall, the vigorous mixing due to boiling and bubbling of liquid through a solid phase and hot air media (continuous bubbles) leads to a fluidizing gas that comes up from the bottom and is uniformly distributed toward the container with the solid mixture acting like a fluidizer. Therefore, the solid particles are continually circulating in swirls and randomly settling back to a bubbling motion. Hence, the gas stream covers each fluidized particle and results in different drying formations such as granulation, capsulation, or coating purposes. Furthermore, the bed is static (packed), and the particles rest on the distributor at low velocities. The fluidization is achieved when the gas stream supports the weight of the whole bed. The minimum fluidization velocity (U_{mf}) was obtained from Ergun (Equation 35); ΔP is the pressure drop; L_t is thickness of particle bed (m); ϵ is the void fraction of bed; μ is viscosity (Pa); v_s is the superficial velocity static bed (m/s), ϕ is a shape factor; d_p is the diameter (m); and ρ is density (kg/m^3), and ρ_f extracted from Archimedes number is the fluid density (kg/m^3).

$$\frac{\Delta P}{L_T} = 150 \frac{(1 - \epsilon)^2}{\epsilon^3} \frac{\mu v_s}{\phi_p^2 d_p^2} + 1.75 \frac{1 - \epsilon}{\epsilon^3} \frac{\rho_f v_s^2}{\phi_p d_p} \quad (\text{Eq.35})$$

Moreover, when the fluidization state is reached by the bed pressure drop, it can be defined by Equation (36); ρ_p is particles density, ρ_g is density of acceleration of gravity (kgm^{-3}), and g is the gravity (m/s^2) (Kunii & Levenspiel, 1991; Mawatari et al., 2003).

$$\frac{\Delta P}{L} = (1 - \epsilon)(\rho_p - \rho_g)g \quad (\text{Eq.36})$$

The terminal velocity of the particles is not reached due to the transfer of particles. Therefore, the terminal free fall velocity is controlled by fluid mechanics and obtained by Equation (37); C_D is an experimentally determined drag coefficient (Kunii & Levenspiel, 1991).

$$u_t = \left[\frac{4d_p(\rho_s - \rho_g)g}{3\rho_g C_D} \right]^{1/2} \quad (\text{Eq.37})$$

The airflow increase affects directly the rate of drying, cracking, and breakage. In contrast, energy consumption and air recirculation fraction decrease the incandescent air rate in fluidized bed dryers (Soponronnarit et al., 2001). To evaluate the functionality of fluidized bed drying on real samples, different drying temperatures (50, 60, and 70°C) and constant flow rate (6 m/s) were tested on blackberry. Homogenous granules were obtained under bubbling fluidization conditions. The drying rate increased by enhancing the drying temperature. The drying time decreased with drying temperature increase (up to 12 min at 70°C). The highest content of fiber, nutrients, phenolic compounds, and antioxidant activity was observed at 60°C (Sozzi et al., 2021), a significant outcome. Table 1 shows more practical examples.

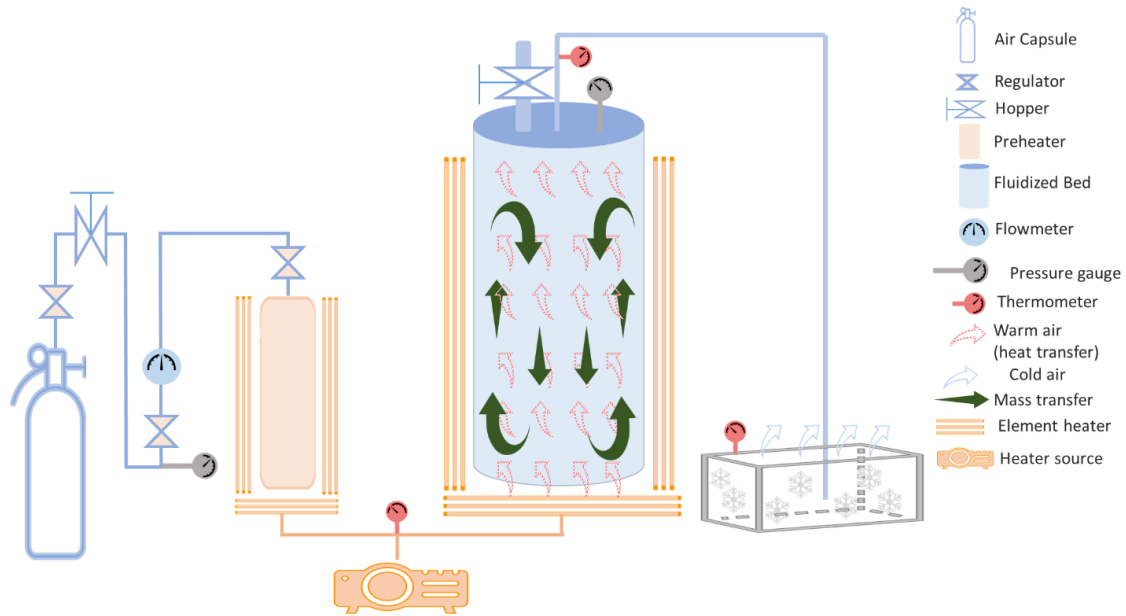


Figure 3- Schematic Fluidized bed dryer.

2.2.6. Dielectric-based Drying Technique

Dielectric drying is not a heat form but uses electromagnetic waves as energy to generate heat in a dielectric material (Mujumdar, 2006). There are two source types involving two drying mechanisms: dipolar relaxation and ionic conduction. The frequency attributed to dielectric drying is in the case of microwaves in the range of 9.15×10^8 and 2.45×10^9 Hz (Land, 2011; Zhang et al., 2017) and the mechanism is explained in Section 2.4.1.

Heat and mass transfer were evaluated by the ionic conduction power per unit volume (ρ_v) by Equation (38), in which E is electrical strength (electrical energy-per unit, voltage), q is the quantity of the electrical charge belonging to each ion, n_i is the number of the ion per unit (ion density), and i_b is the ion mobility.

$$\rho_v = E^2 q n_i i_b \quad (\text{Eq.38})$$

Besides, the dipolar relaxation (dipolar rotation) power was evaluated by Equations (39) and (40); k_{um} is the constant unit of measurement; f is the frequency; ϵ is a relative permittivity constant (relative dielectric); $\tan \delta$ is the dissipation factor; and ϵ'' is the loss factor (Mujumdar, 2006).

$$\rho_v = k_{um} E^2 f \epsilon \tan \delta \quad (\text{Eq.39})$$

$$\rho_v = k E^2 f \epsilon'' \quad (\text{Eq.40})$$

As with other drying techniques, the penetration depth plays a critical role in drying efficiency, since the lack of energy penetration results in a drying limitation of being confined to the surface. Therefore, the penetration depth (D_p) was evaluated by Equation (41); λ_0 is the free space wavelength. This equation was introduced, although having a high loss factor (ϵ'') by Mujumdar (2006).

$$D_p = \frac{\sqrt{\epsilon} \lambda_0}{2\pi \epsilon''} \quad (\text{Eq.38})$$

The increase in electromagnetic waves as energy source can represent a considerable decrease in fossil fuel consumption, improving the sustainability of this drying technique (Land, 2011).

2.2.6.1. Microwave Drying

Microwave (MW) drying utilizes unconventional electric equipment with a frequency in the range 10^8 – 10^{10} Hz. The MW heating mechanism, also known as bulk heating, generates heat in the whole material by converting energy to heat when MW frequency interacts with the material. This causes internal heat generation without specific kinetic barriers to heat transfer. Therefore, the high humidity vaporizes directly, and the decomposition/deformation of phytoconstituents does not occur (Stanisławski, 2005).

The impact of MW s on shortening the drying time and providing a high-quality product has been already proven (Land, 2011). For example, the pear, a good source of volatile compounds, was MW dried at different temperatures (50, 65, and 80°C). Simultaneously, three different conditions were evaluated: constant temperature, combined control, and industrial control. The combined control used a specific heat program “fuzzy logic controller” in which the temperature changed every 4 min following specific rules and there were programmed times for the input and output processes. Industrial control operated just like the combined control with some modifications by a linear control strategy. Experimental results demonstrate that the constant temperature allows for simple but long-lasting operation. The combined control made the process more complex but provided a high-quality final product, more aromatic, within a short time duration. Considering the industrial control as a linear type of combined control, it can be concluded that combined control is able to modify energy consumption to improve drying rate and nutrient content (Li et al., 2021). Meanwhile, MW drying as an individual drying process is not recommended by Zhang et al. (2017) due to quality concerns. Table 1 shows more examples of MW drying.

2.2.6.2. Microwave Fluidization

Microwave fluidization uses MW instead of traditional heat generators in fluidized bed dryers (Section 2.3.6). The electromagnetic radiation is the source of energy, resulting in an internal vibration (energy) that heats up the material and induces the internal moisture from inside to outside (Figure 1). The drying temperature is similar to hot air drying but passes faster, resulting in faster drying. Fortunately, this technique balances dry surface distribution and improves the porosity and texture of the final product, causing internal moisture to be transferred to the environment more quickly (Lv et al., 2019). The MW fluidization technology is declared as an effective, safe, and practical technique for drying fresh vegetables and fruits and for improving sample uniformity and drying efficiency. Decreased nutrient decomposition and improved drying efficiency were observed with the MW fluidization technology compared to the static MW drying and hot air-drying techniques. On the other side, a high-drying quality and uniformity of final products were obtained from subgroups of MW fluidization. Microwave hot-air spouted bed-drying, compressed air pulse-spouted MW vacuum drying, and MW mechanical-vibration fluidization drying were reported as modified MW fluidization drying techniques (combined techniques). However, MW fluidization is not a continuous process, and the distribution and transfer speed of the material need to be improved (Lv et al., 2019). As a practical example, red bell peppers were dried by a MW-assisted fluidized bed dryer using constant and combined conditions with respect to temperature

(40–70°C), power (180–540 W), and air velocity (15–20 m/s), with optimum conditions of MW power (468.04 W), temperature (60.14°C), and air velocity (16.82 m/s). The optimized condition provided higher nutrient contents, phenolic compounds, and antioxidant activity and minimum color changes (Zahoor & Khan, 2021). Table 1 shows more examples on the subject.

2.2.6.3. Microwave Vacuum drying

Microwave with radiofrequency between 300 and 30,000 MHz is used in the MW vacuum drying techniques as a heat source instead of conventional heating systems (Section 2.6.1). As previously mentioned, MW provides uniform energy throughout the material and solvent due to the vaporizing moisture in the inner layer of the material's pores. Besides, MW vacuum drying could be almost 50% faster and more efficient due to combined vacuum drying (high-pressure vacuum) to help remove the moisture. This technique provides products with homogeneous, high-quality texture as well as maintains the material's chemical composition compared to conventional vacuum drying. Vacuum MW drying has been more recently used in food industries for the functions of heating, extracting, drying, and sterilizing (Cui et al., 2004; Mousa & Farid, 2002). As an example, the impact of MW vacuum drying on the quality of oil-free sliced sweet potato was evaluated. The dry sample had minimum water content (low moisture) in less than 30 min. The sliced sample was highly crunchy, with a large amount of puffing, a structure with large pores, no significant color changes, no evidence of burning, and a long shelf-life. The final product confirmed the positive impact of MW vacuum drying on food drying, which helped maintain the food's value and extended its shelf-life (Monteiro et al., 2020a). For more examples, see Table 1.

2.2.6.4. Radio Frequency Drying/Radiation Drying

Radio frequency (RF) uses radiant heat (electromagnetic waves in the range of 1.3×10^6 and 2.7×10^7 Hz) to obtain the operating frequency to heat samples and generate internal heat (Land, 2011; Mujumdar, 2006). This technique is predominantly used as a source of energy or as a pretreatment step before other regular techniques (vacuum drying, fluidized bed drying, spray drying, and heat pump dryer) as well as individual RF dryer systems such as RF dryer WAVEDRY⁺ (STALM radio frequency equipment, S.p.A. Italy). The upper, bottom, or edges and corners of food samples were subjected to electromagnetic waves, produced by the average difference of food dielectric properties and its surrounding media (Figure 4). Depending on the sample (high or low moisture), this technique is combined with other techniques such as rotation or vacuum drying (Jiao et al., 2015). The main differences between RF and MW are frequency ranges from

3 kHz to 300 MHz (RF) and from 300 MHz to 300 GHz (MW), respectively. The RF induces molecular vibration, which creates volumetric heat and causes rapid heating of the foodstuff. Nevertheless, RF produces depth penetration compared to MW and can find better functionality on a large scale (Zhang et al., 2017). Apples, a heat-sensitive food sample, were subjected to low-temperature RF drying. A pretreatment of low humidity air, at 40°C, before RF, provides a considerable enhancement of drying time (up to 37%), a decrease in energy rate (up to 52%), and preserved the phenolic (up to 98%) and flavonoids contents (up to 87%). Therefore, the mentioned drying process can be a promising technique for low-temperature food drying, with a high rate of nutrient preservation and low-energy consumption (Shewale et al., 2021). In conclusion, RF and MW drying are becoming popular in food-processing industries and used as a source of energy and a pretreatment process.

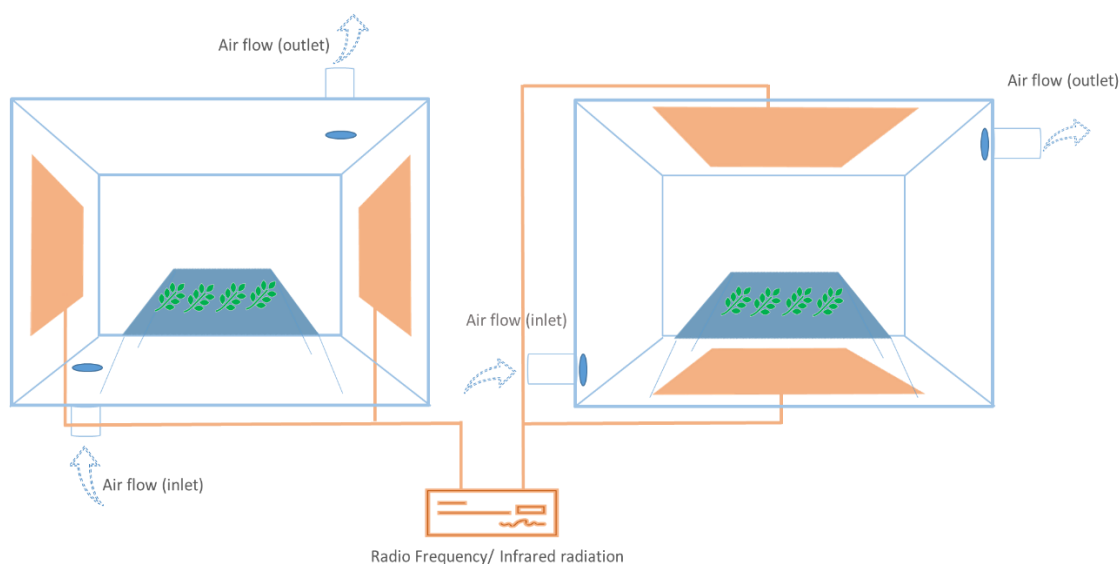


Figure 4- Schematic basic radio frequency and infrared radiation.

2.2.6.5. Infrared Radiation/Far Infrared Radiation

Infrared radiation (IR) wavelengths, around 1–6 μm , interact with the internal layer of the product, promoting the temperature increase and consequently moisture evaporation (Zhang et al., 2017). Also, more recently, the far-infrared radiation (FIR), due to its capacity to allow a uniform heating distribution, is being applied as a drying option. The top, bottom, or left and right of food samples were subjected to IR waves (Figure 4). The FIR drying system smoothly transfers heat through the inner layer (around 1–4 mm) without causing any degradation on the product surface, namely in the case of grains. It must be taken into consideration that FIR drying is faster than the hot air-drying, more expensive and energy-consuming than freeze-drying, and has a higher drying rate compared to conventional drying techniques. The FIR drying technique is used instead

of conventional drying in pharmaceutical and food industries, especially fruit, vegetable, and meat processing, due to less energy cost and higher speed (Land, 2011; Ratseewo et al., 2020). For instance, the effect of drying temperature of FIR drying was evaluated in kiwifruit slices. A significant increase of drying time and drying rate (57.9% and 137.5%, respectively) was observed by enhancing the temperature from 120 to 280°C. The higher FIR temperature provides slices that are more porous and with less moisture (more cavity and porosity). The total phenolics content decreased and total flavonoid and vitamin C contents increased considerably by improving the temperature from 120 to 160°C. At drying temperatures higher than 200°C, all the nutrient content decreased significantly. Therefore, FIR drying is a promising technique to improve the drying time and rate, in optimized conditions, and to preserve the nutrients content (Zeng et al., 2019). For more comparative and similar examples, see Tables 1 and 3.

Table 3 - Examples of food drying applications with special regards to drying temperature.

sample	Drying method	Temperature	Special Condition	Ref
Starch in pasta	Heat drying	50, 70, 80°C	By raise the drying temperature the hardness and brittleness of starched gels formed enhances.	(Masato et al., 2021)
Rice	Infrared radiation (IR) drying	70, 100, 130°C	The drying temperature directly affected the rice grain whiteness, hardness, transparency, and relative crystallinity.	(Timm et al., 2020)
Corn	SKP chamber corn dryer combined with a heat generator TPG-1/25 (new drying method)	38-45°C	Control temperature was set to 39°C at forced air circulation and 20-25°C indoors. Established a new fundamental drying technique with high efficiency with no significant negative impact.	(Mykola et al., 2021)
Tomato	Heat pump drying freeze drying spray drying refractance window drying	40°C - 80°C 140-180°C 97°C	Product obtained from spray dryer and refractive window drying provide similar physicochemical properties. Heat pump drying proved as a mild drying technology (especially chips).	(Jeyaparakash et al., 2020)
Sweet potato snack	Microwave power modulation (microwave vacuum)	60°C	The result achieve a high-quality sweet potato chips.	(Monteiro et al. 2020b)
Potato slice	Microwave and Steam blanching (pretreatments to hot air drying)	70°C	Microwave as pretreatment showed a higher moisture diffusivity and drying rate	(Abano, 2020)
Ginger (<i>Zingiber officinale</i> Roscoe)	hot air drying vacuum drying freeze drying catalytic infrared drying	60°C except freeze drying	The optimum drying was confirmed at 60°C in which improved the physical properties of final product.	(Ren et al., 2021)

Table 3 –Continued -Examples of food drying applications with special regards to drying temperature.

sample	Drying method	Temperature	Special Condition	Ref
Kiwifruit	Hot-air drying	60, 70, 80°C	The efficient drying temperature was confirmed 60°C and the fasted drying time with highest degradation observed from 80°C.	(Tepe et al., 2021)
spinach juice	Spray drying	60°C	Improve the glass transition temperature up to 117.32–127.38 °C.	(Yuksel & Dirim, 2020)
Dried banana puree	Refractance Window	70, 80, 90°C	The efficient drying temperature was confirmed at 90°C with high content of nutrient, low energy consumption and efficient time duration.	(Rajoriya et al., 2021)
Red grape pomace peels	High-temperature drying	160 –200°C	The high-temperature drying provides the high polyphenolic content of pomegranate peel; in contrast, it caused a significant decrease in phenolic of pomegranate seeds.	(Guaita et al., 2021)
Green tea	freeze drying spray drying	-30-40°C 120°C	Freeze drying showed the highest encapsulation efficiency up to 76%. No significant difference s were observed regarding phenolic content from both techniques	(Pasrija et al., 2015)
Garlic	Hot air drying Heat pump drying Vacuum drying Freeze drying Solar cabinet Drying Hot Air Dryer Cum Fluidized Bed Dryer Heat Pump Dryer	40, 50, 60°C 37–40°C 40, 50, 60°C –35°C 53–57°C 40°C 37–40°C	Although an Allicin content were directly depended to thickness of slice and drying temperature, heat pump drying, vacuum drying at 40°C, and freeze drying showed a better retention of Allicin.	(Aware & Thorat, 2011)
Lavender	Shadow drying Convective drying	20–22°C 35°C	Drying at 30 °C enhance the essential oil content up to 18%, and total phenolic content up to 50%	(Salata et al., 2020)
Lavender	Microwave-assisted hydrodistillation	4, 25°C	The highest stability was observed at 25°C	(Uzkuç et al., 2021)
Chamomile (<i>Matricaria chamomilla</i> L.)	Sunlight drying Dried at shadow Oven drying Solar drying Microwave drying	30°C 25°C 40°C 35–40°C 90°C	The highest and lowest content of essential oil obtained from solar drying, and microwave, respectively. Shadow drying successfully preserved the high amount of chemical composition with special attention to essential oil	(Abbas et al., 2021)

* General aspect in term of Fruit and vegetable: contain 85% < water, the drying temperature and technique should affect the flavour, taste, and physiochemical properties of fruit and vegetable.
General aspect in term of Herbal medicine and tea: contain 85% < water, the drying efficiency affected by species, location, drying condition, storage condition.

2.2.6.6. Infrared Radiation/Far Infrared Radiation

Heat transfer can be defined by the Fourier equation for RF and IR. Equation (42) estimates the specific heat, in which $\partial T/\partial t$ is the heat rate ($^{\circ}\text{C/s}$), Q is the RF power converted to thermal energy (W/m^3), and T , in this case, is the processing temperature presented in $^{\circ}\text{C}$ (Hou et al., 2020).

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (k \nabla T) + Q \quad (\text{Eq.42})$$

Equations (43) and (44) evaluated the mass transfer (M_t , m/s). $n_{v,\text{sur}}$ is the water content generated within the sample, removed by diffusion and convention in border conditions ($\text{mol/m}^2\text{s}$); $n_{w,\text{sur}}$ is the water content removed from the border ($\text{mol/m}^2\text{s}$); φ_p is the porosity (dependent on type of moisture/particle); S_g is the gas saturation (dimensionless); S_w is the water saturation (dimensionless); ρ_{av} is the vapor density; and $\rho_{v,\text{amb}}$ is the vapor density of the ambient air (kg/m^3) (Hou et al., 2020).

$$M_t = \frac{(n_{v,\text{sur}} - C_v v) \cdot M_c}{\varphi_p S_g (\rho_{av} - \rho_{v,\text{amb}})} \quad (\text{Eq.40})$$

$$M_t = \frac{n_{w,\text{sur}} M_c}{\varphi_p S_w (\rho_{av} - \rho_{v,\text{amb}})} \quad (\text{Eq.41})$$

2.2.7. Low Temperature Drying Technique

2.2.7.1. Vacuum Drying

Vacuum drying uses a low-temperature direct dryer and is frequently used in the food, agriculture, and pharmaceutical industries. Heat transfer is operated by steam or hot water (not in high temperatures), oxygen, or inert gases such as N_2 , He, or Ar passing through hollow shelves or heat transfer is achieved through a hot plate under high-pressure vacuum conditions (Land, 2011). Although drying temperatures can be carefully controlled, the heat plate causes some material decomposition.

Heat transfer to the material and consequently to the solvent occurs mainly by conduction through a heated surface. Equation (45) calculates the drying process, in which M_l is the mass of solvent (l/ml) and ΔH_{vap} is the vaporization heat, ΔT is the varage tempreture generally in K.

$$Q = MC_p \Delta T + M_l \Delta H_{\text{vap}} \quad (\text{Eq.45})$$

Furthermore, vacuum drying can be difficult to control, needs a large surface (plate), and is a slow process that consumes time (12 to 48 h) and energy. Tray vacuum dryers are the most common in the pharmaceutical industry and are the second stage of the overall drying process. Therefore, vacuum drying compared to innovative drying systems is a lengthy process with high costs of installation and operation (Parikh, 2015). The effect of different vacuum drying temperatures (40–80°C, with 10-degree intervals) was evaluated in Chilean papaya. The sample's water activity decreased up to 0.6 (in the range of stability). The total content of phenolics, protein, and ashes increased compared to fresh sample, and vitamin C and total flavonoids content were preserved totally during the drying process. Moreover, the content of simple sugar increased considerably, while the ratio of glucose/fructose remained unchanged. The total phenolic compounds increased at lower temperatures (40 and 50°C). A slight decrease in the flavonoids content was observed even at low temperatures (40 and 50°C). In contrast, the flavonoids content increased slightly at 80°C. All referred changes were obtained by comparison between dried samples to fresh ones. Finally, the content of vitamin C enhanced at 50 and 60°C, while at other temperatures, a considerable reduction occurred. The antioxidant activity enhanced almost 1.5–2.6 in all samples (Vega-Gálvez et al., 2021). More examples are presented in Table 1.

2.2.7.2. Refractance Window drying

Refractance window drying is a novel mild drying technique, aiming to preserve a high level of phytoconstituents in food products. Heat from warm water (temperature between 30 and 95°C), at atmospheric pressure, is the thermal energy that moves to the material placed on a thin film on a plastic conveyor belt. The product's water evaporates and exits through an upper opening. Then, the free water product moves to a cold water bath and leaves the conveyor. The drying energy consumed is similar to spray drying and definitely higher than freeze-drying (Figure 5) (Bernaert et al., 2019). As an example of application, refractance window drying, at 50, 60, and 70°C for 50 min, was used to evaluate the retention of *Lactococci*, *Lactobacilli*, and acetic acid bacteria in kefir. These microorganisms were highly retained at 50°C. Although the retention of nutrients and phytochemicals at freeze-drying is more significant, refractance window drying at 50°C provides a faster and energy-consuming process with high retention of phytoconstituents (Tontul et al., 2021). Table 3 shows more examples.

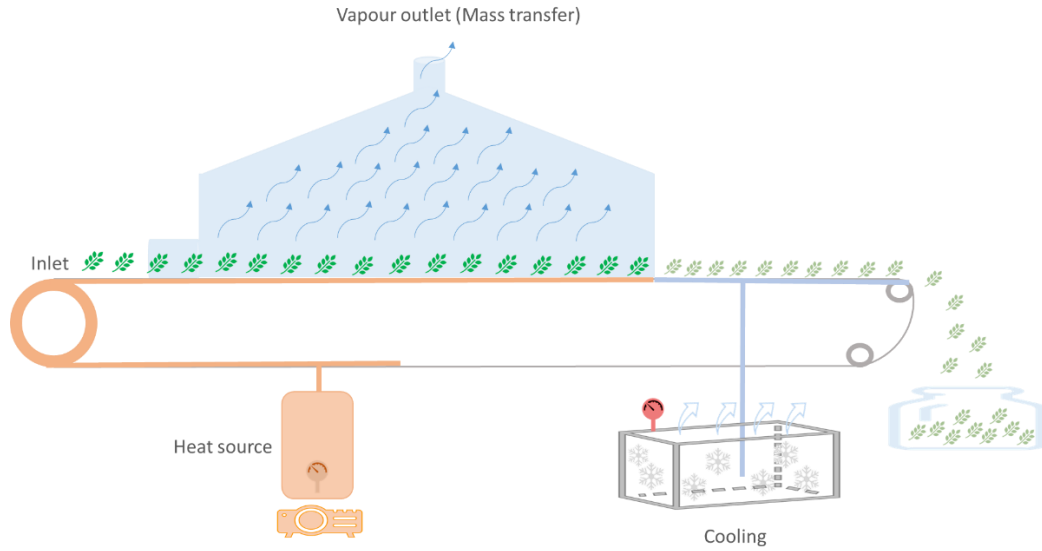


Figure 5- Schematic of basic refractance window dryer.

The mass and heat transfer, as well as the total thermal efficiency, were evaluated for refractance window drying. The procedure occurred in 2 mm of mango pulp (as a real food sample) in contact with water (95°C) for 3–5 min., with natural gas obtained from a steam boiler at high evaporation capacities. The Q_{sp} , is the total sensible heating, and it was determined using Equation (46), in which M_p is the mass factor per unit (kg/h) and H_t is the average specific heat transfer.

$$Q_{sp} = m_p H_t \cdot \Delta T \quad (\text{Eq.46})$$

Considering Reynolds and the Pris and Prandtl numbers, the efficient heat transfer (H_{ab}) was obtained by Equation (47), in which L is the belt length (m), k_∞ is the air thermal conductivity (W/m°C), β is the volumetric thermal expansion coefficient (K^{-1}), T_s and T_∞ are the bottom surface and surrounding air temperatures (°C), μ_{ak} is the air kinetic viscosity (m^2/s), and α is the air thermal diffusivity (m^2/s).

$$H_{ab} = 0.27 k_\infty \frac{g\beta(T_s - T_\infty)L^3}{\mu_{ak}\alpha L} \quad (\text{Eq.47})$$

And finally the total heat efficiency (H_e) were obtained by Eq.48 in which Q_{ev} is the moisture energy evaporation, λ_L is the moisture evaporation constant equal to 2336 kJ/kg, R_d is the drying rate and H_{ti} is the total input energy (heat) (Raghavi et al., 2018).

$$H_e = \frac{Q_{ev} + (\lambda_L \cdot R_d) + Q_{sp}}{H_{ti}} \quad (\text{Eq.48})$$

2.2.7.3. Solar Drying

Solar drying, traditionally used for products such as vegetables, fruits, tobacco, tea, coffee, fruit, nuts, cereals, and rice, needs a low temperature energy (Figure 6) and has played a vital role in human life and technology since ancient times. Solar energy can be converted into two types of energy, electrical and thermal. Electrical solar energy is out of our aim and scope, but thermal solar energy is a subgroup of drying applications. Solar drying does not just depend on solar energy. It can also be applied in combination with other energy sources such as fuel. Besides, solar drying provides higher drying efficiency, uniformity, energy conservation, and less space. Solar drying techniques are effective drying techniques and allow preserving food or pharmaceutical products (VijayaVenkataRaman et al., 2012). Unfortunately, the lack of useful technical information and practical experience has made the solar drying techniques the least publicly used technique but indirect solar drying has become a new approach for food products. This is the case of *Ficus carica* L. samples dried by indirect solar drying (electric auxiliary heater with solar energy), at 60, 70, and 80°C, and two airflows (150 and 300 m³/h). As expected, high drying temperatures together with high speed provided a faster drying process (3.5 h) but with high energy consumption. Henderson and Pabis models were the best prediction factors for heat and mass transfer in the mentioned systems (Hssaini et al., 2021).

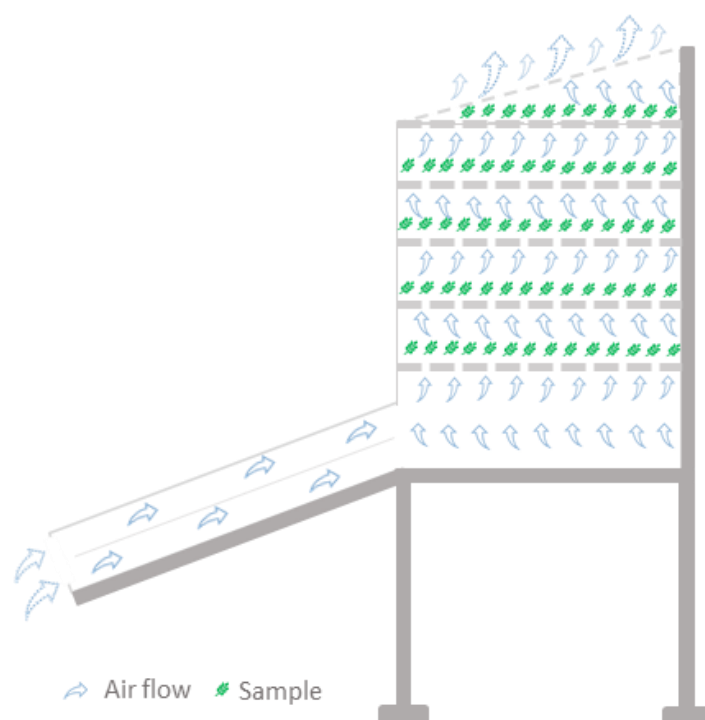


Figure 6- Basic of basic Solar drying (Shadow drying).

2.2.7.4. High Hydrostatic Pressure Drying

High hydrostatic pressure (HHP) drying is a nonthermal drying method mostly used as a pretreatment and applies 100 to 1000 MPa. Dr. Rikimaru Hayashi in 1987 suggested HPP for food processing for the first time for gentle deactivation of spoilage microorganisms (pathogens and enzymes). HPP allows preserving a phytochemical and keeps their biological properties, color, flavor, and texture intact for a long time without additives, synthesized preservatives, or heat application (Figure 7). For food application, HHP utilizes 986.9 atm/1019.7 kgf/cm² (100 MPa) (Koutchma & Marcotte, 2009; Yamamoto, 2017). Although particularly effective to deactivate microorganisms and preserve phytoconstituents, with product appearance remaining unchanged (does not affect color and texture), high pressures can affect slightly the bioactive compounds structure. Often, this technique is used to extract bioactive compounds from food matrices, aiming to optimize their nutrients and phytochemicals composition. Simultaneously, it can generate food with desirable properties, such as preservation of attributes and freshness as well as increasing convenience of handling. HHP not only enhances the efficiency of extraction but also undoubtedly increases the shelf-life stability, the firmness of the final product, improves intestinal absorption as well as improves antioxidant activities (Vega-Gálvez et al., 2011; Yamamoto, 2017). Strawberry slices were subjected to HHP from 50 to 200 MPa, with 50 MPa intervals, as a pretreatment of vacuum-freeze dried. This pretreatment increased the drying time by almost 9–24%. The anthocyanin content increased and protopectin and cellulose decreased with increasing pressure. The content of soluble pectin increased first and then decreased with the increasing of the drying pressure. Therefore, HHP pretreatment is a promising technique to improve product quality and decrease the time and energy consumption involved in a drying process (Zhang et al., 2020). Table 1 presents more examples of this application.

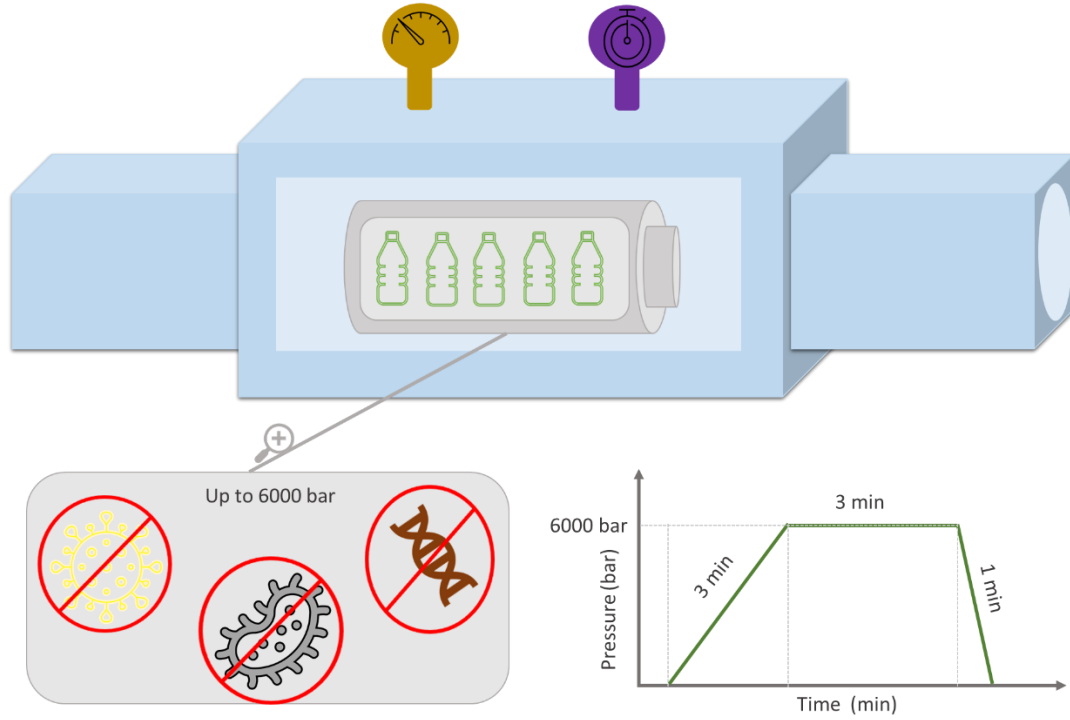


Figure 7- Schematic of high hydrostatic pressure drying.

The classical heat and mass transfers were obtained by the psychrometric approach using the drying rate (in this case, per unit, at a constant rate, during specific time, R_d) (Equation 49), in which A is the heat transfer surface area (m^2) and m_{dm} is the mass of dried material (kg). M_a is the air molar mass, 29 kg/kmol, and dX/dt is the rate of change of moisture content in different times (Ade-Omowaye et al., 2001).

$$R_d = -\frac{m_{dm}}{A \left(\frac{dX}{dt} \right)} \quad (\text{Eq.49})$$

Also Equations (50) and (51) can be used, in which H_t is the heat transfer; H_a and H_s are the humidity of drying air (kg/kg air) and drying air saturation humidity; T and T_s the temperature of drying air, and product bottom surface temperature in K; Δh_{EV} is the latent heat of evaporation (J/kg) (Ade-Omowaye et al., 2001).

$$R_d = k M_a (H_a - H_s) \quad (\text{Eq.50})$$

$$R_d = H_t (T - T_s) / \Delta h_{EV} \quad (\text{Eq.51})$$

2.2.7.5. Freeze Drying

Freeze-drying or lyophilization aims to enhance storage stability and functionality of food and pharmaceutical products (Land, 2011). This technique is an extreme form of

vacuum drying for solvent removal (mostly water) by sublimation from frozen samples. Freeze-drying was described, for the first time, in 1906 (Morais et al., 2016). It is a three-step process, including freezing and primary and secondary drying. During the first step, the solution is frozen between -70 and -80°C such that all components have a crystalline ice structure. The second step (primary drying) removes by sublimation the water from the solution, which causes a probable particle aggregation, especially in emulsion media. The third step removes the remaining water, bound in a crystalline hydrate, or dissolved in an amorphous solid. The drying period depends on the total resistance of water steam to move from the ice-vapor interface to the condenser. Lyophilization protects the phytoconstituents such as proteins, other nutrients, and antioxidants from denaturation, giving rise to products with high storage stability and good ease of transport. On the other hand, the decomposition/deformation of chemical constituents decreases due to the use of low-temperatures. Quick-freezing gives small, numerous ice crystals, while slow freezing results in large and less numerous crystals. Therefore, the fast-freezing process avoids cellular damage. The freezing steps may dramatically affect the properties of the final product. Freeze-drying has several advantages that cannot be ignored, such as: considerably improved product quality by extending shelf-life, a low operating temperature causing minimum degradation and maintaining the nutritional value and volatile and aroma profiles, low energy consumption, less corrosion, and low shipping costs. Although freeze-drying is considered as a user-friendly process, it is an expensive and a slow technique compared to spray dryer (Morais et al., 2016; Shukla, 2011; Wei et al., 2012; Zhang et al., 2017). A considerable decrease in the short-range ordered structure, mesh size, crystalline structure, compactness, and size amorphous were observed in freeze-dried samples, shown by FTIR, small-angle X-ray scattering, and X-ray diffraction (Chi et al., 2020). Table 2 compiles some examples of application of this technique. To evaluate mass transfer (M_{ti} —rate of ice sublimation, g/h per vial), a manometric temperature measurement data described by Tang et al. (2005) was used, at specific conditions between one or half an hour intervals, in which pressure was a function of time at four times per second (Equation 52). A_p is the internal cross-field of vial section (cm^2), P_{ice} is the ice vapor pressure (Torr) at sublimation temperature, R_{ps} is the total of the normalized dry layer and stopper resistance ($\text{cm}^2 \text{ Torr h/g}$).

$$M_{ti} = A_p \frac{P_{ice} - P_c}{R_{ps}} \quad (\text{Eq.52})$$

On the other side, the total heat transfer (H_{tm}) based on manometric temperature measurements was calculated by Equation (53) and ΔH_s is the heat transfer of ice sublimation (cal/g) (Tang et al., 2005)

$$H_{tm} = \Delta H_s M_t \quad (\text{Eq.53})$$

Furthermore, the heat and mass transfer through a nonheated surface (freeze surface) to sublimation media where the produced vapor flows toward the dried layer was calculated by Equation (54) and presented in ($\text{W/m}^2/\text{K}$); T_s and T_{sub} are the plate temperature and sublimation temperature ($^{\circ}\text{C}$), respectively. L and Y are layer thickness and the height of the substance (m), respectively. k_{ice} is the ice thermal conductivity (W/m-K), λ the invisible sublimation of the ice (g/kg/K), P_{sub} and P_c the water vapor pressure at sublimation and condenser temperature (N/m^2), respectively. M_{ts} is the mass transfer from the surface to the material ($\text{kg/s m}^2 \text{ bar}$), β_{cr} is the dried layer constant permeability (kg/s m bar), ρ the density (kg/m^3), M_{wm} is the Moisture content of water to material (kg), λ is the invisible sublimation of the ice presented in (g/KgK), ρ is the Density (kgm^{-3}), and dY/dt is the rate of layer thickness in different times (Jafar & Farid, 2003).

$$q = \frac{T_s - T_{sub}}{\left(\frac{L-Y}{k_{ice}}\right) + 1/H_t} = \lambda \frac{P_{sub} - P_c}{\frac{1}{M_{ts}} + \frac{Y}{\beta_{cr}}} = \rho M_{wm} \lambda \frac{dY}{dt} \quad (\text{Eq.54})$$

Examples of freeze-drying techniques and comparison between freeze and spray dryers are given in Table 2.

2.2.7.6. Electrospray Drying

The electrospray drying technique consists of four main steps that convert the natural polymer-based solution to a dried fiber at room temperature (Figure 8). The drying process operates as follow: an electrostatic force disperses a conductive liquid stream into fine charged droplets. Then the electrostatic energy generates charged droplets by injecting a solution through a high-electric voltage needle; the droplets condense, by solvent evaporation, before the deposition of particles in the substrate, followed by the disintegration of the droplets into dry particles. These are collected in the charge plate (collector) at the end of the process (Figure 8). Meanwhile, the distance between the needle (nozzle) and the collector plate must be accurately adjusted to assure the absolute evaporation of the solvent. Although electrospray was developed to produce ions in spectrometric techniques, nowadays food and pharmaceutical industries are trying to adapt it as a drying technique to obtain high-quality products but in limited volume (He et al., 2011; Zolkepli et al., 2014) (Figure 8). The suitability of electrospray

for the food industry was evaluated, for example, by monitoring the efficiency and activity of probiotics, after protection by soy protein and sunflower oil, and compared to lyophilization. Fortunately, the activity of probiotics after protection by soy protein in both the lyophilized and electrospray followed the same route regarding the improved encapsulation efficiency. By increasing the soy protein content, the viability of probiotics increased and encapsulation efficiency decreased. Both procedures are heat-free, safe, and show a high rate of probiotic protection. Although the electrospray operates at low temperature and voltage, it can easily be used in place of the freeze-dryer due to the functionalities of the final product (Premjit & Mitra, 2021).

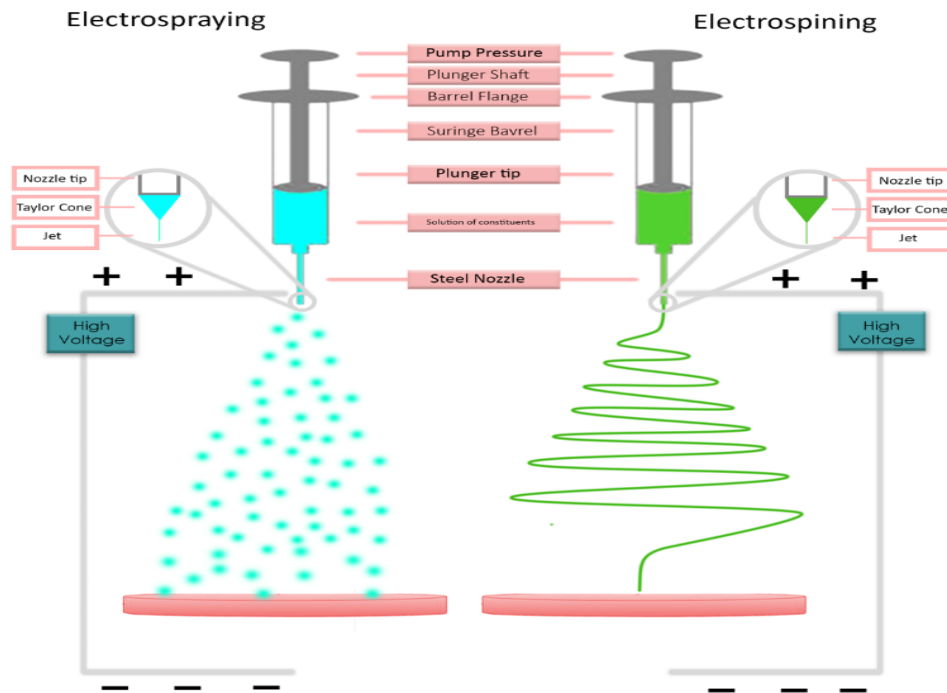


Figure 2- A comparison of electrospinning and electrospraying.

Heat and mass transfer in electrospray drying can be determined theoretically by Lagrangian equation, tracking of single droplets, and evaluated under forced convection. The effect of temperature, initial droplet diameter, and evaporation conditions, which affect the final product quality, can be estimated by the modified equation of mass transfer (M_t) and heat transfer (H_t) reported by Wilhelm et al. (2003) and calculated by Equation (55). d_p is the droplet diameter (m), ρ_a the mass density of air (kg/m^3), U the diffusion coefficient (m^2/s), Sh_{eff} the effective Sherwood number, and m_i the mass transfer of droplet. In Equation (56), k_{∞} is the air thermal conductivity (W/m-K), Nu_{eff} the effective Nusselt number, $T_{i,s}$ the surface temperature of the droplet (K), $T_{g,\infty}$ the temperature of the external dried droplet layer (K), ΔH_v the invisible evaporation heat

(J/kg), and M_{pd} the droplet/particle momentum (kg m/s). The Sh_{eff} , $B_{i,m}$, and Nu_{eff} are dimensionless conditions (Wilhelm et al., 2003).

$$M_t = \pi d_p \rho_a U Sh_{eff} m_i \quad (\text{Eq.55})$$

$$H_t = \pi d_p k_{\infty} Nu_{eff} (T_{i,s} - T_{g,\infty}) - \Delta H_v M_{pd} \quad (\text{Eq.56})$$

In conclusion, electrospray drying can be a promising technique for drying food or pharmaceutical products in the form of nanoparticles giving high yield and efficiency, but with limited output. The procedure can be easily calculated by the mentioned Equations (55) and (56).

2.2.7.7. Electrosprinning

Electrosprinning is a voltage-based technology that occurs due to the electrohydrodynamic phenomenon at high voltage (15 kV). It was developed to produce a fiber with thickness from nanometers to few micrometers and kilometers length, from a liquid media. It aimed at drying a phytoconstituent or drug in the pharmaceutical industry (Figure 8). This technique converts the polymer-based solution to a dried fiber at room temperature (Leidy & Ximena, 2019). Electrosprinning has not yet been approved in the food industry due to the use of a nonfood grade compound such as polypropylene, cellulose, or polycaprolactone, but some food-related researches might be found in this area (Leidy & Ximena, 2019; Liu et al., 2019). The electrosprinning equipment contains the sample container, sample needle syringe, electrosprinning needle, pump, and a high voltage power source. The process starts when the specific voltage causes an electric field between the needle tip and the collector, wherein the solution is pumped with a constant flow rate through a needle (Chronakis, 2010; Leidy & Ximena, 2019). No high temperatures are used and no solvent remains in the dried content. Furthermore, a high-quality product is obtained from electrosprinning due to the high porosity and harmonic morphology of dried samples, but they are not homogeneous. Low weight, high cost, and remarkable surface area per mass unit are observed in electrosprinned-dried samples (Leidy & Ximena, 2019). To determine the heat and mass theories of electrosprinning, an axisymmetric mass transfer, no chemical interaction, a constant temperature inside the nozzle (no gradient), and a trace heat transfer (micro convention) must be taken into account. Therefore, the mass diffusion transfer (kg) is evaluated by axisymmetric Fick's law equation at a radial direction (Equation 57). $R(t)$ is the current nozzle radius, R a universal gas constant equal to 8.3144 (J/mol), T the temperature ($^{\circ}\text{C}$), M_{mw} the molar weight of the solvent, V is the specific volume, and P_{∞} equal to zero, defines the

atmospheric vapor pressure of the solvent, and P_s the solvent concentration at the nozzle surface (can be calculated by Flory–Huggins equation) (Wu et al., 2011).

$$k_g = \frac{R(t) \cdot R \cdot T}{M_{mw} V 1 (P_s - P_\infty)} \quad (\text{Eq.57})$$

Equation (55) provides the nonlinear mass diffusion-transfer, able to predict the accurate drying times and compositional homogeneity in diluted polymer solutions, a fundamental information for better-controlled process of electrospinning (Wu et al., 2011).

As an example, the electrospinning was used to protect cinnamic aldehyde at different concentrations (10%, 20%, 30%, and 40%) by zein in the form of nanofibers. It was proven that increasing the concentration of cinnamic aldehyde also increases significantly the fiber diameter, encapsulation efficiency, loading capacity, and antioxidant activity. Zein nanofibers loaded by cinnamic aldehyde reduced nitrite in sausages, did not have any unfavorable impact on color and porosity, and sausage quality was comparable to the control sample (with 120 ppm nitrite). Therefore, the zein-based nanofiber could be a good alternative to decrease the nitrite content in sausages (Karim et al., 2021).

2.2.8. Continuous Drying / Hybrid Drying Techniques

Continuous drying or hybrid drying is a combination of co-occurring different drying techniques to provide optimized drying conditions and to obtain high-quality food/pharmaceutical products. It is mainly used for large volumes and in a batch process. Hybrid drying or continuous drying can be adjusted like a drying system pretreatment. The most common hybrid technique is the continuous fluid-bed dryers and spray dryers. Besides these, there are several more combinations of techniques compared and discussed in Table 1. For instance, HHP can be applied before vacuum freeze-drying to improve the quality of dried samples (Zhang et al., 2020a, 200b). However, the results demonstrate a noticeable increase in water mobility and production of dried strawberry slices, with a porous morphology and a reducing time process of up to 24% with the anthocyanin content more uniformly distributed. Thereby, the total quality of strawberry slices considerably increased, which revealed a positive effect HHP can have before vacuum freeze-drying for different types of food products. Some examples are given in Table 1.

2.2.9. Drying Pretreatment

Drying pretreatment should be an interesting aspect to improve the quality and functionality of food/dietary supplement products due to the retention of active phytoconstituents in this step. For instance, freeze–thaw cycles were applied before vacuum freeze for drying garlic slices, enhancing their physicochemical properties. Freeze–thaw cycles allowed reducing the remaining water content of the samples, to acquire a porous structure and to improve the retention of flavor and the chemical composition as well as thermal stability and antioxidant activity. The optimized procedure permitted a drying time decrease of about 33%, and lower energy consumption near 15.5%, maintaining the garlic slices quality (Feng et al., 2020). Ultrahigh pressure, ultrasound, and a combination of both methods were applied as drying pretreatments of strawberries by vacuum-freeze drying. A significant decrease in water content of the freeze-dried samples compared to ultrahigh-pressure, ultrasound, and a combination of both methods was verified. Better total properties (enhanced hardness and cross-section areas of the matrix in the dried slices and increased antioxidant activity and anthocyanin, flavonoid, and phenolic contents) were obtained in samples submitted to a combination of ultrahigh-pressure and ultrasound pretreatment (Zhang et al., 2020a). Freeze-drying, hot air drying, vacuum drying, MW vacuum drying, hot air-drying combined with MW vacuum drying, and vacuum drying combined with MW vacuum drying were applied on cabbage to evaluate the changes in the chemical composition and biological activities. Freeze-drying combined with MW vacuum drying and hot air-drying and vacuum drying provided better retention of nutrients and antioxidant activities. On the other hand, freeze-drying took a long time and higher energy consumption compared to the other techniques. Besides, MW vacuum drying combined with hot air-drying provides a uniform honeycomb porous structure with low strength, low energy consumption, short drying time, and higher quality. Consequently, the authors proved that the drying technique can directly influence the preservation of nutrients and stability of natural products. Combining two different drying techniques allows to save energy and preserve the nutrients and the antioxidant activity of a product (Xu et al., 2020b). For examples, see Table 1.

2.2.10. Drying Pretreatment

Drying temperature is another parameter influencing the quality of the final product with special attention to high temperatures and dielectric-based drying techniques. Generally, high temperature is the predominant cause of fragmentation, degradation, and deformation of natural products and directly influences the quality of food products. Fruits and vegetables, herbal medicines and tea, as well as potatoes and sweet potatoes contain almost 85% water, making them perishable products (Mujumdar, 2006). Taking

into account that bound and unbound moisture as discussed in Section 2, unbound moisture is eliminated by evaporation and vaporization, influencing the drying temperature. In terms of evaporation, the process occurs when the moisture vapor of the solid surface is equal to the atmospheric pressure. If we have a heat-sensitive material, the temperature can be lowered by decreasing the atmospheric pressure (vacuum evaporation). When the pressure is higher than the triple point (the thermodynamic condition in which the temperature and pressure of gas, liquid, and solid phases are in equilibrium), moisture evaporation occurs. When the pressure is lower than the triple point, the moisture freezes in the drying chamber and is removed by sublimation just like freeze-drying. The drying rate is constant in these steps (Mujumdar, 2006). In the case of vaporization, drying followed the convection heat transfer by passing the warm vapor or air through the product at constant temperature. The warm air/vapor cools down when in contact with components and the moisture goes out into the environment (in this stage, the drying rate is constant and vaporization of free moisture in the external layer occurs by diffusion). In case of bound moisture, the heat transfer occurs when the moisture content decreased significantly and drying rate falls in seconds. The bound moisture is removed and temperature is settled at the boiling point degree (Mujumdar, 2006). Several researchers studied the impact of temperature on texture and physiochemical properties of the different real samples. For instance, the effect of drying temperatures and storage conditions on total polyphenolic content, including free polyphenols, flavonoids, and anthocyanins of black rice, were investigated. The direct relationship between temperature and total polyphenolic content revealed an apparent reduction with increasing drying temperatures. The polymerization and complexation of phenolic compounds occurs at 60–80°C, with a considerable change in the bioactivity. The reduction of the polyphenolic content occurred even on conventional and vacuum storage conditions, considered as a more efficient procedure (Lang et al., 2019).

Another case study uses collagen, derived from natural sources, as edible artificial casing with a specific modification and drying protocol. Collagen is used in sausage production due to its size and texture uniformity, excellent compatibility with meat, easy production, and low cost. In a study published by Chen et al. (2020) the effect of drying temperatures on the physicochemical composition and microstructure of normal collagen and glutaraldehyde cross-linked collagen casings was discussed. The glutaraldehyde cross-linked dried at 55 to 85°C significantly improved the physicochemical qualities of the collagen casings. It was observed that heat shrinkage and soaking absorbance rates decreased along with an increase in drying temperature; the enhanced mechanical properties of collagen casings and a decrease in drying temperature are justified by

maintaining the triple helix levels. The experimental data from FTIR and XRD revealed no significant changes in the chemical composition of collagen casing, even after a higher cross-linking degree at higher drying temperatures, maintaining the integrity of the triple helix arrangement. On the other side, the thermostability of collagen casings at low temperatures is achieved using low-drying temperatures and glutaraldehyde cross-linking. Consequently, a low drying temperature (about 55°C) causes collagen fiber arrangement along the extrusion direction while higher drying temperatures (around 70 to 85°C) follow a heterogeneous orientation (Chen et al., 2020). The impact of different drying temperatures (25–90°C) on physicochemical and biological properties of sodium–alginate films using glycerol as a plasticizer was evaluated in another study. The glycerol content, sorption isotherm, physical, mechanical, morphological and barrier properties except color were directly related to drying temperatures, as verified by SEM and AFM observations. It was also verified that glycerol content, thickness, elongation at break, water vapor permeability, opacity, and monolayer moisture changed in the opposite way to drying temperatures while drying yield, tensile strength, and elastic modulus changed accordingly. Moreover, the X-ray diffraction revealed the increase in film crystallinity degree with the drying temperature. Meanwhile, the differential thermal gravimetric analyses showed a significant positive impact on film quality characteristics, without any defect by reducing the drying time. Consequently, processes with high-drying temperatures provide compact, nonporous, smooth surface films, which cause a high trenchancy. In terms of performance, drying processes using high temperatures are advised alginate-based samples as a protection (Bagheri et al., 2019). Table 3 presents more examples and practical information on food, with special attention to drying temperatures.

2.2.11. Encapsulation Technique

Encapsulation (particle production) in solid forms is a good alternative to traditional drying processes (Figure 9). Encapsulation coupled to drying techniques converts a liquid into solid product (powder). First introduced in drug delivery, it was then extended to other applications like dietary supplements, food, and cosmetics industries. Some objectives of this technique were to achieve better nutritional retention and efficacy, separate incompatible materials, and mask undesired organoleptic properties like color, taste, or odor. When applied in combination with appropriate drying techniques and drying agents, encapsulation preserves the nutrients and phytoconstituents from decomposition; it provides a controlled and sustained release, which allows decreasing considerably the potential toxicity, reduces dosage, and increases particle stability during storage and handling (Desai & Jin Park, 2005; Augustin & Hemar, 2009).

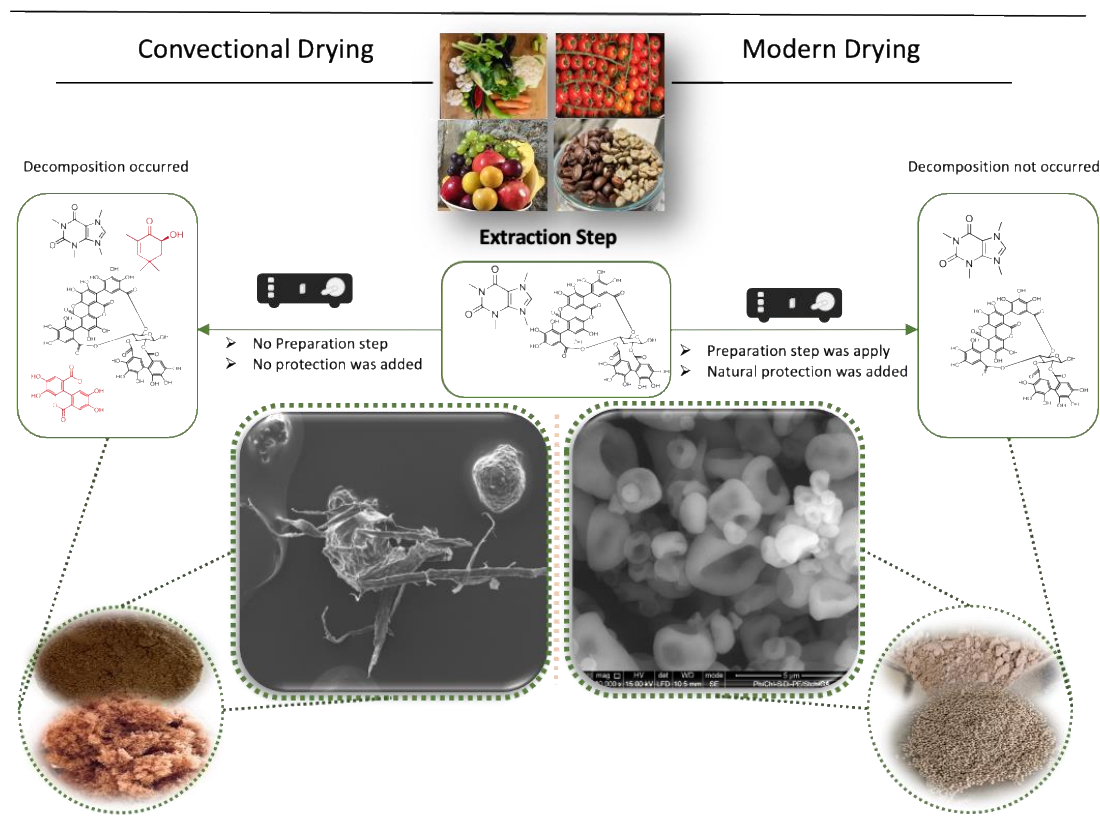


Figure 3- A comparison between modern drying technique like encapsulation and conventional drying technique.

2.2.12. Drying Agent

Drying agent, coating material, and drying adjunct have the same definition as a natural polymer with powdering features used in drying systems or as drying agents. The use of drying agents is a key factor affecting the quality of the final product. Polysaccharides, gums, and proteins such as pectin, sodium alginate, maltodextrin, egg albumin, starch, gum arabic, chitosan, and gelatin are common natural polymers used in foodstuff due to adjunctive drying functionality. The drying agent directly affects the physicochemical quality of the dried sample, because it improves the stability, moisture content, morphology, film forming and emulsifying ability, and release time mechanism. Poor solubility and unwanted color can be considered as some disadvantages of natural polymers (Yinbin et al., 2018).

For instance, pectin and alginate were used to protect nisin by spray drying. These drying agents and the selection of the correct drying process considerably improved stability (production and storage), bioavailability, biological activities, and surface hydrophobicity of this compound. Moreover, the nisin protection with pectin and alginate enhanced its stability during the drying process at high temperatures. Aggregation of nisin protected by pectin was more probable compared to nisin protected by alginate.

Finally, it was proved that alginate could be a better candidate to preserve nisin during a drying process and during storage (Amara et al., 2017). The bioavailability and retention of total phenolics of spent coffee grounds protected by maltodextrin followed by freeze-drying demonstrates the cooperation of maltodextrin with freeze-drying and provides an efficient protection layer that preserves the content of polyphenolic compounds. Furthermore, spray drying produced a capsule with better morphology, but the freeze-dried sample showed more retention of polyphenolics. On the other hand, maltodextrin as a drying agent was a better coating biopolymer to encapsulate those phenolics with a higher loading capacity compared to gum arabic (Ballesteros et al., 2017). Another example is the case of carvacrol drying, using pectin and sodium alginate as drying agents, and spray drying with the inlet temperatures between 100 and 190°C as drying technique. The sample dried at 100°C showed the highest retention of the phytoconstituent and bulk density. The lowest hygroscopicity, encapsulation efficiency as well as dissolution time were also observed at this temperature. It was verified that to increase the encapsulation efficiency, higher inlet temperatures are required. However, this condition causes a considerable decrease in antioxidant activity but an increase in antimicrobial activity. The SEM images showed small particles with a spherical smooth surface in the samples dried at 100–130°C. Thus, enhancing the inlet air provides a considerable increase in dissolution time, hygroscopicity, and encapsulation efficiency. A significant decrease in moisture content, bulk density, and retention efficiency was observed. The lower temperature range, between 100 and 130°C, provided a higher antimicrobial (lower MIC) and antioxidant properties (higher DPPH value) for encapsulated powder (Sun et al., 2020).

Another study used egg albumin, as drying agent of caffeine, for spray drying (encapsulation). The spray-dried samples at less than 120°C are more spherical, while at higher temperatures, crumpled particles were obtained, due to denaturation of albumin. Moreover, at higher inlet temperatures, more particles are produced, while at low temperatures, lower moisture contents were observed. Differential scanning calorimetry (DSC) spectra confirms the significant denaturation of protein at high temperatures, for the spray-dried samples and the most amorphous caffeine microcapsules (Tan et al., 2020).

Modified starch (drying agent) was used to protect red cabbage and red chicory extracts (70% ethanol) dried by spray drying. The experimental results showed a considerable increase in polyphenolic thermal stability, around 44%–55% and 20%–30%, respectively, and significant stability against light and pH conditions. Moreover, the

chemical degradation of both species, especially red cabbage was noticed. Eventually, the encapsulation using modified starch and dried with a spray dryer seems to be a proper drying technique for a food product, food nutrient, and/or natural colorant (Zanoni et al., 2020). Due to the growing interest in meat alternatives, pea protein derivatives from *Pisum sativum* L. were selected as promising protein alternatives. Gluten-free, high nutritional content, low cost, and high sustainability are properties contributing to its attractiveness. Despite all positive properties, this product has low solubility. Solid dispersion-based spray drying technique was applied to pea protein, using a combination of gum arabic and maltodextrin as drying agents, to improve solubility and cover undesirable taste and color. Different percentages of the carriers were used and analyzed by SEM, FTIR, and XRD. A considerable increase in solubility of pea protein isolate (PPI) was observed, due to electrostatic interaction, as well as specific hydrogen bonds with the natural carriers. Meanwhile, the positive impact of drying agent on the solubility and stability of pea protein significantly improved its functionality in food products (Lan et al., 2019). Other researches described the use of chickpea protein, covered by high methoxylated pectin, by sequential adsorption and layer-by-layer protection technique to stabilize a microemulsion that was based on buriti oil. The microemulsion was spray dried to obtain a powder with high stability, bioavailability, and solubility. The chickpea protein successfully preserved the microemulsion of buriti oil, being stable for about 6 months. Furthermore, no changes were observed in the oil droplet size and spray dried powder, with a high encapsulation efficiency due to poor surface oil content (Moser et al., 2020). The effect of different percentages of maltodextrin, gum Arabic, and inulin as drying agents on maqui extract stability and chemical retention were evaluated after freeze-drying. The features and content of the natural polymers directly affected the color, stability, and bioactivity of encapsulated maqui extract. Maltodextrin and inulin provided more efficiency compared to gum arabic. An efficient cover of the maqui powder was obtained with 10% of polysaccharides, providing a similar color to the natural powder even after 60 days of storage at 25°C. Samples protected with inulin showed a higher antioxidant activity after 60 days of storage, while the positive impact of a maltodextrin-based particle on retention of the polyphenolic compound was verified. Therefore, the stable powder could be used as a natural colorant in cosmetics, dairy food products, and food supplements by controlling the process (Romero-González et al., 2020). In another case of study, Geranpour et al. (2020) confirmed that the type of drying agent (carriers/wall material) and drying technique (freeze/spray drying) are factors that could undoubtedly modify the final dried product (Geranpour, et al., 2020).

2.2.6. Retention of Phytoconstituents/Nutrients

The retention of bioactive compounds in a food product has paramount importance for its successful consumer acceptance in the global market. This is especially important when these compounds are responsible for biological properties, organoleptic characteristics, or functional features that add value to the product. Some examples to explain the importance of conserving nutrients/phytoconstituents in food products will be discussed here. Nowadays, there is an increasing demand for fruits and vegetables in the world market. Due to the short lifespan of fresh fruits and vegetables, enhancing the quality of dried products has become an industrial concern in the competitive worldwide market. Therefore, applying the most appropriate drying technique after optimization will provide a high-quality product with a high shelf-life and free of synthetic preservatives. For instance, a combination of three methods to preserve red beetroot (*Beta vulgaris* L.) and avoiding the use of an unnatural preservative was evaluated. The free convection at 50–70°C and forced convection at 40°C combined with 315W MW were used as drying methods. Differences in betalain and polyphenol contents and antioxidant activity were the parameters used to choose the adequate method. The combined approach was more effective in preserving phytochemicals than the conventional method, due to minimizing the drying time and enhancing the drying rate. The results can be extended to different fruits and vegetables aiming to preserve a phytochemical structure in a safe and green mode (Nistor et al., 2017).

The impact of different drying systems on the chemical composition and antioxidant activity of residual polysaccharides from *Chimonobambusa quadrangularis* sprouts was described by Chen et al. (2019). Freeze-drying compared to vacuum drying, hot air-drying, and spray drying showed the best retention stability of different hydro and liposoluble compounds as well as antioxidant activity (Chen et al., 2019).

The effect of five drying systems in phytochemical and biological activities of robusta coffee beans was reported by Dong et al. (2017). The five drying techniques were compared taking into account the volatile and fatty acid profiles. Room temperature drying, heat pump drying, and solar drying follow a similar behavior, whereas hot air-drying and freeze-drying are considerably distinctive. Freeze-drying showed more preservation of monounsaturated fatty acids, fats, and organic acids; for hot air-drying, an improvement of amino acids and polyunsaturated fatty acids contents was noticed; and for the heat-pump drying, a higher percentage of volatiles was noticed (Dong et al., 2017).

The effect of drying on retention of total polyphenolic, anthocyanins, flavonoids, vitamins C and B, as well as antioxidant properties in different types of berries by three drying techniques (hot air-drying, freeze-drying, and refractance window drying) were tested. The freeze-dried samples presented a better retention of polyphenolic, anthocyanins, vitamins C and B, and antioxidant capacity. Refractance window drying technique was more effective in retaining vitamin B. Hot air-drying was not a suitable technique for any sample. Subsequently, the freeze-dried blueberries, tart cherries, strawberries, and cranberries showed better quality than the other samples dried by refractance window and hot air-drying methods (Nemzer et al., 2018).

Another point of interest of drying techniques is the effective preservation of food-grade colors enabling the use of the dried products as color additives in food industries. Taking into account the consumer awareness about synthetic compounds, preserving a food colorant by a safe and green technology has recently increased the attention of food producers. For instance, spray drying, electrospraying, and freeze-drying are common drying techniques used to preserve a food colorant (Boer et al., 2019). Different types of drying techniques on fruits, vegetables, and flowers with natural sources of pigments are described focused on their functionality and retention of these compounds. Freeze-drying is a promising drying technique for the preservation of natural colorants, with high yield and high retention of active phytoconstituents (Różyło, 2020). Betacyanin pigments, a red-purple food colorant extracted from *Opuntia stricta* fruit juice, dried by spray drying with an inlet temperature of 160°C and 10% w/v content of extract, produced a high color strength and nonsticky powder with 58% yield. Dried samples of red-purple color showed be stable during one month on yogurt, on a soft drink, and stored at 25°C. As a result, spray drying was introduced as an appropriate technique for preserving healthy natural food colorants in commercialized food samples (Obón et al., 2009). Furthermore, *Amaranthus* betacyanin pigments were dried using maltodextrin as drying agent and spray drying under the inlet temperature of 180°C and outlet temperature of 96°C. A suitable preservation of pigment for almost 63 weeks was reported (Cai & Corke, 2000). Moreover, curcumin is a natural sharp food colorant with high content of phytoconstituents and high antioxidant activity. Nevertheless, its low solubility and stability limit its functionality in the food and pharmaceutical industries. Porous starch and gelatin were used as drying agents to protect curcumin by a spray dryer at 190°C of inlet temperature. Curcumin loaded on the mixture of starch and gelatin presented higher stability against light, heat, pH as well as a considerable increase in solubility, which made it highly useful in food industries (Wang et al., 2009).

Essential oils (EOs) and vegetable oils are other food-related sources that need accurate preservation. EOs are from different parts of aromatic plants and result from their secondary metabolism. Vegetable oils are products from the primary metabolism of plant seeds. So, both are oils with different effects and applications in the food industry. EOs are antioxidant and antimicrobial compounds providing good preservation, but due to their low solubility and stability, their industrial application is limited. Encapsulation provides, to the sensitive natural compounds, a functional cover against oxidative stress and undesirable interactions, an optimized release, and improved solubility and stability in the mouth, stomach, and small intestine. Therefore, antimicrobial agents from natural products covered by food-grade carriers and dried by freeze-drying or spray drying could be used as food preservatives and significantly enhance the quality of food products. Delshadi et al. (2020) described the potential protection of phytoconstituents by drying in the form of encapsulation, to improve the functionality of vegetable oils and EOs in food products. These authors reported efficient drying methods (freeze or spray drying) and drying agents (proteins, lipids and carbohydrates) to encapsulate EOs and vegetable oils safely and concluded that they are two major influential factors of the functional and nutritional properties of food products (Delshadi et al., 2020).

2.2.13. Controlled Release

Controlled release was first introduced in drug delivery/therapy. Then it was extended to other applications like dietary supplements and food and cosmetics industries to achieve the better nutritional absorption and lower toxicity. Phytoconstituents, nutrients, and drugs with protection (drying agents) are released, at a predetermined rate, in a specified time. In general, encapsulation combining drying agents and drying methods is used in food industries to protect the bioactive compounds from decomposition. It can also be used successfully for encapsulation of probiotics and other nonpathogen bacteria, providing sustained and controlled release and producing high-quality food products. Therefore, the type of drying technique and agent directly influence the release kinetics and time. Thus, the quality of the dried samples directly affects the bioactive compounds release and should be optimized to give a high-quality final product (Ray et al., 2016). For instance, Loughrill et al. (2019) evaluated the drying method and carrier effect on the release of docosahexaenoic acid (DHA, omega-3-fatty acid). The pH-dependent polymer hydroxyl-propyl-methyl-cellulose-acetate-succinate (HPMCAS) and DHA solid-lipid nanoparticles (SLN) were the carriers to protect DHA and spray drying (encapsulation) was the drying method used. Then the release behavior of both samples was compared, via acidic media and phosphate buffer (pH 6.8). The DHA spray-dried sample showed more stability compared to the SLN sample, taking into account

experimental data. As for release time, 2 h for acidic media and fast release for buffer phosphate were observed. It can be concluded that the encapsulated powder protects the odor and taste of DHA. However, additional studies on storage stability and consolidation of the encapsulated powder into the food products are needed (Loughrill et al., 2019).

Flavonoids, like naringenin and quercetin, were loaded on polymeric carrier and dried by spray drying to improve the physicochemical activities for functionalization in food and pharmaceutical industries. The results demonstrated that high contents of flavonoid were released (more than 80%) in simulated intestinal fluid. It was also reported that the drying agent plays a vital role in modulating the release in intestinal fluid. Moreover, it was proved that encapsulation by spray drying significantly improved the shelf-life until 12 months (retention of active component), the dissolution, and provided a high release rate at pH 1.0. Therefore, it can be concluded that encapsulation could be a promising technique for controlled release of the active components and can be expanded to various food and pharmaceutical products to design a smart product (Sansone et al., 2011).

2.2.14. Storage Stability

Improving the storage stability of food and pharmaceutical products based on a green formulation without incorporating preservatives becomes a challenge. The harmful effects of normal preservatives in the food, pharmaceutical, and cosmetic industries cannot be forgotten. So, a safe solution to overcome these concerns and considerably enhance the quality of the final product must be considered. To answer to this challenge, the stability of flaxseed protein hydrolysates during and after spray drying of liposomal nanocarriers coated by chitosan was improved, aiming their functionalization for food and pharmaceutical applications. SEM, FTIR, and DLS revealed the physical morphology, particle size, and zeta potential of flaxseed protein coating with chitosan. These techniques can provide a sustained release mechanism, long-time storage stability, high bioavailability, and high chemical composition retention as well as maintain the antioxidant properties. Finally, lipid cover proved to be a suitable candidate for improving desired properties of flaxseed protein. The availability of chitosan-coated liposomal layer as drying agent considerably enhances the total properties of flaxseed protein as well as improves the total stability (Sarabandi & Jafari, 2020). In another example, four drying techniques were applied to xylooligosaccharides in the presence of drying agents (maltodextrin and gum arabic) to evaluate their physicochemical properties, microstructure, and storage stability. The improvement of their

physicochemical properties was obtained by vacuum drying. However, spray drying revealed to be the best drying system for preserving the final product with a high storage stability, taking into account its drying efficiency. Transition temperature and water activity reduction were obtained with vacuum drying and an optimized amount of drying agent. Freeze-drying was recommended as a cost-effective technique at an industrial scale to preserve a long-lasting food product (Zhang et al., 2019).

2.2.15. Impact of Drying System on Food Staff-Examples

The discussion of the impact of different drying techniques on the physicochemical properties of phytoconstituents provides an appropriate overview of the quality of natural sources, as well as their inherent cost and application capacity in large-scale food industries. The effect of drying methods on the chemical composition of three types of rice pigments was determined. The chemical evaluation showed results directly affected by the type of drying technique. The FIR showed a significant enhancement on total phenolic content and consequently in antioxidant activity. Besides, this technique enhances also flavonoids and glucoside flavonoids content as well as the anthocyanin and tocopherols total content. On the other hand, hot air-drying decreased the amino acids content. The changes in chemical composition, due to the drying techniques used, revealed hot air and FIR might have a considerable impact on the chemical composition (Ratseewo et al., 2020).

Rheometer, SEM, X-Ray diffractometer, and visco-amylograph are equipment used to analyze the physicochemical properties of green banana flour. Experimental results revealed that freeze-drying and hot air-drying techniques directly affect particle size, morphology, starch resistance, and hydration of green banana flour with water. Although both techniques are useful in retaining the total starch content of green banana, it directly influences the starch resistance and hydration. The XRD diffraction revealed that banana starch morphology persists intact after both drying processes. Finally, it was concluded that although both techniques showed some differences, the main result is almost the same and both techniques could be useful for drying green banana flour (Ahmed et al., 2020). An optimized drying system can be available based on the advantages and disadvantages of different drying systems and agents, as well as the chemical composition of the food or pharmaceutical product.

2.2.16. Recent Improved Application of Food Product Drying

2.2.16.1. Freeze-dried Vegetable Snack

A freeze-dried vegetable snack with sufficient porosity, enough strength, low water content, and low shrinkage was produced and commercialized. The mixtures of locust bean gum with xanthan gum and sodium alginate combined with calcium lactate were used to obtain this customer-attractive product. While the type of drying agent directly affects the physicochemical properties of the final product, high porosity and strength were obtained when sodium alginate was used as drying agent. On the other hand, the energy consumption per product unit was evaluated and 32% of the energy consumption was due to the heating plate used during the formation of the hydrocolloid gels. Overall, 22–32% of electrical energy consumption belongs to freeze-drying (Cieurzyńska et al., 2020).

2.2.16.2 Protein for Encapsulate/Drying of Food Product

Proteins play a valuable role in preserving a food product safely and reliably because fats, oils, natural colorants, phytosterols, total nutrients, vitamins, fibers, and probiotic bacteria can be encapsulated by protein. Meanwhile, high-performance stability, quality, and efficiency are properties offered by a biopolymer-based encapsulation system. Protein-based encapsulation is a suitable choice for the positively charged polymer. The chemical reactivity and structure of proteins limit suitable drying technologies, allowing only spray drying and coacervation. In terms of performance, protein functionality in the spray-drying method must be combined with starch and gum. However, both coacervation and spray-drying techniques need an emulsion as a feed solution to preserve the folding of protein against deformation caused by heat. The protein-based spray drying technique was typically used for nonflavoring food ingredients due to a particular reaction between protein and flavoring compounds, thereby changing product flavor.

Furthermore, the high viscosity of protein provides strong protection to flavoring compounds, which is not acceptable in food industries. Reducing the amount of protein to have a final product with more flavor is a desirable approach. As noted, protein is not a suitable drying agent or carrier of flavoring in food industries. However, there are some alternatives of positively charged polymers, like chitosan, used instead of protein, to optimize a drying technique to preserve both proteins and flavors as drying agents and active phytoconstituents. Therefore, coacervation with protein is not a suitable option for flavorful and fragrant materials. In this case, a protein-based carrier for highly unsaturated edible oils, nonflavoring food ingredients, supplements, and drugs should be an appropriate option. In terms of performance, the high encapsulation efficiency of unsaturated edible oils revealed the positive impact of protein on such systems, which

causes a small amount of oil potentially on the surface of the particle to oxidize. However, the adequate formulation of protein carriers can provide a good barrier to prevent undesirable oxidation, resulting in a longer shelf-life of unstable oils. Finally, spray drying and coacervation techniques, based on protein, could be a promising method for encapsulating food products due to their food functionality (Reineccius, 2019).

2.2.16.3 Encapsulation of Food Preservatives

Microbial and fungal contamination occurs in food products due to different reasons and is a major cause of food degradation. The improvement of natural food quality and its safety became challenging in recent technologies. The relative resistance of microbial and fungal species and the toxic base of conventional preservatives justify the need for strong antifungal and antimicrobial preservatives such as antifungal peptides and proteins. They must follow the novel delivery systems and highlight the requirement for new approaches to increase the shelf life and food quality. Natural antifungal peptides are significantly efficient against food-related fungi and play an essential role in natural food preservation. Thaumatin-like proteins, thionins, defensins, lipid transfer proteins, glycine-rich peptides, 2S albumins, puroindolines, 7S globulins, hevein-like peptides, knottins, protease inhibitors, α -hairpin, and vertebrate sources like cathelicidin, bovine myeloid antimicrobial peptide (BMAP), mammalian defensins, histatins, frog skin, thanatin, insect defensins, tachystatins, and killer toxins can be used as natural preservatives in food applications (Thery et al., 2019). The use of a peptide or protein without a cover or protection, for a long time, may cause a deformation/degradation and loss of functionality. Intensive research on novel technologies to fight against probable contamination and improving food quality showed the critical role of a drying agent and an appropriate drying technique to increase the functionality of these promising structures as natural food preservatives. Therefore, suitable encapsulation techniques to protect natural antimicrobial/antifungal preservatives and release them along the time, in food product package, are advisable. Meanwhile, the drying agent/technique provides a sustained/controllable release with long stability during storage, causing consequently an increase in shelf-life. On the other hand, the positive effect of food protein encapsulation was verified and allows clarifying various categories, taking into account the type of drying agent, particle size and distribution, encapsulation efficiency, features and purity of wall material, wall ratio, drying technique, production condition, gastric and storage stability, and release time and mechanism (Mohan et al., 2015). Therefore, encapsulation of food preservatives following specific standards, rules, and different safety restrictions on food products showed significant advantages.

2.2.17. Food Microorganisms

Food microorganisms are from two main groups: microorganisms responsible for food spoilage (discoloration, breakdown, agglomeration, fungi growing, deformation, etc.), and microorganisms responsible for improving the food quality called fermenting microorganisms (probiotics, yeast, baking powder, sourdough, etc.) (Blackburn, 2006). The fermenting microorganisms can be probiotic and biogenic. Probiotic microorganisms are added directly to food products and act in the digestive system, being responsible for several functions, such as preventing or controlling food intolerance and allergic responses to food. Biogenic is one that indirectly synthesizes metabolites during the ingestion process (Gobbetti et al., 2010). Considering the information on all subgroups in Section 2, it can be concluded that the developed drying techniques decrease the moisture and result in the deactivation of food spoilage microorganisms and in the preservation of its nutrients. The complete definition and functionality of these technologies has already been explained in Sections 2.3–2.7. Some drying techniques were theoretically based on encapsulation (natural base protection), such as spray drying, freeze-drying (under some conditions), fluid bed drying, and electrospray drying.

Briefly, any drying technique that provides particles and fibers can be categorized as an encapsulation technique. These technologies can play a vital role in protecting a beneficial food microorganism, with particular attention to probiotic microorganisms (Chávarri et al., 2012). For example, in food industries, sodium caseinate protects the probiotic bacteria *Lactobacillus* in the drying step. In addition, the low melting point of the fat present acts as a heat shield. To prove this, considerable survival of encapsulated bacteria protected by a fat with a low melting point was observed. In other words, encapsulating probiotic bacteria with the protection of low melting fat and sodium caseinate can significantly prevent their deactivation and provide adequate food preservation functionality. Furthermore, a sustained release of probiotic bacteria can preserve a food product (Liu et al., 2015).

2.3. Conclusion

In conclusion, hybrid drying, drying pretreatments, and drying with encapsulation are promising drying improvements. It should be taken into account that the combination of two or three different drying techniques can significantly enhance the quality of the final product. Subsequently, a novel drying pretreatment can substantially enhance the retention of active components and improve porosity and homogeneity. Overall, a good drying technique considerably improves the general properties of food and

pharmaceutical products. The use of a drying agent directly affects the protection and preservation of active components and provide a product with a high shelf-life.

It can be concluded that, according to recent innovations, spray drying is the most efficient technique in terms of energy consumption, fast production, safe and readily available equipment, and high-quality final product. Meanwhile, the drying technologies offer a controlled/sustained release that considerably improves storage stability, in the lack of general preservatives. On the other hand, optimizing a drying technique and using an appropriate drying agent should be a promising step in preserving probiotic bacteria and antimicrobial preservatives, either effective for phytoconstituents bioavailability or storage stability.

Furthermore, High Hydrostatic Pressure (HHP) is a non-thermal drying method that promotes a gentle deactivation of spoilage microorganisms (pathogens and enzymes), aiming to preserve phytochemicals and keep their biological properties, color, flavor and texture, for a long time, without using synthetic preservatives or other additives. This technique could easily improve the overall properties of food and pharmaceutical products and be useful in the respective industries.

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Author Contributions

Faezeh Fathi: conceptualization; investigation; methodology; writing—original draft; writing—review and editing. **Samad N. Ebrahimi:** conceptualization; writing—review and editing; supervision. **Luís Carlos Matos:** writing—review and editing. **Maria Beatriz**

Prior Pinto Oliveira: conceptualization; funding acquisition; project administration; supervision. **Rita C. Alves:** visualization; writing—review and editing; supervision.

Declaration of competing interest

The authors declare no conflict of interest.

2.4. References

- Abano, E., (2020). Microwave and blanching pretreatments for hot air drying of orange-fleshed sweet potato slices (*Ipomoea batatas*). *International Journal of Food Science*, 2020. 1-12. <https://doi.org/10.1155/2020/8872429>
- Abbas, A. M., Seddik, M. A., Gahory, A.-A., Salaheldin, S., & Soliman, W. S. (2021). Differences in the Aroma Profile of Chamomile (*Matricaria chamomilla* L.) after Different Drying Conditions. *Sustainability*, 13(9), 5083. <https://doi.org/10.3390/su13095083>
- Ade-Omowaye, B., Rastogi, N., Angersbach, A., & Knorr, D. (2001). Effects of high hydrostatic pressure or high intensity electrical field pulse pre-treatment on dehydration characteristics of red paprika. *Innovative Food Science & Emerging Technologies*, 2(1), 1-7. [https://doi.org/10.1016/S1466-8564\(00\)00022-9](https://doi.org/10.1016/S1466-8564(00)00022-9)
- Ahmed, J., Thomas, L. & Khashawi, R. (2020). Influence of hot-air drying and freeze-drying on functional, rheological, structural and dielectric properties of green banana flour and dispersions. *Food Hydrocolloids*, 99, 105331. <https://doi.org/10.1016/j.foodhyd.2019.105331>
- Amara, C. B., Kim, L., Oulahal, N., Degraeve, P., & Gharsallaoui, A. (2017). Using complexation for the microencapsulation of nisin in biopolymer matrices by spray-drying. *Food chemistry*, 236, 32-40. <https://doi.org/10.1016/j.foodchem.2017.04.168>
- Augustin, M. A. & Hemar, Y. (2009). Nano- and micro-structured assemblies for encapsulation of food ingredients. *Chemical Society Reviews*, 38(4), 902–912. <https://doi.org/10.1039/B801739P>
- Aware, R., & Thorat, B. (2011). Garlic under various drying study and its impact on allicin retention. *Drying Technology*, 29(13), 1510-1518. <https://doi.org/10.1080/07373937.2011.578230>
- Bagheri, F., Radi, M. & Amiri, S. (2019). Drying conditions highly influence the characteristics of glycerol-plasticized alginate films. *Food Hydrocolloids*, 90, 162–171. <https://doi.org/10.1016/j.foodhyd.2018.12.001>
- Ballesteros, L. F., Ramirez, M. J., Orrego, C. E., Teixeira, J. A. & Mussatto, S. I. (2017). Encapsulation of antioxidant phenolic compounds extracted from spent coffee grounds by freeze-drying and spray-drying using different coating materials. *Food Chemistry*, 237, 623–631. <https://doi.org/10.1016/j.foodchem.2017.05.142>
- Bernaert, N., Van Droogenbroeck, B., Van Pamel, E. & De Ruyck, H. (2019). Innovative

- refractance window drying technology to keep nutrient value during processing. *Trends in Food Science & Technology*, 84, 22–24. <https://doi.org/10.1016/j.tifs.2018.07.029>
- Blackburn, C. D. V. (2006). *Food spoilage microorganisms* (1rd ed). Woodhead Publishing.
- Broadhead, J., Edmond Rouan, S. K. & Rhodes, C. T. (1992). The spray drying of pharmaceuticals. *Drug Development and Industrial Pharmacy*, 18(11–12), 1169–1206. <https://doi.org/10.3109/03639049209046327>
- Caccavale, P., De Bonis, M. V., & Ruocco, G. (2016). Conjugate heat and mass transfer in drying: A modeling review. *Journal of food Engineering*, 176, 28–35. <https://doi.org/10.1016/j.jfoodeng.2015.08.0311>
- Cai, Y. Z. & Corke, H. (2000). Production and Properties of Spray-dried *Amaranthus Betacyanin* Pigments. *Journal of Food Science*, 65(7), 1248–1252. <https://doi.org/10.1111/j.1365-2621.2000.tb10273.x>
- Chávarri, M., Marañón, I., & Villarán, M. C. (2012). Encapsulation technology to protect probiotic bacteria. In Everlon, E. C. (Ed.), *Probiotics* (pp. 501–540). IntechOpen.
- Chen, C., Liu, F., Yu, Z., Ma, Y., Dounglas Goff, H. D. & Zhong, F. (2020). Improvement in physicochemical properties of collagen casings by glutaraldehyde cross-linking and drying temperature regulating. *Food Chemistry*, 318, 126404. <https://doi.org/10.1016/j.foodchem.2020.126404>
- Chen, G., Li, C., Wang, S., Mei, X., Zhang, H., Kan, J. (2019). Characterization of physicochemical properties and antioxidant activity of polysaccharides from shoot residues of bamboo (*Chimonobambusa quadrangularis*): Effect of drying procedures. *Food Chemistry*, 292, 281–293. <https://doi.org/10.1016/j.foodchem.2019.04.060>
- Chi, C., Li, X., Zhang, Y., Miao, S., Chen, L., Li, L. & Liang, Y. (2020). Understanding the effect of freeze-drying on microstructures of starch hydrogels. *Food Hydrocolloids*, 101, 105509. <https://doi.org/10.1016/j.foodhyd.2019.105509>
- Chronakis, I. S. (2010). Micro-/Nano-Fibers by Electrospinning Technology: *Micro-Manufacturing Engineering and Technology* (pp. 264–286). Denmark, National Food Institute. <https://doi.org/10.1016/B978-0-8155-1545-6.00016-8>
- Ciurzyńska, A., Marczak, W., Lenart, A. & Janowicz, M. (2020). Production of innovative freeze-dried vegetable snack with hydrocolloids in terms of technological process and carbon footprint calculation. *Food Hydrocolloids*, 108, 105993. <https://doi.org/10.1016/j.foodhyd.2020.105993>
- Colak, N. & Hepbasli, A. (2009). A review of heat pump drying: Part 1 – Systems, models and studies. *Energy Conversion and Management*, 50(9), 2180–2186. <https://doi.org/10.1016/j.enconman.2009.04.031>
- Coronel-Aguilera, C. P., San Martín-González, M. F., (2015). Encapsulation of spray dried β -carotene emulsion by fluidized bed coating technology. *LWT - Food Science and Technology*. 62(1), 187–193. <https://doi.org/10.1016/j.lwt.2014.12.036>

- Cui, Z.-W., Xu, S.-Y. & Sun, D.-W. (2004). Microwave–vacuum drying kinetics of carrot slices. *Journal of Food Engineering*, 65(2), 157–164. <https://doi.org/10.1016/j.jfoodeng.2004.01.008>
- Dantas, D., Pasquali, M. A., Cavalcanti-Mata, M., Duarte, M. E. & Lisboa, H. M. (2018). Influence of spray drying conditions on the properties of avocado powder drink. *Food Chemistry*, 266, 284–291. <https://doi.org/10.1016/j.foodchem.2018.06.016>
- Boer, F. Y. d., Imhof, A. & Velikov, K. P. (2019). Encapsulation of colorants by natural polymers for food applications. *Coloration Technology*, 135(3), 183–194. <https://doi.org/10.1111/cote.12393>
- Delshadi, R., Bahrami, A., Tafti, A. G., Barba, F. J. & Williams, L. L. (2020). Micro and nano-encapsulation of vegetable and essential oils to develop functional food products with improved nutritional profiles. *Trends in Food Science & Technology*, 104, 72-83. <https://doi.org/10.1016/j.tifs.2020.07.004>
- Desai, K. G. H. & Jin Park, H. (2005). Recent Developments in Microencapsulation of Food Ingredients. *Drying Technology*, 23(7), 1361–1394. <https://doi.org/10.1081/DRT-200063478>
- Morais, A. R. D. V., Alencar, É. D. N., Xavier Júnior, F. H., Oliveira, C. M. De, Marcelino, H. R., Barratt, G., Fessi, H., Egito, E. S. T. Do & Elaissari, A. (2016). Freeze-drying of emulsified systems: A review. *International Journal of Pharmaceutics*, 503(1–2), 102–114. <https://doi.org/10.1016/j.ijpharm.2016.02.047>
- Dong, W., Hu, R., Chu, Z., Zhao, J. & Tan, L. (2017). Effect of different drying techniques on bioactive components, fatty acid composition, and volatile profile of robusta coffee beans. *Food Chemistry*, 234, 121–130. <https://doi.org/10.1016/j.foodchem.2017.04.156>
- Du, J., Gao, L., Yang, Y., Guo, S., Chen, J., Omran, M., & Chen, G. (2020). Modeling and kinetics study of microwave heat drying of low grade manganese ore. *Advanced Powder Technology*, 31(7), 2901-2911. <https://doi.org/10.1016/j.appt.2020.05.013>
- El-Messery, T. M., Altuntas, U., Altin, G. & Özçelik, B. (2020). The effect of spray-drying and freeze-drying on encapsulation efficiency, in vitro bioaccessibility and oxidative stability of krill oil nanoemulsion system. *Food Hydrocolloids*, 106, 105890. <https://doi.org/10.1016/j.foodhyd.2020.105890>
- Farid, M. (2019). Heat and mass transfer in food processing. In M. Kutz (Ed.), *Handbook of Farm, Dairy and Food Machinery Engineering* (pp. 439-460). <https://doi.org/10.1016/B978-0-12-814803-7.00017-8>
- Feng, Y., Ping Tan, C., Zhou, C., Yagoub, A. E. G. A., Xu, B., Sun, Y., Ma, H., Xu, X. & Yu, X. (2020). Effect of freeze-thaw cycles pretreatment on the vacuum freeze-drying process and physicochemical properties of the dried garlic slices. *Food Chemistry*, 324, 126883. <https://doi.org/10.1016/j.foodchem.2020.126883>
- Gao, Y., Yang, X., Chu, L., Zhang, Y., & Li, Q. (2021). Experimental investigation and thin-layer modelling of cassava slice drying. *Journal of Thermal Analysis and Calorimetry*, 1-9. No issue no volume <https://doi.org/10.1007/s10973-020-10401-X>

- Geranpour, M., Assadpour, E. & Jafari, S. M. (2020). Recent advances in the spray drying encapsulation of essential fatty acids and functional oils. *Trends in Food Science & Technology*, 102, 71–90. <https://doi.org/10.1016/j.tifs.2020.05.028>
- Gobbetti, M., Cagno, R. D., & De Angelis, M. (2010). Functional microorganisms for functional food quality. *Critical reviews in food science and nutrition*, 50(8), 716-727. <https://doi.org/10.1080/10408398.2010.499770>
- Guaita, M., Panero, L., Motta, S., Mangione, B., & Bosso, A. (2021). Effects of high-temperature drying on the polyphenolic composition of skins and seeds from red grape pomace. *LWT*, 145, 111323. <https://doi.org/10.1016/j.lwt.2021.111323>
- Halonen, A. (2020). Numerical modelling of heat losses and economic optimisation of insulation thickness in process industry pipelines. Master thesis. <http://urn.fi/URN:NBN:fi:tuni-202002282419>
- He, Y., Huang, Y., Wang, W. & Cheng, Y. (2011). Integrating micromixer precipitation and electrospray drying toward continuous production of drug nanoparticles. *Chemical Engineering Journal*, 168(2), 931–937. <https://doi.org/10.1016/j.cej.2011.01.092>
- Hoskin, R. T., Xiong, J., Esposito, D. A. & Lila, M. A. (2019). Blueberry polyphenol-protein food ingredients: The impact of spray drying on the in vitro antioxidant activity, anti-inflammatory markers, glucose metabolism and fibroblast migration. *Food Chemistry*, 280, 187–194. <https://doi.org/10.1016/j.foodchem.2018.12.046>
- Hou, L., Zhou, X., & Wang, S. (2020). Numerical analysis of heat and mass transfer in kiwifruit slices during combined radio frequency and vacuum drying. *International Journal of Heat and Mass Transfer*, 154, 119704. <https://doi.org/10.1016/j.ijheatmasstransfer.2020.119704>
- Hssaini, L., Ouaabou, R., Hanine, H., Razouk, R., & Idlimam, A. (2021). Kinetics, energy efficiency and mathematical modeling of thin layer solar drying of figs (*Ficus carica* L.). *Scientific reports*, 11(1), 1-21. <https://doi.org/10.1038/s41598-021-01688-3>
- Hung, P. V. & Duy, T. L. (2012). Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. *International Food Research Journal*, 19(1), 327–332.
- Inada, K. O. P., Nunes, S., Martinez-Blazquez, J. A., Tomás-Barberán, F. A., Perrone, D., & Monteiro, M. (2020). Effect of high hydrostatic pressure and drying methods on phenolic compounds profile of jaboticaba (*Myrciaria jaboticaba*) peel and seed. *Food chemistry*, 309, 125794. <https://doi.org/10.1016/j.foodchem.2019.125794>
- Ivanova, M., Katrandzhiev, N., & Dospatliev, L. (2020). Using some mathematical moldes in modeling mashroom drying. *International Journal of Applied Mathematics*, 33(1), 109-124. <http://dx.doi.org/10.12732/ijam.v33i1.9>
- Jafar, F., & Farid, M. (2003). Analysis of heat and mass transfer in freeze drying. *Drying Technology*, 21(2), 249-263. <https://doi.org/10.1081/DRT-120017746>
- Jafari, S. M., Assadpoor, E., He, Y., Bhandari, B. (2008). Encapsulation efficiency of food flavours and oils during spray drying. *Drying Technology*, 26(7), 816-835. <https://doi.org/10.1080/07373930802135972>

- Jeyapragash, S., Heffernan, J. E., Driscoll, R. H., & Frank, D. C. (2020). Impact of drying technologies on tomato flavor composition and sensory quality. *LWT*, 120, 108888. <https://doi.org/10.1016/j.lwt.2019.108888>
- Jiao, Y., Shi, H., Tang, J., Li, F., & Wang, S. (2015). Improvement of radio frequency (RF) heating uniformity on low moisture foods with Polyetherimide (PEI) blocks. *Food research international*, 74, 106-114. <https://doi.org/10.1016/j.foodres.2015.04.016>
- Jiang, H., Zhang, M., Fang, Z., Mujumdar, A. S. & Xu, B. (2016). Effect of different dielectric drying methods on the physic-chemical properties of a starch–water model system. *Food Hydrocolloids*, 52, 192–200. <https://doi.org/10.1016/j.foodhyd.2015.06.021>
- Jittanit, W., & Angkaew, K. (2020). Effect of superheated-steam drying compared to conventional parboiling on chalkiness, head rice yield and quality of chalky rice kernels. *Journal of Stored Products Research*, 87, 101627. <https://doi.org/10.1016/j.jspr.2020.101627>
- Jylhä, P., Halmemies, E., Hellström, J., Hujala, M., Kilpeläinen, P., & Brännström, H. (2021). The effect of thermal drying on the contents of condensed tannins and stilbenes in Norway spruce (*Picea abies* [L.] Karst.) sawmill bark. *Industrial Crops and Products*, 173, 114090. <https://doi.org/10.1016/j.indcrop.2021.114090>
- Karim, M., Fathi, M., & Soleimani-Zad, S. (2021). Nanoencapsulation of cinnamic aldehyde using zein nanofibers by novel needle-less electrospinning: Production, characterization and their application to reduce nitrite in sausages. *Journal of food Engineering*, 288, 110140. <https://doi.org/10.1016/j.jfoodeng.2020.110140>
- Koutchma, T. & Marcotte, M. (2009). *Agriculture and Agri-Food Canada*.
- Kunii, D., & Levenspiel, O. (1991). *Fluidization engineering* (2nd ed). Butterworth-Heinemann.
- Land, V. C. M. (2011). *Drying in process industry*. Hoboken, New Jersey, US: John Wiley & Sons.
- Lan, Y., Xu, M., Ohm, J.-B., Chen, B. & Rao, J. (2019). Solid dispersion-based spray-drying improves solubility and mitigates beany flavour of pea protein isolate. *Food Chemistry*, 278, 665–673. <https://doi.org/10.1016/j.foodchem.2018.11.074>
- Lang, G. H., Lindemann, I. da S., Ferreira, C. D., Hoffmann, J. F., Vanier, N. L. & de Oliveira, M. (2019). Effects of drying temperature and long-term storage conditions on black rice phenolic compounds. *Food Chemistry*, 287, 197–204. <https://doi.org/10.1016/j.foodchem.2019.02.028>
- Le, K. H., Tsotsas, E., & Kharaghani, A. (2018). Continuum-scale modeling of superheated steam drying of cellular plant porous media. *International Journal of Heat and Mass Transfer*, 124, 1033-1044. <https://doi.org/10.1016/j.ijheatmasstransfer.2018.04.032>
- Leidy, R. & Maria Ximena, Q-C. (2019). Use of electrospinning technique to produce nanofibres for food industries: A perspective from regulations to characterisations. *Trends in Food Science and Technology*, 85, 92–106.

<https://doi.org/10.1016/j.tifs.2019.01.006>

- Li, J., Li, Z., Li, L., Song, C., Raghavan, G., & He, F. (2021). Microwave drying of balsam pear with online aroma detection and control. *Journal of Food Engineering*, 288, 110139. <https://doi.org/10.1016/j.jfoodeng.2020.110139>
- Li, Y., Xu, S. Y. & Sun, D-W. (2007). Preparation of garlic powder with high allicin content by using combined microwave-vacuum and vacuum drying as well as microencapsulation. *Journal of Food Engineering*, 83(1), 76–83. <https://doi.org/10.1016/j.jfoodeng.2007.02.057>
- Liu, H., Gong, J., Chabot, D., Miller, S. S., Cui, S. W., Ma, J., Zhong, F. & Wang, Q. (2015). Protection of heat-sensitive probiotic bacteria during spray-drying by sodium caseinate stabilized fat particles. *Food Hydrocolloids*, 51, 459–467. <https://doi.org/10.1016/j.foodhyd.2015.05.015>
- Liu, Y., Ramakrishna, S., Mohideen, M., & Li, K. (2019). Formation of fibrous structure and influential factors in melt electrospinning: *Melt Electrospinning* (pp. 21–90). <https://doi.org/10.1016/b978-0-12-816220-0.00003-8>
- Loughrill, E., Thompson, S., Owusu-Ware, S., Snowden, M. J., Douroumis, D. & Zand, N. (2019). Controlled release of microencapsulated docosahexaenoic acid (DHA) by spray-drying processing. *Food Chemistry*, 286, 368–375. <https://doi.org/10.1016/j.foodchem.2019.01.121>
- Lv, W., Li, D., Lv, H., Jin, X., Han, Q., Su, D. & Wang, Y. (2019). Recent development of microwave fluidization technology for drying of fresh fruits and vegetables. *Trends in Food Science & Technology*, 86(17), 59–67. <https://doi.org/10.1016/j.tifs.2019.02.047>
- Maalal, O., Prat, M., & Lasseux, D. (2021). Pore network model of drying with Kelvin effect. *Physics of Fluids*, 33(2), 027103. <https://doi.org/10.1063/5.0035651>
- Mahiuddin, M., Khan, M. I. H., Kumar, C., Rahman, M. M. & Karim, M. A. (2018). Shrinkage of Food Materials During Drying: Current Status and Challenges. *Comprehensive Reviews in Food Science and Food Safety*, 17(5), 1113–1126. <https://doi.org/10.1111/1541-4337.12375>
- Masato, O., Kentaro, M., Tatsuro, M., Akio, F., Yukako, H., & Yasuki, M. (2021). Effects of drying temperature on the properties of starch in pasta. *LWT*, 145, 111171. <https://doi.org/10.1016/j.lwt.2021.111171>
- Mawatari, Y., Tatemoto, Y., & Noda, K. (2003). Prediction of minimum fluidization velocity for vibrated fluidized bed. *Powder Technology*, 131(1), 66–70. [https://doi.org/10.1016/S0032-5910\(02\)00323-6](https://doi.org/10.1016/S0032-5910(02)00323-6)
- Mezhericher, M., Levy, A. & Borde, I. (2008). Modelling of particle breakage during drying. *Chemical Engineering and Processing: Process Intensification*, 47(8), 1404–1411. <https://doi.org/10.1016/j.cep.2007.06.018>
- Mohan, A., Rajendran, S. R. C. K., He, Q. S., Bazinet, L. & Udenigwe, C. C. (2015). Encapsulation of food protein hydrolysates and peptides: a review. *RSC Advances*, 5(97), 79270–79278. <https://doi.org/10.1039/C5RA13419F>

- Monteiro, R. L., de Moraes, J. O., Domingos, J. D., Carciofi, B. A. M., & Laurindo, J. B. (2020). Evolution of the physicochemical properties of oil-free sweet potato chips during microwave vacuum drying. *Innovative Food Science & Emerging Technologies*, 63, 102317. <https://doi.org/10.1016/j.ifset.2020.102317>
- Monteiro, R. L., Gomide, A. I., Link, J. V., Carciofi, B. A., & Laurindo, J. B. (2020). Microwave vacuum drying of foods with temperature control by power modulation. *Innovative Food Science & Emerging Technologies*, 65, 102473. <https://doi.org/10.1016/j.ifset.2020.102473>
- Moser, P., Nicoletti, V. R., Drusch, S. & Brückner-Gühmann, M. (2020). Functional properties of chickpea protein-pectin interfacial complex in buriti oil emulsions and spray dried microcapsules. *Food Hydrocolloids*, 107, 105929. <https://doi.org/10.1016/j.foodhyd.2020.105929>
- Mousa, N. & Farid, M. (2002). Microwave vacuum drying of banana slices. *Drying Technology*, 20(10), 2055–2066. <https://doi.org/10.1081/DRT-120015584>
- Mujumdar, A. S. (2006). *Handbook of industrial drying* (3rd ed). CRC press. <https://doi.org/10.1201/9781420017618>
- Mykola, K., Victor, K., Julia, K., & Oleg, S. (2021). Influence of a New Energy-Saving Drying Method on the Quality of Corn Seeds. *American Journal of Agriculture and Forestry*, 9(1), 1-6. <https://doi.org/10.11648/j.ajaf.20210901.11>
- Neikov, O. D., & Yefimov, N. A. (2009). *Handbook of non-ferrous metal powders: technologies and applications* (1st ed). Elsevier.
- Nemzer, B., Vargas, L., Xia, X., Sintara, M. & Feng, H. (2018). Phytochemical and physical properties of blueberries, tart cherries, strawberries, and cranberries as affected by different drying methods. *Food Chemistry*, 262, 242–250. <https://doi.org/10.1016/j.foodchem.2018.04.047>
- Nistor, O.-V., Seremet (Ceclu), L., Andronoiu, D. G., Rudi, L. & Botez, E. (2017). Influence of different drying methods on the physicochemical properties of red beetroot (*Beta vulgaris* L. var. *Cylindra*). *Food Chemistry*, 236, 59–67. <https://doi.org/10.1016/j.foodchem.2017.04.129>
- Obón, J. M., Castellar, M. R., Alacid, M. & Fernández-López, J. A. (2009). Production of a red-purple food colorant from *Opuntia stricta* fruits by spray drying and its application in food model systems. *Journal of Food Engineering*, 90(4), 471–479. <https://doi.org/10.1016/j.jfoodeng.2008.07.013>
- Parikh, D. M. (2015). Vacuum Drying: Basics and application: Chemical Engineering. 122(4), 48-54.
- Pasrija, D., Ezhilarasi, P., Indrani, D., & Anandharamakrishnan, C. (2015). Microencapsulation of green tea polyphenols and its effect on incorporated bread quality. *LWT-Food Science and Technology*, 64(1), 289-296. <https://doi.org/10.1016/j.lwt.2015.05.054>
- Pattanayak, B., Mohapatra, S. S., & Das, H. C. (2019). Mathematical modelling for low temperature batch drying of paddy using fluidised bed technology. *International Journal of Mathematical Modelling and Numerical Optimisation*, 9(1), 89-103.

<https://doi.org/10.1504/IJMMNO.2019.096919>

- Plazzotta, S., Calligaris, S. & Manzocco, L. (2019). Structure of oleogels from κ-carrageenan templates as affected by supercritical-CO₂-drying, freeze-drying and lettuce-filler addition. *Food Hydrocolloids*, 96, 1–10. <https://doi.org/10.1016/j.foodhyd.2019.05.008>
- Premjit, Y., & Mitra, J. (2021). Optimization of Electrospray-Assisted Microencapsulation of Probiotics (*Leuconostoc lactis*) in Soy Protein Isolate-Oil Particles Using Box-Behnken Experimental Design. *Food and Bioprocess Technology*, 14(9), 1712–1729. <https://doi.org/10.1007/s11947-021-02670-7>
- Raghavi, L., Moses, J., & Anandharamakrishnan, C. (2018). Refractance window drying of foods: A review. *Journal of food Engineering*, 222, 267–275. <https://doi.org/10.1016/j.jfoodeng.2017.11.032>
- Rajoriya, D., Bhavya, M., & Hebbar, H. U. (2021). Impact of process parameters on drying behaviour, mass transfer and quality profile of refractance window dried banana puree. *LWT*, 145, 111330. <https://doi.org/10.1016/j.lwt.2021.111330>
- Ratseewo, J., Meeso, N. & Siriamornpun, S. (2020). Changes in amino acids and bioactive compounds of pigmented rice as affected by far-infrared radiation and hot air drying. *Food Chemistry*, 306, 125644. <https://doi.org/10.1016/j.foodchem.2019.125644>
- Ray, S., Raychaudhuri, U. & Chakraborty, R. (2016). An overview of encapsulation of active Loughrill compounds used in food products by drying technology. *Food Bioscience*, 13, 76–83. <https://doi.org/10.1016/j.fbio.2015.12.009>
- Reineccius, G. (2019). Use of proteins for the delivery of flavours and other bioactive compounds. *Food Hydrocolloids*, 86, 62–69. <https://doi.org/10.1016/j.foodhyd.2018.01.039>
- Ren, Z., Yu, X., Yagoub, A. E. A., Fakayode, O. A., Ma, H., Sun, Y., & Zhou, C. (2021). Combinative effect of cutting orientation and drying techniques (hot air, vacuum, freeze and catalytic infrared drying) on the physicochemical properties of ginger (*Zingiber officinale* Roscoe). *LWT*, 144, 111238. <https://doi.org/10.1016/j.lwt.2021.111238>
- Reyes, A., Cerón, S., Zúñiga, R. & Moyano, P. (2007). A comparative study of microwave-assisted air drying of potato slices. *Biosystems Engineering*, 98(3), 310–318. <https://doi.org/10.1016/j.biosystemseng.2007.07.006>
- Romero-González, J., Shun Ah-Hen, K., Lemus-Mondaca, R. & Muñoz-Fariña, O. (2020). Total phenolics, anthocyanin profile and antioxidant activity of maqui, *Aristotelia chilensis* (Mol.) Stuntz, berries extract in freeze-dried polysaccharides microcapsules. *Food Chemistry*, 313, 126115. <https://doi.org/10.1016/j.foodchem.2019.126115>
- Rózyło, R. (2020). Recent trends in methods used to obtain natural food colorants by freeze-drying. *Trends in Food Science & Technology*, 102, 39–50. <https://doi.org/10.1016/j.tifs.2020.06.005>
- Salata, A., Buczkowska, H., & Nurzyńska-Wierdak, R. (2020). Yield, essential oil content,

- and quality performance of *Lavandula angustifolia* leaves, as affected by supplementary irrigation and drying methods. *Agriculture*, 10(12), 590. ; <https://doi.org/10.3390/agriculture10120590>
- Sanchez, V., Baeza, R., Galmarini, M. V., Zamora, M. C., Chirife, J., (2013). Freeze-Drying Encapsulation of Red Wine Polyphenols in an Amorphous Matrix of Maltodextrin. *Food and Bioprocess Technology*, 6(5), 1350-1354. <https://doi.org/10.1007/s11947-011-0654-z>
- Sansone, F., Picerno, P., Mencherini, T., Villecco, F., D'Ursi, A. M., Aquino, R. P. & Lauro, M. R. (2011). Flavonoid microparticles by spray-drying: Influence of enhancers of the dissolution rate on properties and stability. *Journal of Food Engineering*, 103(2), 188–196. <https://doi.org/10.1016/j.jfoodeng.2010.10.015>
- Sarabandi, K. & Jafari, S. M. (2020). Effect of chitosan coating on the properties of nanoliposomes loaded with flaxseed-peptide fractions: Stability during spray-drying. *Food Chemistry*, 310, 125951. <https://doi.org/10.1016/j.foodchem.2019.125951>
- Senadeera, W., Adiletta, G., Önal, B., Di Matteo, M., & Russo, P. (2020). Influence of different hot air drying temperatures on drying kinetics, shrinkage, and colour of persimmon slices. *Foods*, 9(1), 101. <https://doi.org/10.3390/foods9010101>
- Shewale, S. R., Rajoriya, D., Bhavya, M., & Hebbar, H. U. (2021). Application of radiofrequency heating and low humidity air for sequential drying of apple slices: Process intensification and quality improvement. *LWT*, 135, 109904. <https://doi.org/10.1016/j.lwt.2020.109904>
- Shukla, S. (2011). Freeze Drying Process: a Review. *International Journal of Pharmaceutical Sciences and Research*, 2(12), 3061–3068. [https://doi.org/http://dx.doi.org/10.13040/IJPSR.0975-8232.2\(12\).3061-68](https://doi.org/http://dx.doi.org/10.13040/IJPSR.0975-8232.2(12).3061-68)
- Sinnott, R. (2014). *Chemical engineering design* (2rd ed). Elsevier.
- Sivakumar, R., Saravanan, R., Elaya Perumal, A. & Iniyan, S. (2016). Fluidized bed drying of some agro products – A review. *Renewable and Sustainable Energy Reviews*, 61, 280–301. <https://doi.org/10.1016/j.rser.2016.04.014>
- Smaniotto, F., Prosapio, V., Zafeiri, I. & Spyropoulos, F. (2020). Freeze drying and rehydration of alginate fluid gels. *Food Hydrocolloids*, 99, 105352. <https://doi.org/10.1016/j.foodhyd.2019.105352>
- Soponronnarit, S., Swasdisevi, T., Wetchacama, S. & Wutiwiwatchai, W. (2001). Fluidised bed drying of soybeans. *Journal of Stored Products Research*, 37(2), 133–151. [https://doi.org/10.1016/S0022-474X\(00\)00015-1](https://doi.org/10.1016/S0022-474X(00)00015-1)
- Sozzi, A., Zambon, M., Mazza, G., & Salvatori, D. (2021). Fluidized bed drying of blackberry wastes: Drying kinetics, particle characterization and nutritional value of the obtained granular solids. *Powder Technology*, 385, 37-49. <https://doi.org/10.1016/j.powtec.2021.02.058>
- Stanisławski, J. (2005). Drying of Diced Carrot in a Combined Microwave–Fluidized Bed Dryer. *Drying Technology*, 23(8), 1711–1721. <https://doi.org/10.1081/DRT-200065129>

- Sun, X., Cameron, R. G. & Bai, J. (2020). Effect of spray-drying temperature on physicochemical, antioxidant and antimicrobial properties of pectin/sodium alginate microencapsulated carvacrol. *Food Hydrocolloids*, 100, 105420. <https://doi.org/10.1016/j.foodhyd.2019.105420>
- Tan, S., Zhong, C. & Langrish, T. (2020). Encapsulation of caffeine in spray-dried micro-eggs for controlled release: The effect of spray-drying (cooking) temperature. *Food Hydrocolloids*, 108, 105979. <https://doi.org/10.1016/j.foodhyd.2020.105979>
- Tang, X. C., Nail, S. L., & Pikal, M. J. (2005). Freeze-drying process design by manometric temperature measurement: design of a smart freeze-dryer. *Pharmaceutical research*, 22(4), 685-700. <https://doi.org/10.1007/s11095-005-2501-2>
- Taşeri, L., Aktaş, M., Şevik, S., Gülcü, M., Uysal Seçkin, G. & Aktekeli, B. (2018). Determination of drying kinetics and quality parameters of grape pomace dried with a heat pump dryer. *Food Chemistry*, 260, 152–159. <https://doi.org/10.1016/j.foodchem.2018.03.122>
- Tepe, F. B., Tepe, T. K., & Ekinci, A. (2021). Impact of air temperature on drying characteristics and some bioactive properties of kiwi fruit slices. *Chemical Industry and Chemical Engineering Quarterly*, 15(1), 1-56. <https://doi.org/10.2298/CICEQ210126026T>
- Thery, T., Lynch, K. M. & Arendt, E. K. (2019). Natural Antifungal Peptides/Proteins as Model for Novel Food Preservatives. *Comprehensive Reviews in Food Science and Food Safety*, 18(5), 1327–1360. <https://doi.org/10.1111/1541-4337.12480>
- Timm, N. d. S., Lang, G. H., Ferreira, C. D., Pohndorf, R. S., & de Oliveira, M. (2020). Infrared radiation drying of parboiled rice: Influence of temperature and grain bed depth in quality aspects. *Journal of Food Process Engineering*, 43(4), e13375. <https://doi.org/10.1111/jfpe.13375>
- Tontul, I., Ergin, F., Eroğlu, E., Küçükçetin, A., & Topuz, A. (2021). The impact of refractance window drying conditions on the physical and microbiological properties of kefir powder. *Food Bioscience*, 43, 101317. <https://doi.org/10.1016/j.fbio.2021.101317>
- Tuncak, C., & Doymaz, İ. (2020). Performance analysis and mathematical modelling of banana slices in a heat pump drying system. *Renewable Energy*, 150, 918-923. <https://doi.org/10.1016/j.renene.2020.01.040>
- Turkiewicz, I. P., Wojdyło, A., Tkacz, K., Lech, K., Michalska-Ciechanowska, A. & Nowicka, P. (2020). The influence of different carrier agents and drying techniques on physical and chemical characterization of Japanese quince (*Chaenomeles japonica*) microencapsulation powder. *Food Chemistry*, 323(April), 126830. <https://doi.org/10.1016/j.foodchem.2020.126830>
- Uzkuç, N. M. Ç., Uzkuç, H., Berber, M. M., Kuzu, K. T., Toğay, S. Ö., Hoşoğlu, M. İ., Toklucu, A. K., Kurt, S. B., Sahiner, N., Yüceer, Y. K. (2021). Stabilisation of lavender essential oil extracted by microwave-assisted hydrodistillation: Characteristics of starch and soy protein-based microemulsions. *Industrial Crops and Products*, 172, 114034. <https://doi.org/10.1016/j.indcrop.2021.114034>

- Van Khanh, P., & Hang, T. T. T. Evaluation of Heat and Mass Transfer Efficiency of Milk Spray Drying by Computational Simulation Tool. *Journal of Mechanical Engineering Research and Developments*, 44(2), 251-259.
- Vega-Gálvez, A., Poblete, J., Rojas-Carmona, R., Uribe, E., Pastén, A., & Goñi, M. G. (2021). Vacuum drying of Chilean papaya (*Vasconcellea pubescens*) fruit pulp: effect of drying temperature on kinetics and quality parameters. *Journal of food science and technology*, 58, 3482-3492. <https://doi.org/10.1007/s13197-021-05005-8>
- Vega-Gálvez, A., Uribe, E., Perez, M., Tabilo-Munizaga, G., Vergara, J., Garcia-Segovia, P., Lara, E. & Di Scala, K. (2011). Effect of high hydrostatic pressure pretreatment on drying kinetics, antioxidant activity, firmness and microstructure of Aloe vera (*Aloe barbadensis* Miller) gel. *LWT - Food Science and Technology*, 44(2), 384–391. <https://doi.org/10.1016/j.lwt.2010.08.004>
- VijayaVenkataRaman, S., Iniyar, S. & Goic, R. (2012). A review of solar drying technologies. *Renewable and Sustainable Energy Reviews*, 16(5), 2652–2670. <https://doi.org/10.1016/j.rser.2012.01.007>
- Wang, H. C., Zhang, M. & Adhikari, B. (2015). Drying of shiitake mushroom by combining freeze-drying and mid-infrared radiation. *Food and Bioproducts Processing*, 94, 507–517. <https://doi.org/10.1016/j.fbp.2014.07.008>
- Wang, Y., Lu, Z., Lv, F. & Bie, X. (2009). Study on microencapsulation of curcumin pigments by spray drying. *European Food Research and Technology*, 229(3), 391–396. <https://doi.org/10.1007/s00217-009-1064-6>
- Wei, S., Wang, Z., Wang, F., Xie, W., Chen, P., & Yang, D. (2019). Simulation and experimental studies of heat and mass transfer in corn kernel during hot air drying. *Food and Bioproducts Processing*, 117, 360-372. <https://doi.org/10.1016/j.fbp.2019.08.006>
- Wei, W., Mo, C., & Guohua, C. (2012). Issues in freeze drying of aqueous solutions. *Chinese Journal of Chemical Engineering*, 20(3), 551-559. [https://doi.org/10.1016/S1004-9541\(11\)60218-8](https://doi.org/10.1016/S1004-9541(11)60218-8)
- Wilhelm, O., Mädler, L., & Pratsinis, S. E. (2003). Electrospray evaporation and deposition. *Journal of Aerosol Science*, 34(7), 815-836. [https://doi.org/10.1016/S0021-8502\(03\)00034-X](https://doi.org/10.1016/S0021-8502(03)00034-X)
- Wu, X.-F., Salkovskiy, Y., & Dzenis, Y. A. (2011). Modeling of solvent evaporation from polymer jets in electrospinning. *Applied Physics Letters*, 98(22), 223108. <https://doi.org/10.1063/1.3585148>
- Xu, X., Zhang, L., Feng, Y., ElGasim A Yagoub, A., Sun, Y., Ma, H. & Zhou, C. (2020). Vacuum pulsation drying of okra (*Abelmoschus esculentus* L. Moench): Better retention of the quality characteristics by flat sweep frequency and pulsed ultrasound pretreatment. *Food Chemistry*, 326, 127026. <https://doi.org/10.1016/j.foodchem.2020.127026>
- Xu, Y., Xiao, Y., Lagnika, C., Li, D., Liu, C., Jiang, N., Song, J. & Zhang, M. (2020). A comparative evaluation of nutritional properties, antioxidant capacity and physical characteristics of cabbage (*Brassica oleracea* var. Capitata var L.) subjected to

- different drying methods. *Food Chemistry*, 309, 124935. <https://doi.org/10.1016/j.foodchem.2019.06.002>
- Yamamoto, K. (2017). Food processing by high hydrostatic pressure. *Bioscience, Biotechnology and Biochemistry*, 81(4), 672–679. <https://doi.org/10.1080/09168451.2017.1281723>
- Yinbin, L., Wu, L., Weng, M., Tang, B., Lai, P. & Chen, J. (2018). Effect of different encapsulating agent combinations on physicochemical properties and stability of microcapsules loaded with phenolics of plum (*Prunus salicina* lindl.). *Powder Technology*, 340, 459–464. <https://doi.org/10.1016/j.powtec.2018.09.049>
- Yuksel, H., & Dirim, S. N. (2020). Application of the agglomeration process on spinach juice powders obtained using spray drying method. *Drying Technology*, 39(1), 19–34. <https://doi.org/10.1080/07373937.2020.1832515>
- Zahoor, I., & Khan, M. A. (2021). Microwave assisted fluidized bed drying of red bell pepper: Drying kinetics and optimization of process conditions using statistical models and response surface methodology. *Scientia Horticulturae*, 286, 110209. <https://doi.org/10.1016/j.scienta.2021.110209>
- Zanoni, F., Primiterra, M., Angeli, N. & Zoccatelli, G. (2020). Microencapsulation by spray-drying of polyphenols extracted from red chicory and red cabbage: Effects on stability and color properties. *Food Chemistry*, 307, 125535. <https://doi.org/10.1016/j.foodchem.2019.125535>
- Zeng, Y., Liu, Y., Zhang, J., Xi, H., & Duan, X. (2019). Effects of far-infrared radiation temperature on drying characteristics, water status, microstructure and quality of kiwifruit slices. *Journal of Food Measurement and Characterization*, 13(4), 3086–3096. <https://doi.org/10.1007/s11694-019-00231-3>
- Zhang, L., Liao, L., Qiao, Y., Wang, C., Shi, D., An, K. & Hu, J. (2020)a. Effects of ultrahigh pressure and ultrasound pretreatments on properties of strawberry chips prepared by vacuum-freeze drying. *Food Chemistry*, 303, 125386. <https://doi.org/10.1016/j.foodchem.2019.125386>
- Zhang, L., Qiao, Y., Wang, C., Liao, L., Shi, D., An, K., Hu, J., Wang, J. & Shi, L. (2020)b. Influence of high hydrostatic pressure pretreatment on properties of vacuum-freeze dried strawberry slices. *Food Chemistry*, 331, 127203. <https://doi.org/10.1016/j.foodchem.2020.127203>
- Zhang, L., Qiu, J., Cao, X., Zeng, X., Tang, X., Sun, Y. & Lin, L. (2019). Drying methods, carrier materials, and length of storage affect the quality of xylooligosaccharides. *Food Hydrocolloids*, 94, 439–450. <https://doi.org/10.1016/j.foodhyd.2019.03.043>
- Zhang, M., Bhandari, B., Fang, Z. (2017). *Handbook of Drying of Vegetables and Vegetable Products*, Boca Raton, US: Taylor & Francis Group.
- Zolkepli, N. K., Abu Bakar, N. F., Naim, M. N., Anuar, N. & Abu Bakar, M. R. (2014). Nanoparticle preparation of Mefenamic acid by electrospray drying, 1586, 113–118. <https://doi.org/10.1063/1.48>

PART II | EXPERIMENTAL SECTION

Chapter 3

Antimicrobial Multi-Component Lipid-Based Nanoemulsion of *Eucalyptus globulus* and *Mentha piperita* as Natural Preservative

Antimicrobial Multi-Component lipid-based Nanoemulsion of *Eucalyptus globulus* and *Mentha piperita* as Natural Preservative

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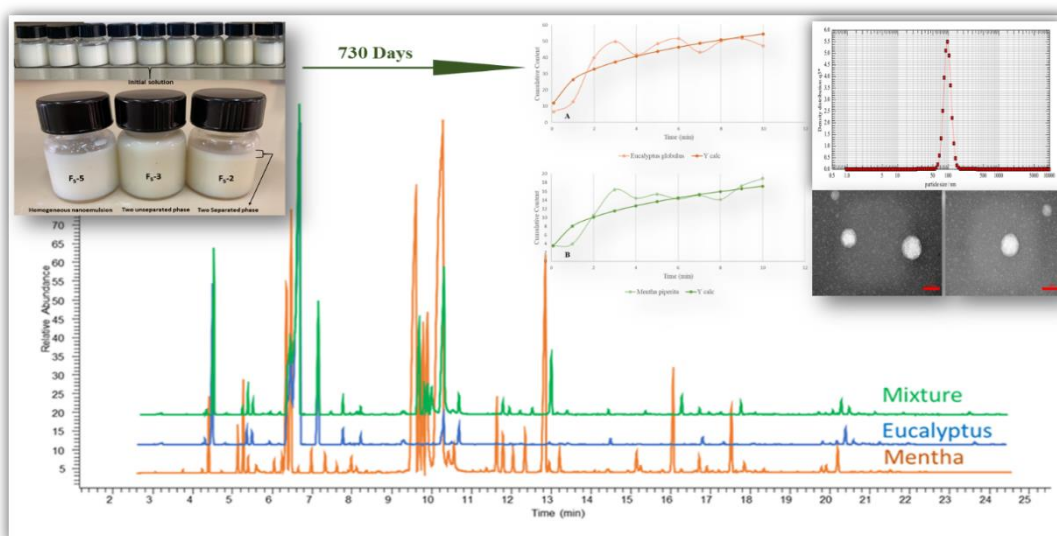
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3.1. Abstract

Natural preservatives were predominantly developed to eliminate the use of synthetic preservatives for food and agriculture industries. Multi-component nanoemulsions (MCN) with significant antibacterial activities should be proper candidates as a natural preservative. This research aimed to formulate a multi-component phospholipid-based nanoemulsion (PNE) with prolonged shelf-life loaded by a mixture of *Eucalyptus globulus* and *Mentha piperita* essential oils (EOs). The effects of different chemical and mechanical conditions (ultrasonication) on the stability of MCN were evaluated. The emulsions were monitored from day 1 to 730, and stable formulae were investigated for around two years. The Zeta potential confirmed the anionic surface charge. Dynamic light scattering and transmission light scattering confirmed the droplet size range between 93.40-100.00 nm. The homogeneous formula was stable for more than 730 days. Ultrasonication treatment and 1% v/v of food-grade castor oil as co-emulsifiers were critical in fabricating stable formulae. Minimum inhibitory concentration (MIC) values of 31, 8, and 63 µg/ml were observed against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella enteritidis*, respectively. The results showed a significant reduction in MIC values of 32-, 250-, 15-times against *E. coli*, *S. aureus* and *S. enteritidis*, respectively, compared to an original mixture of essential oils. The mass transfer followed a case-I transfer (Fick law diffusion) evaluated by Koresmeyer-Peppas kinetic model ($R^2= 0.88$). In conclusion, MCN could be a promising candidate for natural preservatives functionalizing in the food, agriculture and cosmetic industries.

Keywords: Encapsulation, Nanoemulsion, Lecithin, Ultrasonication, Shelf life.

3.2. Graphical Abstract



3.3. Main text

3.3.1. Introduction

Natural-based preservatives were predominantly developed to eliminate the use of synthetic preservatives, associated with potential adverse side effects. Natural food and agricultural preservatives are being extensively formulated for their potential antimicrobial properties against food^[1, 2] and plant pathogens (green pesticides) with particular attention to seeds and cereals^[3], for industrial applications, and for their vital role in human health and safety. To address these issues, natural-based preservatives were developed, with minimum toxicity and side effects, and easy to use. They were formulated to preserve crops in agriculture, and food products, from bacterial contamination/degradation, a significant worldwide concern.

Nanoemulsions (NEs), also known as mini-emulsions, are produced by a combination of oil, water, emulsifier and co-emulsifier, with a particle size range ≤ 100 nm. They have vast applications in the pharmaceutical, cosmetic and food industries.^[4] NEs are thermodynamic and kinetically stable, non-toxic, non-irritant, easy to use and provide a sustained and controlled release due to increased particles surface area on a nano-size scale.^[5] These nanoemulsions have potentially a superior physical stability (shelf life), a performance which attracts significant interest for food application.^[6,7]

Meanwhile, ultrasonic emulsification, an energy-efficient method, produces NEs by cavitation within the solution medium, resulting in a small droplet size. The occurrence of cavitation during sonication originates a nano-sized droplet providing more stability, wide surface area and prolonged shelf life. This technique has been frequently used for the fabrication of green-based nanoemulsions with low polydispersity.^[4,8]

Indeed, the use of antimicrobial nanoemulsions to protect the physicochemical properties of foods without affecting their phytoconstituents value has been previously proven.^[6] *Eucalyptus globulus* and *Mentha piperita* essential oils (EOs) were selected as the antibacterial agents with a significant enhancement on repulsive activities.^[9] *E. globulus* EO is a great source of 1,8-cineole (around 45-65%) and is commonly used in pharmaceutical and food products as an antimicrobial agent.^[10,11] Also, antibacterial properties of *Mentha piperita* were proved in nanoemulsion form, previously.^[12] Therefore, a combination of *E. globulus* and *M. piperita* loaded on a phospholipid-based multi-component nanoemulsion (MCN) could be a new approach to obtain natural food and agriculture preservatives not only of interest as an ingredient but also as a preparation technique. It must take into consideration that the ensuing product would be

biologically and environmentally safe and healthy with minimum side effects and maximum antibacterial properties.

Thus, the primary purpose of this work was to optimize and investigate the impact of different parameters (ultrasonication duration, emulsifiers content and castor oil content) in a decrease in droplet size, and improve a shelf-life quality improvement and antibacterial properties of phospholipid-based MCNs. Therefore, producing a natural preservative for food and agriculture industries by optimizing the combination of co-emulsifier and mechanical emulsification condition to improve a shelf-life quality in the MCN system were reported for the first time in this study.

3.3.2. Material and Methods

3.3.2.1. Materials and Equipments

Tween 80 was purchased from Biochem (Chemopharma, France). Lecithin was supplied by Acros, Thermo Fisher Scientific company (Geel, ANTWERP Belgium). *E. globulus* and *M. piperita* EOs were kindly supplied by the Vasha Darou Pars Herbal Pharmaceutical Co. (Qom, Iran). Pharmaceutical/food-grade castor oil was purchased from the Oilseed company Karaj (Tehran, Iran). Distilled water was used to prepare all aqueous solutions and obtained from a Millipore water purification system (Merck Millipore). A Mettler Toledo AG245 analytical balance (Columbus, OH, USA) was used for technical weight measurement. A 20 kHz with 1200 w probe ultrasonic model (UH-1200) from Behin Tamin Ahura (Esfahan, Iran) was endowed with a 20 mm diameter probe was used for MCNs preparation. A Dynamic Light Scattering (DLS) equipment (Nanophox Sympatec GmbH, Claushtal, Germany) was used for analyzing particle size.

3.3.2.2. GC-FID and GC-MS analysis

The EOs chemical constituents were evaluated by gas chromatography using a Thermoquest-Finnigan gas chromatography apparatus equipped with a flame ionization detector (FID) and a Rtx-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The carrier gas was set to a constant flow rate of 1.1 mL/min. The oven temperature was set up at 60°C for 1 min, then extended to 250°C at a rate of 4°C /min, and then held for 10 min at 4°C /min. The injector and detector temperatures (FID) were adjusted to 250°C and 280°C, respectively.

Gas chromatography-mass spectrometry (GC-MS) analysis was performed in a Thermoquest-Finnigan Trace GC-MS (ThermoQuest, Manchester, UK) instrument

equipped with an Rtx-5 fused silica column (30 m × 0.25 mm i.d., film thickness 0.25 µm). The oven was programmed to increase from 60°C to 250°C at a rate of 4°C/min, and then kept at 250°C for 10 min; transfer line temperature was 250°C. A quadrupole mass spectrometer scanned the fracture over the 45-465 amu, using an ionizing voltage of 70 eV and an ionization current of 150 µA.

3.3.2.3. Identification of EO components

The retention times on the DB-5 column, obtained under temperature-programmed conditions, allowed the identification of the n-alkanes (C6 - C24) of the *E. globulus* and *M. piperita* oils. The chemical composition of the EOs was recognized by comparison of the reference mass spectra library (Wiley 7.0) with the obtained mass spectra. Quantitative assessments were performed using the FID area percentages.

3.3.2.4. Nanoemulsion preparation

NEs were prepared by ultrasonic homogenization based on a top-down procedure.^[13] The HLB value (Equations 1, 2) was calculated for all the formulae under studies (Table 1).

$$(1) \text{ Percentage of emulsifier } A = \frac{100 (\text{Desired HLB Value} - \text{Lowest HLB})}{(\text{Highest HLB} - \text{Lowest HLB})}$$

$$(2) \text{ Percentage of emulsifier } B = 100 - \text{Percentage of emulsifier } A$$

E. globulus, and *M. piperita*, castor oil, and tween 80 were used as the oil phase. Table 1 shows the precise amount of each ingredient used in the fabrication of individual formulations with considering the stable milky dispersion range, which was set to 8-10.^[13] Formulae with HLB values of 8 were discarded due to instability during sampling and preparation. **Table 1**, clarifies the leading formulation and their subgroups, with particular attention to the variety between similar units, as well as chemical and mechanical modification applied to each formula. To be precise, lecithin and tween 80 functions as emulsifiers and castor oil function as co-emulsifier were classified and calculated according to the hydrophilic-lipophilic balance (HLB) Equations 1 and 2, discussed in Table 1.

The preparation procedure was operated as follows: a pharmaceutical/food-grade castor oil and tween 80 were mixed in a 10 ml glass flask and stirred at 800-1000 rpm for 10 min at 40°C until a homogeneous oil phase was formed (M.A). The mixture of M.A was cool down to 40°C. Lecithin was added to M.A at 40°C, stirred at 800-1000 rpm for

20-30 min, and then stirred again for more than 30 min at 30°C to achieve a uniform bright lecithin-based yellow oil phase without any spots, droplets or bubbles. *E. globulus* and *M. piperita* were prepared beforehand (M.B) with a specific ratio mentioned in Table1. The M.B was added at 30°C to M.A and stirred for 20 min. Distilled water was added to the oil phase at 30°C, and stirred for 20 min. The emulsion was finally dispersed using an ultrasonic homogenization (60% amplitude) for 20 min.^[14] The amounts of each ingredient are presented in Table 1. Eleven different formulations were selected from a series of preliminary experiments, those were used for further physicochemical studies.

Table 1. Amount of each ingredient used in the production of NE.

Formulation Series			Essential oil (%)		HLB value	Emulsifier (%)		Co-emulsifier	Water (%)	Ultrasound condition		
			Total	<i>E. globulus</i>		<i>M. piperita</i>	Total	Tween 80			Lecithin	Castor oil (%)
1	F ₁	F ₁ -0	25	17.51	7.50	9	10	8.572	1.428	0	68	20 min
2	F ₂	F ₂ -0		17.51	7.50			8.572	1.428	1	67	3x 20 min
3	F ₃	F ₃ -0		17.51	7.50	10	10	7.143	2.857	0	68	20 min
4	F ₄	F ₄ -0		17.51	7.50			7.143	2.857	1	67	3x 20 min
5		F ₅ -1		17.51	7.50	9	5	4.286	0.714	0.01	73	20 min
6		F ₅ -2		17.51	7.50			4.286	0.712	0.5	72	20 min
7	F ₅	F ₅ -3		17.51	7.50			4.286	0.712	0.75	72	3x 20 min
8		F ₅ -4		17.51	7.50			4.286	0.714	1	72	20 min
9		F ₅ -5		17.51	7.50			4.286	0.712	1	72	3x 20 min
10	F ₆	F ₆ -0		17.51	7.50			0.712	4.286	1	72	3x 20 min
11	F ₇	F ₇ -0	10	7.00	3.00	9	1.5	0.857	0.712	1	84	3x 20 min

* HLB: in case of milky dispersion, hydrophilic-lipophilic balance ranges between 8 and 10. Designed formulations with an HLB value of 8 were not stable and discarded in the initial step.

3.3.2.5. Particle size distribution and surface charge

The Particle size distribution of NEs were evaluated with Dynamic Light Scattering (DLS) equipment (Nanophox Sympatec GmbH, Claushtal, Germany). Before measurement, a small volume of the NE was dispersed in deionized water to ensure a specific particle distribution (200-1000 Kcps). The particle size was measured in triplicate and each analysis took about 90-100 seconds. The surface shape morphology was just estimated using the Sauter mean diameter (SMD) divided by volume median diameter (VMD) values. The Zeta potential (surface charge) was evaluated by NANO-flex II (surface charge reader), colloid metrix GmbH, Meerbusch, Germany for the stable sample at 780 nm.

3.3.2.6. Nanoemulsion stability

The stability test was performed at room temperature by keeping all the samples on the laboratory shelf. After the first month, stability was assessed by subjecting samples to 0, 20, 30, 40, 50°C, under ambient humidity and centrifuged for 30 min at 3500 rpm. The stable samples (with no apparent phase separation) were kept for a longer monitorization period till 730 days (2 years) by monitoring the particle size distribution. Moreover, the thermodynamic stability of the stable formula was evaluated by centrifugation at 5000 rpm for 15 min, following through cycling of heating and cooling (at 40°C to 25°C and -20°C to 25°C) under ambient humidity to set the stability criteria.^[15]

3.3.2.7. Transmission Electron Microscopy (TEM)

The evaluation of the sphericity and morphology of the stable formulation was performed by transmission electron microscopy (TEM). A 20 µL sample was spotted on a carbon film coated on a 300 mesh copper grid (Agar) for 2 min. The residue was absorbed with filter paper. Samples were negatively stained with a 20 µL drop of 2% w/v uranyl acetate for 1-2 min and the grid was dried with air. A Zeiss EM900 transmission electron microscope, operating at an accelerating voltage of 80 kV, was used for grid analysis.

3.3.2.8. Antibacterial activity

The most stable formula (F₅-5) was selected to evaluate antibacterial activity. Determination of the minimum inhibitory concentration (MIC) by broth micro-dilution method was performed according to the methodology of Ghaderi *et al.*^[16] The bioassays

were carried out against three different bacterial pathogens *Escherichia coli* ATCC 1390, *Staphylococcus aureus* ATCC 1431, and *Salmonella enteritidis* PTCC1609, which were obtained from the Academic Center for Education, Culture, and Research (ACECR, Tehran, Iran). *E. globulus* and *M. piperita* Eos separately, a mixture of both EOs and formula F₅₋₅ were highly diluted in a 96 well plate containing Mueller Hinton Broth (MHB) medium. Bacterial cells were added to each case up to a concentration of about 5×10^6 CFU/ml⁻¹. All plates were incubated at 37°C for 20 hrs. The MIC values were determined considering the lowest concentrations of sample able to inhibit the visible growth of bacteria. All experiments were carried out in triplicate.

3.3.2.9. Control release assay

The release profile of the stable MCN (F₅₋₅) was performed with 200µl of F₅₋₅ and 10 ml of hexane as solvent. The release monitoring was recorded for eleven minutes and six intervals (recorded every one minutes) and analyzed by Thermoquest-Finnigan Trace GC-MS (ThermoQuest, Manchester, UK) instrument with the same protocol explained in Section 2.2.. The release profiles were determined by evaluating the amount of menthol and 1,8-cineole over a specific time, at room temperature and under agitation in hexane.

3.3.2.10. Kinetic release model

The release model was evaluated by Korsmeyer-Peppas kinetic to investigate the type of mechanism associated, assessing the parameter n. (Equation 3).

$$(3) \quad F_t/F_{\infty} = F_{KP} t^n$$

In which; " F_t/F_{∞} " justified the amount of active compound released until time "t", " F_{KP} " is the Korsmeyer-Peppas constant, "t" is a value of time, and "n" is the released exponent (estimating a different release mechanism). Hence, "n" evaluated the release mechanism I, by specific data range; if " $n < 0.43$ ", case-I mass transfer occurs (diffusion following Fickian law). If " $n > 0.43$ ", mass transfer follows non-Fickian models. Accordingly, if " $0.43 < n < 0.85$ ", anomalous mass transfer (combination of Fickian diffusion and swelling release (case II)) is operated If "n" is equals to 0.85, Case-II mass transfer (zero-order kinetic controlled swelling and relaxation of polymer matrix) occurs. And finally, if $n > 0.85$, super case-II mass transfer takes place.^[17, 18]

3.3.3. Result and Discussion

3.3.3.1. Chemical composition of EOs

The chemical composition of the essential oils extracted from *E. globulus* and *M. piperita* as well as mixture of (70:30) was analyzed by GC-FID and GC-MS (Figure 1). The major chemical components of eucalyptus oil were: 1,8-cineole (56.6%), p-cymene (15.4%), α -pinene (11.7%), γ -terpinene (9.3%), *E*-citral (2.5%), α -terpineol (1.1%), and globulol (1.1%). Meanwhile, the major chemical components of peppermint contained menthol (34.6%), menthone (19.2%), menthyl acetate (8.9%), 1,8-cineole (7.7%), p-cymene (5.7%), neo-menthol (5.2%), iso-menthone (3.6%), menthofuran (3.1%), and β -caryophyllene (2.8%). Twenty-six major compounds were found in the mixture of EOs (*E. globulus*:*M. piperita*) following the ratio of 70:30, Table 2. The main compounds were observed 1,8-cineole (40.5%), menthol (13.2%), p-cymene (9.5%), α -pinene (8.1%), menthone (6.8%), and γ -terpinene (6.3%) (Table 2, Figure 1). Our findings differed slightly from those described by Emamjomeh *et al.*^[19] in term of chemical compounds with particular attention to 1,8-cineole (59.08%). Moreover, the current data is in acceptance with report of Taherpour *et al.*^[20] in term of *M. piperita* with slight differences and special attention to menthol (29.38%). The slight difference between the literature and the current experimental might due to background genetic, region, and condition of a plant, which significantly affects the type and percentage of phytoconstituents.^[21]

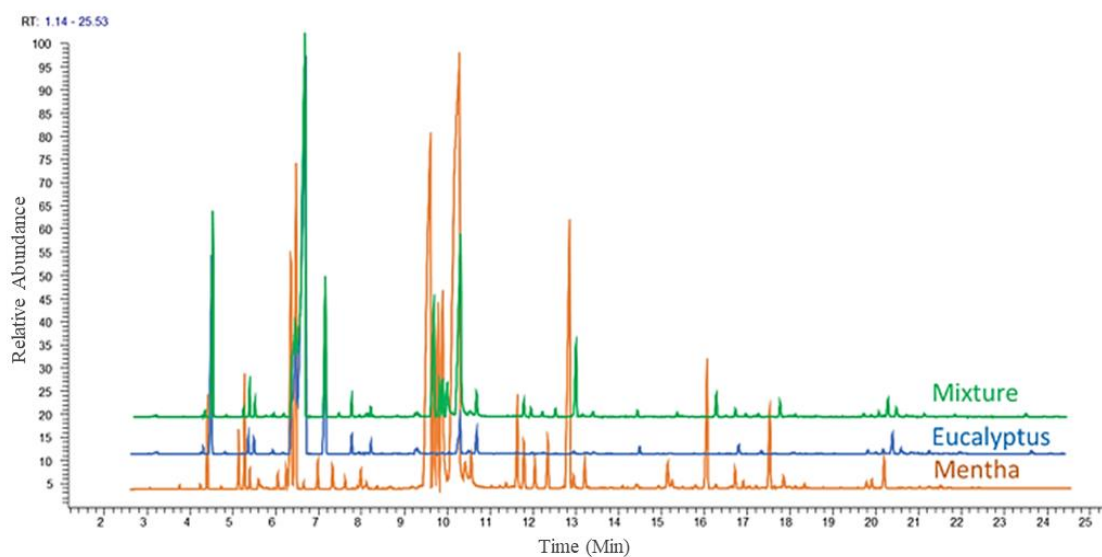


Figure 1. Total ion current (TIC) GC-MS chromatograms from essential oils of *E. globulus*, *M. piperita* and their mixture (70:30).

Table 2. Chemical composition of *M. piperita* and *E. globulus* essential oils.

No.	Compounds	RI	<i>E. globulus</i> (%)	<i>M. piperita</i> (%)	Mixture* (% ^a)
1	α -pinene	932	11.7	1.1	8.1
2	sabinene	970	0.7	1.6	1.0
3	myrcene	989	0.6	0.6	0.5
4	<i>p</i> -cymene	1021	15.4	5.7	9.5
5	1,8-cineole	1027	56.6	7.7	40.5
6	γ -terpinene	1056	9.3	-	6.3
7	α -terpinene	1012	0.8	-	0.5
8	linalool	1088	0.4	-	0.3
9	menthone	1146	-	19.2	6.8
10	menthofuran	1157	-	3.1	1.1
11	iso-menthone	1160	-	3.6	1.3
12	neo-menthole	1161	-	5.2	1.6
13	Exo-isocitral	1140	2.5	-	0.8
14	menthol	1167	-	34.6	13.2
15	α -terpineol	1186	1.1	-	0.3
16	neo-isomenthole	1185	-	0.4	0.8
17	pulegone	1235	-	1.7	0.5
18	<i>p</i> -mentha-6.8-dien-2-one	1244	-	0.8	0.3
19	menthyl acetate	1296	-	0.8	0.3
20	iso-menthyl acetate	1307	-	8.9	3.2
21	α -terpinyl acetate	1345	-	-	0.3
22	β -caryophyllene	1427	-	2.8	0.9
23	germacrene D	1490	-	1.7	0.5
24	globulol	1590	1.1	-	0.7
25	veriflorol	1596	-	0.5	0.3

*Mixture of *E. globulus* and *M. piperita* essential oils (70:30). ^a Quantification of compounds was done by running GC-FID and calculation of the relative area percentages analysis without the use of correction factors.

3.3.3.2. Particle size distribution and Surface charges

The particle size distribution of the multi-component nanoemulsions (MCNs) were monitored for 730 days (two years) and reported in Table 3. Simultaneously, the average particle size was measured at the end of days 15, 30, 90, 180, 360, 540, 730 for formulae with a homogeneous milky texture still visible. The particle size was varied between 19.88-1034.15 nm for all formulae under studies. Formulae with an HLB value of 9 had a smaller particle than the formula with an HLB value of 10. Formula F₅-2 exhibited the biggest particle size, which was deformed after 180 days. In contrast, formula F₅-5 showed a normal particle size from day zero to 730 days with SMD/VMD (a morphology criterion) of almost one, which explains the tendency to present a spherical form for 730 days Table 3.

The particle size was decreased by increasing the castor oil content and ultrasonication condition in all samples under study, especially to series F₅. The castor

oil content and ultrasonication were improved stepwise from formula F₅-1 to F₅-3, resulting in optimizing the particle size in formula F₅-3 (174.68 nm). In contrast, formula F₅-4 was deformed due to decreasing ultrasonication conditions up to just one cycle of 20 min at preparation steps. Formula F₅-5 with 1% castor oil, 3×20 min achieved an optimized particle size of 93.40 nm and spherical shape regarding a morphology criterion (SMD/VMD: 1.002). The result reported by Pratap-Singh *et al.*^[22] proved the size of particles decreased by increasing the duration of the ultrasonication process which is confirmed our experimental result.

In another point of view, formulae F₅-3 and F₅-5 were ultrasonicated three times, 20 min each with the highest castor oil content as a co-emulsifier comparatively to other formula under study, exhibited an optimized nano-size particle size for at least 540 days (Table 3, Figure 2, 3). The result is in accordance with reported data by Eko *et al.*^[23] in term of particle size, 135.0-181.1nm. They reported the use of castor oil in the oil phase produces a homogeneous nanoemulsion.

The anionic surface charges was reported on anionic charge in range of -19.13 ± 0.2 For formula F₅-5. Our resut is in accordance with result reported from Fathi *et al.* regarding athe nionic charge of nano particles. The following factor may dur to existence of lecithin as an emulsifier which exhibited the anionic charge to surface of particle.^[24]

Table 3. Stability test based on particle size (nm) and morphology criterion of a particle over 730 days.

Formulation Series			Days and Particle size (nm)														Appearance
			15 th	SMD/VMD	30 th	SMD/VMD	90 th	SMD/VMD	180 th	SMD/VMD	360 th	SMD/VMD	540 th	SMD/VMD	730 th	SMD/VMD	
1	F ₁	F ₁ -0	UN	-	-	-	-	-	-	-	-	-	-	-	-	-	TSP
2	F ₂	F ₂ -0	-	-	UN	-	-	-	-	-	-	-	-	-	-	-	TSP
3	F ₃	F ₃ -0	UN	-	-	-	-	-	-	-	-	-	-	-	-	-	TSP
4	F ₄	F ₄ -0	307.04	1.57	UN	-	-	-	-	-	-	-	-	-	-	-	TSP
5		F ₅ -1	19.88	1.21	26.83	1.60	159.02	1.60	UN	-	-	-	-	-	-	-	TSP
6		F ₅ -2	61.65	7.04	1034.15	6.57	450.97	19.94	441.82	85.00	UN	-	-	-	-	-	APT
7	F ₅	F ₅ -3	43.44	1.31	95.32	1.00	106.58	1.10	111.97	1.04	174.68	1.01	UN	-	-	-	TUSP
8		F ₅ -4	21.94	3.74	28.54	4.63	23.91	6.08	53.97	9.28	UN	-	-	-	-	-	TSP
9		F ₅ -5	168.95	1.031	151.05	1.00	145.75	1.738	140.60	1.00	129.13	1.001	95.69	1.02	93.40	1.002	HME
10	F ₆	F ₆ -0	UN	-	-	-	-	-	-	-	-	-	-	-	-	-	TSP
11	F ₇	F ₇ -0	54.26	1.23	66.20	1.25	UN	-	-	-	-	-	-	-	-	-	TSP

* UN, unstable; F, formulation; TSP, two separate phases; TUSP, two unseparated phases; APT, aggregation of powder on top; HME, homogeneous milky emulsion; -, unstable sample: not a suitable candidate. SMD/VMD is a morphology criterion of a particle, which explains the tendency to present a spherical or oval shape. If the SMD/VMD criterion tends to be one, or near one, the particle tends to have a spherical shape. On the other hand, if the SMD/VMD criterion is higher than one, the particle tends to oval shape instability.

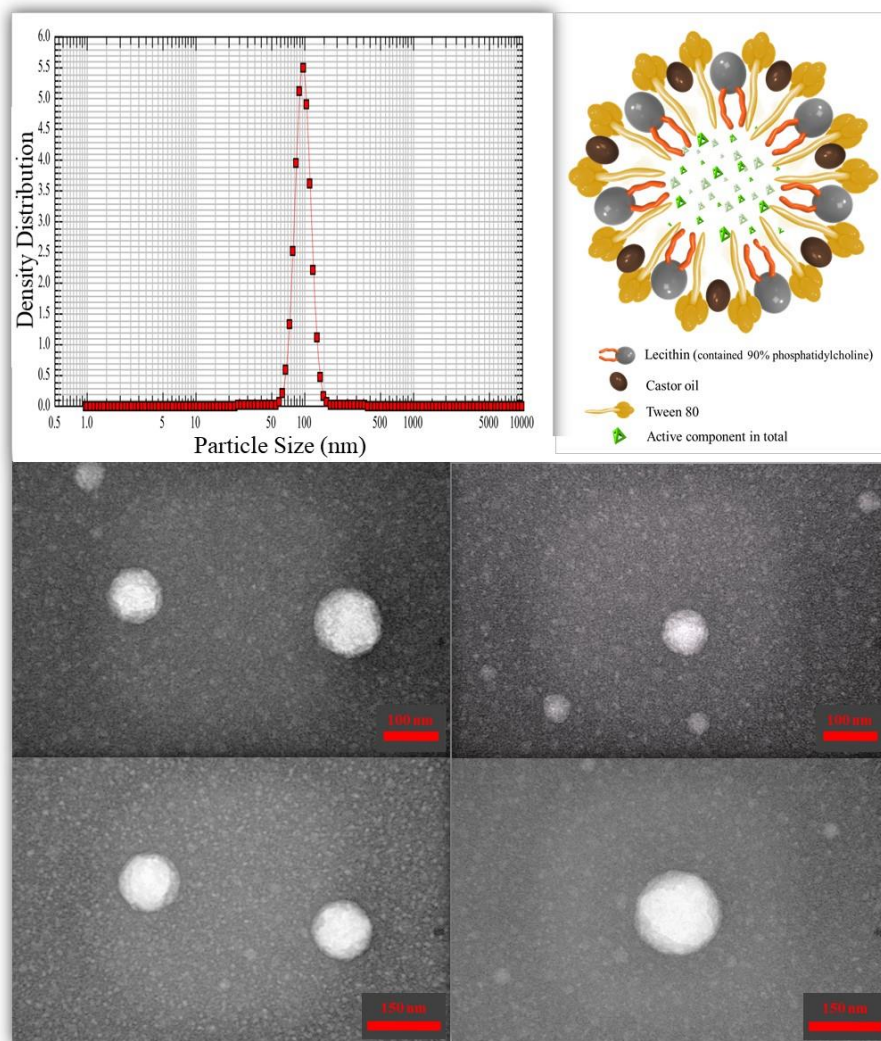


Figure 2. Schematic model of some initial MCNs. Formula F₅-5 has one stable homogeneous phase, while sample F₅-3 has two unseparated phases (small oil spots are obviously visible), and sample F₅-2 has two separated phases.

3-3-3-3. Nanoemulsion stability

The nanoemulsion preparation steps and ratio are presented in Table 1. Differences in particles size resulted in improved stability of all formulae, as shown in Table 3. All formulae were categorized by the HLB value of milky dispersions (8-10), the total content of essential oil and the total content of emulsifier and co-emulsifier, as shown in Table 1. The stability of nanoemulsions was monitored for 730 days (two years), similar to Section 3.2. During this period, the samples were monitored visually to detect any change in phase separation or aggregation, which directly affect particle size and morphology criterion changes. The particle size and SMD/VMD criteria confirmed the stability/instability of formulae under study over time. A significant change in particle size makes the NE unstable, resulting in the visualization of two separate phases or oil droplet appearance in the top of NE media (Figure 2). Meanwhile, the formulas F₁-0, F₂-0, F₃-0,

F₄-0, and F₆-0 were unstable in the early stages and just a subgroup of formula F₅-0 and F₇-0 was stable for more days (long shelf-life).

Regarding F₅ series, formulae F₅-1, F₅-2 and F₅-3 were optimized with 0.01%, 0.5%, 0.75% of castor oil, respectively, resulting in improved stability up to 540 days by enhancing the ultrasonication and castor oil content. In terms of formula F₅-4, although the castor oil content provides the highest chemical stability, decreasing the ultrasonication condition reduces the mechanical stability of NE up to 180 days (Table 3). Furthermore, according to the HLB criteria (Table 1), the most stable samples were obtained from the HLB value of 9 with 5% emulsifier in total, ultrasonicated three times, 20 min each, and 1% castor oil. Moreover, all formulae showed significant stability when enhancing the mechanical condition (ultrasonication) and castor oil content. Formula F₅-5 was the most stable formula, overcoming 730 days of stability without showing any phase separation or evidence of milky phase segregation, and best kinetic stability, mainly due to the nano-size particle stability (Table 3). Specifically, the critical role of ultrasonication on the homogeneity and stability of NEs was already mentioned by other researchers.^[25,26] Furthermore, F₅-5 had an HLB value of 9, which remained kinetically stable under mechanical and thermal stress conditions (heating, cooling shaking, and centrifugation) at day 730. The role of HLB value on production of stable nanoemulsions in a two-phase emulsifier system proven previously^[27] which is in agree with the experiment results.

In conclusion, a considerable increase in stability was gained in the formula containing 1% of castor oil and subjected to ultrasonication, which provoked a strong correlation on the production of smaller particles (due to cavitation of oil in water), confirmed by sample F₅-5 (Table 3). By the way, the positive impact of castor oil in a high concentration (3-11)% to stabilize nanoemulsion was also proven previously. The authors reported stability for just three months of storage at room temperature.^[23] Meanwhile, optimizing the minimum amount of castor oil with ultrasonication, providing stability over 730 days (two years), is a new approach demonstrated in the current study.

Indeed, similar research has shown that eucalyptus oil-based-NE with 6% of EOs and 12% of surfactant were only stable for 90 days even in high percentage of emulsifier.^[28] Based on the literature, the stability evaluations were performed for two months,^[29] three months^[23] and a maximum of 6 months^[30,31] for most of the studies. Therefore, it must consider that the deformation/instability of nanoemulsions was probable after the mentioned time. Meantime, evaluation of stability over two years should be an excellent proof of the best fabrication of MCN both in methodology, and amount and proportion of

ingredients. To the best of our knowledge, the individual role of pharmaceutical/food-grade castor oil and ultrasonication on the shelf-life stability of NEs are being described for the first time. Moreover, it could be concluded that a critical micellar concentration (CMC) was overcome by the concentration of emulsifiers in the mixture.

3.3.3.4. Shape and morphology

The size and morphology were confirmed by TEM (Transmission Electron Microscopy) in accordance with the results measured by DLS (Section 3.2.). TEM image showed a good spherical droplet shape with an average particle size of 100 nm and 150 nm associated with F₅-5 and F₅-3, respectively. Therefore, the TEM images confirmed the DLS measurements data. According to TEM images, the droplets had a circular and round surface with a significant repulsion between droplets (Figure 3). The results justify the good correlation of oil phase, emulsifier ratio, and ultrasonication condition in droplet size, resulting in good homogeneity between droplets in nanoemulsion. The TEM images proved to provide trustable data to evaluate the shape, size and morphology of droplets in nanoemulsion and indicate smaller droplet size.^[32] It could also be concluded that a stable nanoemulsion with a smaller droplet size was obtained when the equilibrium occurs at the hydrophile-lipophile balance (HLB)^[32] which is in accordance with our data exhibiting the HLB value of 9.

Figure 3. Size distribution of sample F₅-5 (image A), TEM picture of F₅-5 (100 nm, image B), and TEM picture of sample F₅-3 (150 nm, image C).

3.3.3.5. Antimicrobial activity

The antibacterial activity was studied for F₅-5 and assessed by measuring the MIC. The MIC values obtained with a mixture of *M. piperita* and *E. globulus* against *E. coli*, *S. aureus*, and *S. enteritidis* were 1×10^3 , 2×10^3 , 1×10^3 µg/ml, respectively. In turn, the MIC values of F₅-5 against the same bacteria were 31, 8, 63 µg/ml, respectively. The results revealed a significant reduction in MIC values against *E. coli*, *S. aureus* and *S. enteritidis* of 32, 250, 15-fold, respectively. Additionally, a significant increase in the antibacterial activity of formula F₅-5 has been demonstrated (Table 4). The antibacterial activity of EOs of *E. globulus*^[28], and *M. piperita*^[33] loaded on nanoemulsion media has already been described in the literature in a single form.

Nevertheless, the effective insecticidal activity of a mixture of *E. globulus* and *M. piperita* in nanoemulsion was reported previously.^[34] However, a lack of information

regarding the subsequent mixture was tangible in natural food and agriculture literature. Concludingly, a significant reduction in MIC values against *E. coli*, *S. aureus* and *S. enteritidis* should be a pleasant proof of considerable improvement on bactericidal properties of nanoemulsion with a mixture of *E. globulus* and *M. piperita* compared to literature.

Table 4. Antibacterial activity of free *E. globulus* and *M. piperita* compared to the mixture of both essential oils and formula F₅-5 (µg/ml).

	<i>E. globulus</i>	<i>M. piperita</i>	EOs (MIX)	F ₅ -5	Fold reduction in MIC
<i>E. coli</i>	8×10 ³	50	1×10 ³	31	32
<i>S. aureus</i>	8×10 ³	50	2×10 ³	8	256
<i>S. enteritidis</i>	8×10 ³	1×10 ³	1×10 ³	63	16

Mix of EOs; *E. globulus* and *M. piperita* (70:30). *E. coli*, ATCC 25922, *S. aureus*, ATCC25923, *S. enteritidis* PTCC1609

3.3.3.6. Controlled release profiles

The release profiles of formula F₅-5 were investigated by monitoring menthol associated with *M. piperita* and 1,8-cineole associated with *E. globulus*. Therefore, *M. piperita* had a faster release (in three minutes), while the *E. globulus* was the slowest release (in six minutes) in the same conditions. Although evaluating the multi-component nanoemulsion is a big challenge due to variety in phytoconstituents, Korsmeyer-Peppas provide a good fitting for both *E. globulus* and *M. piperita* (R^2 , 0.88). Moreover, both release patterns followed Case-I mass transfer - Fick law diffusion ($n < 0.43$). Generally, our experimental data revealed that the release of constituents belonging to *M. piperita*, like menthol and menthone, was faster in the four-first fractions with constant and smooth release profiles. The compounds belonging to *E. globulus* were gradually and inconstantly released from the nanoemulsion capsule slower than the ones from *M. piperita* (Table 5). Our result follows Lei *et al.* [35], who used the Korsmeyer-Peppas for mass transfer in carvacrol nanoemulsion-based composite films to avoid food contamination and enhance the shelf life of food products. They reported the majority of carvacrol (up to 95%) released following a diffusion-controlled process over 10 minutes.

Another study in the same area also confirmed the use of the Korsmeyer-Peppas kinetic model to evaluate a release profile.^[35, 36]

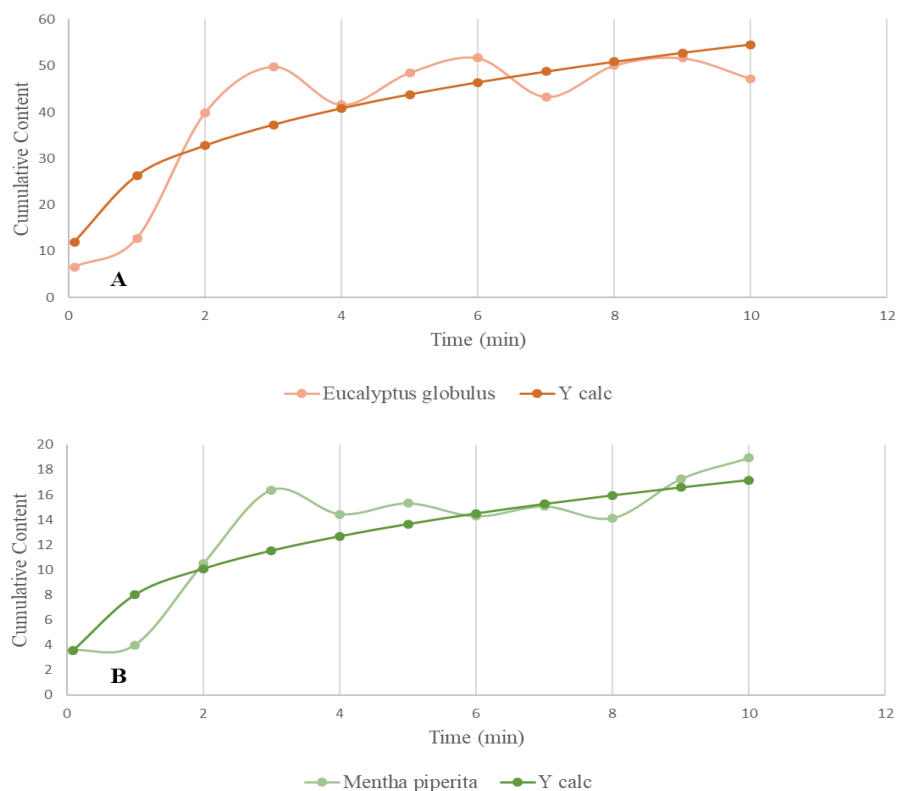


Figure 4. Release curve of sample F₅₋₅ in case of *E. globulus* (image A) and in case of *M. piperita* (image B).

Table 5. The release mechanism value of *E. globulus* and *M. piperita*

	Stability time	K _{KP}	R ²	n	Mass Transfer
<i>E. globulus</i>	6.00	26.28	0.88	0.32	Case-I mass transfer
<i>M. piperita</i>	3.00	8.04	0.88	0.33	Case-I mass transfer

*Stability time; the time in which the release starts to stabilize.

3.3.4. Conclusion

The study aimed of to formulate natural-based preservatives, containing a minimum dosage of emulsifier and co-emulsifier, based on ultrasonication of multi-component antibacterial agents. Formula F₅-5 exhibited a high shelf-life with ≤ 100 nm particle size and 1.002 SMD/VMD criteria, which was selected as an optimized formula. This sample was formulated by using a minimum amount of emulsifier (5%), small castor oil content (1%), and preparation procedures based on ultrasonication conditions. It must be taken into consideration that the natural-based preservatives contained a combination of *E. globulus* and *M. piperita* EOs with a significant reduction in MIC values (32-, 250-, 15-times fold against *E. coli*, *S. aureus* and *S. enteritidis*, respectively) compared to an original mixture of both EOs. Such approach was reported for the first time in this study. Moreover, the synergic effect of a small amount of castor oil and ultrasonic homogenization, even in the presence of a minimum amount of emulsifier, significantly improves the stability of multi-component nanoemulsion for almost two years. The results showed in this study could set guidelines for developing natural-based MCNs functionalized as preservatives with a prolonged shelf life and minimum amount of emulsifier optimized by mechanical ultrasonic homogenization and aid of just 1% of co-emulsifier, in this case, food-grade castor oil.

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3.4. References

- [1] Moghimi, R.; Ghaderi, L.; Rafati, H.; Aliahmadi, A.; McClements, D. J. Superior antibacterial activity of nanoemulsion of *Thymus daenensis* essential oil against *E. coli*. *Food Chem.* **2016**, *194*, 410–415. DOI: <https://doi.org/10.1016/j.foodchem.2015.07.139>
- [2] Liu, Q.; Huang, H.; Chen, H.; Lin, J.; Wang, Q. Food-grade nanoemulsions: preparation, stability and application in encapsulation of bioactive compounds. *Molecules* **2019**, *24*, 4242. DOI: <https://doi.org/10.3390/molecules24234242>
- [3] Kumari, S.; Kumaraswamy, R. V.; Choudhary, R.C.; Sharma, S. S.; Pal, A.; Raliya, R.; Biswas, P.; Saharan, V. Thymol nanoemulsion exhibits potential antibacterial activity against bacterial pustule disease and growth promotory effect on soybean. *Sci. Rep.* **2018**, *8*, 1-12. DOI: <https://doi.org/10.1038/s41598-018-24871-5>
- [4] Shahavi, M. H.; Hosseini, M.; Jahanshahi, M.; Meyer, R. L.; Darzi, G. N. Clove oil nanoemulsion as an effective antibacterial agent: Taguchi optimization method. *Desalin. water Treat.* **2016**, *57*, 18379-18390. DOI: <https://doi.org/10.1080/19443994.2015.1092893>
- [5] Augustin, M. A; Hemar Y. Nano- and micro-structured assemblies for encapsulation of food ingredients. *Chem. Soc. Rev.* **2008**, *38*, 902–912. DOI: <https://doi.org/10.1039/B801739P>
- [6] Severino, R.; Vu, K. D.; Donsi, F.; Salmieri, S.; Ferrari, G.; Lacroix, M. Antibacterial and physical effects of modified chitosan based-coating containing nanoemulsion of mandarin essential oil and three non-thermal treatments against *Listeria innocua* in green beans. *Int. J. Food Microbiol.* **2014**, *191*, 82-88. DOI: <https://doi.org/10.1016/j.jfoodmicro.2014.09.007>
- [7] Anton N; Benoit J. P; Saulnier P. Design and production of nanoparticles formulated from nano-emulsion templates-A review. *J. Control Release.* **2008**;128,185–199. DOI: <https://doi.org/10.1016/j.jconrel.2008.02.007>
- [8] Jaiswal, M; Dudhe, R; Sharma, P. K. Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech.* **2015**, *5*, 123–127. DOI: <https://doi.org/10.1007/s13205-014-0214-0>
- [9] Mohammadi, R.; Khoobdel, M.; Talebi, A.A.; Negahban, M.; Norani, M.; Moradi, M.; Dehghan, O. *In vivo* evaluation of the repellency effects of nanoemulsion of *mentha piperita* and *eucalyptus globulus* Essential Oils against mosquitoes. *Open Biotechnol. J.* **2020**, *14*. DOI: <https://doi.org/10.2174/1874070702014010145>
- [10] Bachir, R. G; Benali, M.. Antibacterial activity of the essential oils from the leaves of *eucalyptus globulus* against *escherichia coli* and *staphylococcus aureus*. *Asian Pac. J. Trop. Biomed.* **2012**, *2*, 739–742. DOI: [https://doi.org/10.1016/S2221-1691\(12\)60220-2](https://doi.org/10.1016/S2221-1691(12)60220-2)
- [11] Lima, T.S.; Silva, M.F.S.; Nunes, X.P.; Colombo, A.V.; Oliveira, H.P.; Goto, P.L.; Blanzat, M.; Piva, H.L.; Tedesco, A.C.; Siqueira-Moura, M.P. Cineole-containing nanoemulsion: Development, stability, and antibacterial activity. *Chem. Phys. Lipids* **2021**, *239*, 105113. DOI:

<https://doi.org/10.1016/j.chemphyslip.2021.105113>

- [2] Osanloo, M.; Abdollahi, A.; Valizadeh, A.; Abedinpour, N. Antibacterial potential of essential oils of *Zataria multiflora* and *Mentha piperita*, micro-and nano-formulated forms. *Iran. J. Microbiol.* **2020**, 12, 43.
- [13] Panigrahi, K. C; Patra, C. N; Jena, G. K; Ghose, D; Jena, J; Panda, S. K; Sahu, M. Gelucire: A versatile polymer for modified release drug delivery system. *Futur J. Pharm. Sci.* **2018**, 4, 102–108. DOI: <https://doi.org/10.1016/j.fjps.2017.11.001>
- [14] Liang, R.; Xu, S.; Shoemaker, C.F.; Li, Y.; Zhong, F.; Huang, Q. Physical and antimicrobial properties of peppermint oil nanoemulsions. *J. Agric. Food Chem.* **2012**, 60, 7548-7555. DOI: <https://doi.org/10.1021/jf301129k>
- [15] Fryd, M. M; Mason, T. G. Advanced Nanoemulsions. *Annu. Rev. Phys. Chem.* **2012**, 63, 493–518. DOI: <http://doi.org/10.1146/annurev-physchem-032210-103436>
- [16] Ghaderi L, Moghimi R, Aliahmadi A, McClements DJ, Rafati H. Development of antimicrobial nanoemulsion-based delivery systems against selected pathogenic bacteria using a thymol-rich *Thymus daenensis* essential oil. *J. Appl. Microbiol.* **2017**, 123, 832–840. DOI: <https://doi.org/10.1111/jam.13541>
- [17] Costa, P.; Lobo, J. M. S. Modeling and comparison of dissolution profiles. *Eur J Pharm Sci.* **2001**, 13, 123-133. DOI: [https://doi.org/10.1016/S0928-0987\(01\)00095-1](https://doi.org/10.1016/S0928-0987(01)00095-1)
- [18] Permanadewi, I.; Kumoro, A.; Wardhani, D.; Aryanti, N. Modelling of controlled drug release in gastrointestinal tract simulation. *J. Phys. Conf. Ser.* 1295, 2019; 012063. DOI: <https://doi.org/10.1088/1742-6596/1295/1/012063>
- [19] Emamjomeh, L.; Imani, S.; Talebi Jahromi, K.; Moharramipour, S. Nanoencapsulation enhances the contact toxicity of *Eucalyptus globulus* Labill and *Zataria multiflora* Boiss essential oils against the third instar larvae of *Ephestia kuehniella* (Lepidoptera: Pyralidae). *International Journal of Pest Management* 2021, 1-9. DOI: <https://doi.org/10.1080/09670874.2020.1871529>
- [20] Taherpour, A. A.; Khaef, S.; Yari, A.; Nikeafshar, S.; Fathi, M.; Ghambari, S. Chemical composition analysis of the essential oil of *Mentha piperita* L. from Kermanshah, Iran by hydrodistillation and HS/SPME methods. *J. Anal. Sci. Technol.* **2017**, 8, 1-6. DOI: <https://doi.org/10.1186/s40543-017-0122-0>
- [21] Zeinali, H.; Arzani, A.; Razmjoo, K.; Rezaee, M.B. Evaluation of oil compositions of Iranian mints (*Mentha* ssp.). *J. Essent. Oil Res.* **2005**, 17, 156-159. DOI: <https://doi.org/10.1080/10412905.2005.9698863>
- [22] Pratap-Singh, A.; Guo, Y.; Ochoa, S.L.; Fathordoobady, F.; Singh, A. Optimal ultrasonication process time remains constant for a specific nanoemulsion size reduction system. *Sci. Rep.* **2021**, 11, 1-12. DOI: <https://doi.org/10.1038/s41598-021-87642-9>
- [23] Prasetyo, B.E.; Maruhawa, S.M.; Laila, L. Formulation and physical evaluation of castor oil based nanoemulsion for diclofenac sodium delivery system. *Res. J. Pharm. Technol.* **2018**, 11, 3861-3865. DOI: <https://doi.org/10.5958/0974->

- [24] Fathi, F.; Ebrahimi, S.N.; Valadão, A.I.G.; Andrade, N.; Costa, A.S.G.; Silva, C.; Fathi, A.; Salehi, P.; Martel, F.; Alves, R.C.; Oliveira, M.B.P.P. Exploring Gunnera tinctoria: From Nutritional and Anti-Tumoral Properties to Phytosome Development Following Structural Arrangement Based on Molecular Docking. *Molecules* **2021**, 26, 5935. DOI: <https://doi.org/10.3390/molecules26195935>
- [25] Hashtjin, A. M; Abbasi, S. Optimization of ultrasonic emulsification conditions for the production of orange peel essential oil nanoemulsions. *J. Food. Sci. Technol.* **2015**, 52, 2679–2689. DOI: <https://doi.org/10.1007/s13197-014-1322-x>
- [26] Kentish, S; Wooster, T. J; Ashokkumar, M; Balachandran, S; Mawson, R; Simons, L. The use of ultrasonics for nanoemulsion preparation. *Innov. Food. Sci. Emerg. Technol.* **2008**; 9, 170–175. DOI: <https://doi.org/10.1016/j.ifset.2007.07.005>
- [27] Pagar, K. R; Darekar, A. B. Nanoemulsion: A new concept of Delivery System. *Asian. J. Res. Pharm. Sci.* **2019**, 9, 39. DOI: <https://doi.org/10.5958/2231-5659.2019.00006.7>
- [28] Sugumar, S; Ghosh, V; Nirmala, M. J; Mukherjee, A; Chandrasekaran, N. Ultrasonic emulsification of eucalyptus oil nanoemulsion: Antibacterial activity against *Staphylococcus aureus* and wound healing activity in Wistar rats. *Ultrason. Sonochem.* **2014**, 21, 1044–1049. DOI: <https://doi.org/10.1016/j.ultsonch.2013.10.021>
- [29] Smejkal, G.B.; Ting, E.Y.; Nambi Arul Nambi, K.; Schumacher, R.T.; Lazarev, A.V. Characterization of Astaxanthin Nanoemulsions Produced by Intense Fluid Shear through a Self-Throttling Nanometer Range Annular Orifice Valve-Based High-Pressure Homogenizer. *Molecules* **2021**, 26, 2856. DOI: <https://doi.org/10.3390/molecules26102856>
- [30] Benelli, G.; Pavoni, L.; Zeni, V.; Ricciardi, R.; Cosci, F.; Cacopardo, G.; Gendusa, S.; Spinozzi, E.; Petrelli, R.; Cappellacci, L. Developing a highly stable Carlinia acaulis essential oil nanoemulsion for managing Lobesia botrana. *Nanomaterials* **2020**, 10, 1867. DOI: <https://doi.org/10.3390/nano10091867>
- [31] Darooee, M.; Akbari, V.; Taheri, A. Inhibition of aldehyde dehydrogenase by furazolidone nanoemulsion to decrease cisplatin resistance in lung cancer cells. *Ther. Deliv.* **2021**, 12, 611-625. DOI: <https://doi.org/10.4155/tde-2020-0130>
- [32] Mossa, A.-T.H.; Mohafrash, S.M.; Ziedan, E.-S.H.; Abdelsalam, I.S.; Sahab, A.F. Development of eco-friendly nanoemulsions of some natural oils and evaluating of its efficiency against postharvest fruit rot fungi of cucumber. *Ind. Crops. Prod.* **2021**, 159, 113049. DOI: <https://doi.org/10.1016/j.indcrop.2020.113049>
- [33] de Carvalho, R. J; de Souza, G. T; Pagán, E; García-Gonzalo, D; Magnani, M; Pagán, R. Nanoemulsions of Mentha piperita L. essential oil in combination with mild heat, pulsed electric fields (PEF) and high hydrostatic pressure (HHP) as an alternative to inactivate Escherichia coli O157: H7 in fruit juices. *Innov. Food. Sci. Emerg. Technol.* **2018**, 48, 219–227. DOI: <https://doi.org/10.1016/j.ifset.2018.07.004>
- [34] Pinto, M.L.; Vella, L.; Agrò, A. Adulticidal activity of essential oils of *Mentha piperita*

L., *Cupressus sempervirens* L., and *Eucalyptus globulus* Labill. against the tomato leafminer *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae). *J. Entomol. Zool. Stud.* **2020**, 8, 1721-1728.

- [35] Lei, K.; Wang, X.; Li, X.; Wang, L. The innovative fabrication and applications of carvacrol nanoemulsions, carboxymethyl chitosan microgels and their composite films. *Colloids Surf. B* **2019**, 175, 688-696. DOI: <https://doi.org/10.1016/j.colsurfb.2018.12.054>
- [36] Algahtani, M.S.; Ahmad, M.Z.; Ahmad, J. Nanoemulsion loaded polymeric hydrogel for topical delivery of curcumin in psoriasis. *J. Drug. Deliv. Sci. Technol.* **2020**, 59, 101847. DOI: <https://doi.org/10.1016/j.jddst.2020.101847>

Chapter 4

Exploring *Gunnera tinctoria*: from Nutritional and Anti-tumoral Properties to Phytosome Development Following Structural Arrangement Based on Molecular Docking

Exploring *Gunnera tinctoria*: From Nutritional and Anti-tumoral Properties to Phytosome Development Following Structural Arrangement Based on Molecular Docking

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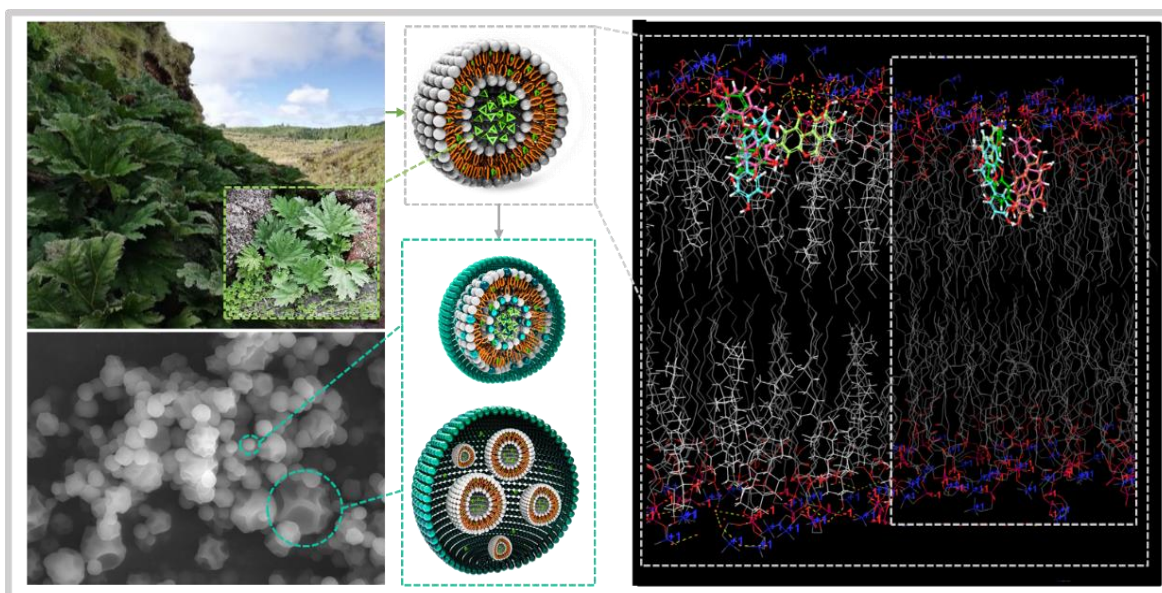
4.1. Abstract

Gunnera tinctoria, an underexplored invasive plant found in Azores, Portugal, was studied regarding its nutritional, antioxidant and antitumoral properties. Higher antioxidant activity was found in baby leaves, followed by adult leaves and inflorescences. A phenolic fraction of the plant was enriched using adsorbent resin column chromatography (Diaion™ HP20LX, and Releite EXA90). Antitumoral effects were observed with the enriched fractions in breast (MCF-7) and pancreatic (AsPC-1) cancer cell lines, being more pronounced in the latter. To improve protection and membrane absorption rates of phenolic compounds, nano-phytosomes and cholesterol-conjugated phytosomes coated with natural polymers were loaded with the enriched fraction. The particles were characterized and their physiochemical properties were evaluated and compared. All samples presented anionic charge and

nanometer size in relation to the inner layer and micrometer size regarding the external layers. In addition, the molecular arrangement of phenolics within both types of phytosomes were studied for the first time by molecular docking. Polarity and molecular size were key factors on the molecular arrangement of the lipid bilayer. In conclusion, *G. tinctoria* showed to be an interesting source of nutrients and phenolic compounds with anti-tumoral potential. Moreover, phytosome loading with these compounds can increase their stability and bioavailability having in view future applications.

Keywords: Bioactive compounds; Phytosome; Secondary layer; Molecular docking; Bilayer arrangement.

4.2. Graphical Abstract



4.3. Main Text

4.3.1. Introduction

Natural plant sources produce multiplied decisive effects on human health attributed to potent therapeutic properties [1]. *Gunnera tinctoria*, a highly competitive plant named Nalca, belongs to the Gunneraceae family [2] and to the subgenus panke (molina) Schindler [3]. It is cultivated in South America [4] being typically found in São Miguel Island, Azores, Portugal, as an invasive plant which is seen as a threat against endemic cultures [5]. However, in native areas, like Chile, *G. tinctoria* has been used as a part of food; flavour, salads, sweets or ice creams [6]. Dysentery, diarrhea, circulatory and urinary disorders, high respiratory tract diseases, and gynecological and obstetric problems, are some examples where *G. tinctoria* is used in folk medicine [7]. Moreover, antioxidant capacity, dermal anti-inflammatory, antifungal activity, and improvement of endothelial function are recent

therapeutic activities that have been reported for *G. tinctoria*, as well [8]. Based on this, this plant emerges as a particularly interesting natural source of bioactive compounds.

Regrettably, cancer is a major cause of death in both developed and developing countries. It is a group of diseases involving abnormal cell proliferation with the potential to spread to other parts of the body. Despite the efforts aiming to improve all treatment methods, early diagnosis and novel and advanced therapeutic intervention are still needed. Besides, plant extracts are mostly rich in bioactive compounds, especially phenolic compounds (PCs), which possess many anticarcinogenic properties, including inhibition of cancer cell proliferation, tumor growth, angiogenesis, metastasis and inflammation, as well as pro-apoptotic effects. So, it is vital to continue to search for anticancer agents/compounds in plants to find potential therapeutic targets that can be safe and reduce the side effects induced by chemotherapy [9].

Moreover, considering the high structural sensitivity and low bioavailability of natural products, especially PCs [10], the search for appropriate natural-based techniques to improve the low bioavailability and stability of such compounds, preventing their decomposition through the digestive system or during storage, is indispensable. Thereupon, encapsulation by natural polymers can provide a natural protection to PCs, improving their bioavailability, bioaccessibility, and bioactivity of such structures [11]. Moreover, encapsulation of natural products based on lipid carriers similar to those of cell membrane can improve desirable features and cover undesirable properties of natural products, attracting the interest of potential consumers.

In this work, the nutritional composition of different parts of *Gunnera tinctoria*, namely, root, inflorescence, baby and adult leaf were analyzed in terms of total protein, fat, insoluble and soluble fiber, and available carbohydrates contents. Total phenolics and flavonoids and the antioxidant activity of *G. tinctoria* crude extracts (using DPPH• scavenging and ferric reducing antioxidant power (FRAP) assays) have been also evaluated for the different parts of the plant. Afterwards, a phenolic enriched fraction of *G. tinctoria* was encapsulated based on a lipid layer (nano-phytosome/cholesterol-conjugated phytosome) and coated by binary polymeric layers. The physiochemical properties of the micro/nanoparticles obtained were characterized. Furthermore, the molecular arrangement of the lipid layers were analysed by membrane generator (MemGen). The molecular interaction between the lipid layer and phenolic compounds were investigated by computational simulation regarding molecular modeling (Docking), typically used to create the active binding site of ligand to receptor especially in protein, [12,13]. Indeed, this study presents for the first time the molecular arrangement of phenolic compounds within phytosomes layer by molecular docking.

Besides, the variant formation of the polymeric coating layer was estimated schematically throughout a phytosome layer for the first time, as well.

4.3.2. Results and Discussion

4.3.2.1. Nutritional Composition

Table 1 describes the nutritional composition of different parts of *G. tinctoria*. Adult leaves presented significantly higher ($p < 0.05$) contents of total fiber (44 g/100 g dry weight (dw)) and protein (14 g/100 g dw) compared to the other parts of the plant. In terms of insoluble fiber, there were no significant differences ($p > 0.05$) between adult leaves (40 g/100 g dw) and inflorescences (39 g/100 g dw), both being richer than baby leaves (32 g/100 g dw) and roots (10 g/100 g dw). The soluble fiber contents were quite similar for adult and baby leaves (4-5 g/100 g dw), both higher than inflorescences and roots (~2 g/100 g dw). In terms of available carbohydrates, roots presented the highest content (~80 g/100 g dw) compared to the other parts of the plant (30-44 g/100 g dw). According to the European Commission, a fiber-rich food must contain at least 6 g of fiber/100 g or 3 g of fiber/100 kcal [14]. Based on this, all the freeze-dried parts of the plant can be described as sources of fiber, especially adult and baby leaves. In contrast, inflorescences were the richest in total minerals (11 g/100 g dw) and fat (~8 g/100 g) compared to the other samples in study. Also, for a food being considered a source of protein, 12% of its energy value should come from protein. And to be considered a rich source of protein, this value should be at least 20% (EC, 2006). Based on the protein content obtained for dried adult leaves, they could be also considered an alternative source of protein.

Our findings differed slightly from those described by Zamorano *et al.* [15], who reported a lower crude fiber value (12 g/100 g dw) and a higher carbohydrate content (59 g/100 g dw) for *G. tinctoria* leaves. However, ash, fat and total protein contents were in similar ranges. The differences in carbohydrate and total fiber could be due to differences in several influencing factors, such as degree of leaf maturation, soil or edaphoclimatic conditions to which the plants were subjected.

Table 1. Nutritional composition of different parts of *G. tinctoria* and comparison with other vegetables.

Test	Roots	Inflorescences	Baby Leaves	Adult Leaves
Moisture	61.20 ± 0.11 ^d	91.35 ± 0.06 ^a	90.55 ± 0.10 ^b	85.95 ± 0.08 ^c
Ash	5.13 ± 0.05 ^d	11.37 ± 0.12 ^a	7.13 ± 0.06 ^c	9.96 ± 0.12 ^b
Protein	2.53 ± 0.05 ^d	8.79 ± 0.31 ^c	10.55 ± 0.23 ^b	13.62 ± 0.85 ^a
Fat	0.03 ± 0.00 ^d	7.96 ± 0.13 ^a	1.14 ± 0.16 ^c	2.10 ± 0.23 ^b
Total Fiber	12.62 ± 0.53 ^d	41.31 ± 0.55 ^b	36.54 ± 0.43 ^c	44.19 ± 0.32 ^a
Insoluble Fiber	10.42 ± 0.25 ^c	39.24 ± 1.21 ^a	31.55 ± 1.49 ^b	39.94 ± 0.93 ^a
Soluble Fiber	2.21 ± 0.78 ^b	2.07 ± 0.66 ^b	4.98 ± 1.06 ^a	4.25 ± 1.26 ^a
Available Carbohydrates	79.75 ± 0.59 ^a	30.76 ± 0.78 ^c	44.78 ± 0.46 ^b	30.24 ± 1.60 ^c

The results are expressed in g/100 g dry weight (except for moisture: g/100 g fresh weight). In each line, different small letters represent significant differences between samples ($p < 0.05$).

4.3.2.2. Antioxidant Profile

According to the data presented in Table 2, baby leaves present a significantly higher ($p < 0.05$) content of total phenolics (308 mg GAE/g) compared to the other parts of the plant under study. In turn, roots contain the highest amount of total flavonoids (30 mg CE/g). The antioxidant activity of the samples was evaluated using two different methods (FRAP and DPPH• inhibition). Baby leaves showed a considerably higher antioxidant activity ($p < 0.05$), by both methods, compared to the other parts of the plant. In contrast, roots presented the lowest antioxidant activity ($p < 0.05$). No significant differences ($p > 0.05$) were found between the antioxidant activity of adult leaves and inflorescences. Zamorano *et al.* [15] reported a total of 10.7 g GAE/100 g sample in the case of total phenolics, 111.9 g ascorbic acid equivalents/100 g in the FRAP method and 83.3 mg TE/100 g in the DPPH• inhibition method, regarding adult leaves of *G. tinctoria*. However, our results cannot be directly compared due to the different extraction methods used. While, in our study, an equitable ethanol/water mixture was used for antioxidant assays, in the study conducted by Zamorano *et al.* [15], the authors used water with 1% HCl.

Table 2. Total Phenolics Content (TPC), Total Flavonoids Content (TFC), Ferric Reducing Antioxidant Power (FRAP), and DPPH• scavenging ability (SA) of different parts of *G. tinctoria*.

Test	Roots	Inflorescences	Baby Leaves	Adult Leaves
TPC (mg GAE/g)	115.4 ± 3.0 ^d	176.2 ± 3.8 ^b	308.0 ± 2.7 ^a	151.4 ± 13.8 ^c
TFC (mg CE/g)	30.1 ± 1.06 ^a	13.76 ± 0.59 ^c	11.86 ± 0.28 ^d	18.28 ± 0.66 ^b
FRAP (mmol FSE/g)	5677 ± 165 ^c	6427 ± 205 ^b	13153 ± 214 ^a	6437 ± 276 ^b
DPPH• SA(mg TE/g)	220.9 ± 13.3 ^c	542.3 ± 86.1 ^b	705.0 ± 26.1 ^a	515.2 ± 65.9 ^b

In within each line, different small letters represent significant differences between samples ($p < 0.05$). GAE, gallic acid equivalents; CE, catechin equivalents; FSE, ferrous sulfate equivalents; TE, Trolox equivalents; SA, scavenging ability.

4.3.2.3. Obtention of Phenolic-Enriched Extracts of *G. tinctoria* leaves

4.3.2.3.1. Total Phenolic Content

Although baby leaves presented an overall better antioxidant profile, the *G. tinctoria* adult leaves (GuT) were selected to continue the assays, based on their good nutritional profile, good antioxidant activity and significantly higher abundance in nature, which is of importance considering future applications.

The sample was extracted with hydroethanolic solvent (GuT₁) and ultrasonication with water (GuT₂), being then enriched using Diaion™ HP20LX (Di) and Releite EXA90 (Re) adsorbent resins. The phenolic-enriched fraction of GuT was confirmed based on the total content of phenolic compounds and total flavonoids content, as described in Table 3. The highest extraction yield was achieved with the hydroethanolic extraction (GuT₁, 58.0%), and the highest enrichment was obtained with Diaion™ HP20LX (GuT₁-Di₁, 38.6%). Accordingly, the highest flavonoid content was also observed for GuT₁-Di₁ (43.1 mg/L). Moreover, total phenolics increased in relation to crude extract (GuT₁). In what concerns to GuT₁-Re₁, the enrichment yield was 36.1% and the total phenolics content was 77.8 mg/L with a 2.23-fold enrichment. A significant enhancement in phenolics was also observed for all the other samples under study. However, the lower extraction and enrichment yields obtained in the second series (GuT₂: 15.3%; GuT₂-Di₁: 21.0%; GuT₂-Re₁: 22.00%), allowed us to select GuT₁-Di₁ as the best sample/process to achieve higher extraction and enrichment yields, and higher richness in phenolics compounds (Table 3). These results are in accordance with those already published by Davoodi *et al.* [16], in which the phenolic fraction of *Satureja khuzistanica* was purified by Diaion HP20 regarding pre-preparation of phenolic extract for open column chromatography and HPLC to find a new natural scaffold. Phenolic enrichment was also confirmed in another study with a similar protocol using *Punica granatum* peel [17]. The EXA-118 adsorbent resin was also successfully employed in the purification of

anthocyanins and hydroxycinnamic acids from a citrus by-product, as well [18]. Additionally, the use of adsorbent resin column chromatography in the purification of food ingredients as a substantial modern source of economic technology was previously proved in food area to obtain high purity end products [19].

Table 3. Results of extraction and enrichment for the different fractions.

Sample	Code	Yield	TFC (mg CE/L)	ER	TPC (mg GAE/L)	ER
Hydroethanolic Extract	GuT ₁	58.00	24.74 ± 0.43 ^c	-	34.93 ± 1.53 ^e	-
E-GuT of Di	GuT ₁ -Di ₁	38.55	43.14 ± 0.25 ^a	1.74	82.82 ± 4.02 ^b	2.37
DF-GuT of Di	GuT ₁ -Di ₂	DC*	01.26 ± 0.43 ^g	0.05	3.05 ± 0.06 ^f	0.09
E-GuT of Re	GuT ₁ -Re ₁	36.11	21.26 ± 0.87 ^{cd}	0.86	77.84 ± 0.76 ^c	2.23
DF-GuT of Re	GuT ₁ -Re ₂	DC*	02.42 ± 0.25 ^f	0.10	3.01 ± 0.18 ^f	0.10
Ultrasonication Extract	GuT ₂	15.29	09.96 ± 0.00 ^e	-	41.84 ± 2.47 ^d	-
E-GuT of Di	GuT ₂ -Di ₁	21.00	18.51 ± 0.25 ^d	1.86	80.38 ± 2.07 ^{bc}	1.92
DF-GuT of Di	GuT ₂ -Di ₂	DC*	02.57 ± 0.43 ^f	0.26	3.29 ± 0.70 ^f	0.10
E-GuT of Re	GuT ₂ -Re ₁	22.00	37.78 ± 0.43 ^b	3.79	87.40 ± 2.22 ^a	2.09
DF-GuT of Re	GuT ₂ -Re ₂	DC*	01.70 ± 0.43 ^g	0.17	0.93 ± 0.34 ^g	0.04

In each column, different small letters represent significant differences between samples ($p < 0.05$). DC, discarded; TFC, total flavonoids content; CE, catechin equivalents; TPC, total phenolics content; GAE, gallic acid equivalents; ER, Enrichment ratio. E-GuT; Enriched fraction of GuT. DF-GuT; discarded fraction of GuT: discarded fractions is undesirable compounds such as sugars and chlorophyll.

4.3.2.4. Bioassays

Gallic acid, ellagic acid, catechin, epicatechin, and quercetin have been described as major phenolics of *G. tinctoria* [8, 20], and all of them are widely known for their antioxidant properties. Based on the hypothesis that the phenolic-enriched fraction of *G. tinctoria* would exert antitumoral activities in breast and pancreatic cancer cell lines, their effects on cell viability, culture growth and proliferation were studied. Considering cell viability (Figures 1A and 1D) in the lowest concentration, after 24 h, none of extracts was able to exert a cytotoxic activity on both cell lines. Observing the results from the MCF-7 cell line in the highest extract concentration (1mg/mL), it is possible to note a cytotoxic tendency induced by almost all extracts except GuT₁-Di₁, although with no statistical differences compared to control. However, regarding the AsPC-1 pancreatic cancer cell line (Figure 1D), all samples except GuT₂ at (1 mg/mL) had a marked significative effect upon cell viability. In particular, GuT₁-Re₁ (307% of control) and GuT₂-Re₁ (250% of control) exerted a strong cytotoxic activity on this line. Nevertheless, these samples were passed over due to low enrichment yield and the phenolic content in Section 2.3.1.

Concerning culture growth (by SRB assay), after 24 h, almost all extracts in both concentrations were able to reduce the MCF-7 cell mass (Figure 1B). The reduction in culture growth was more marked by GuT₁ and GuT₂ in both concentrations. Data regarding SRB assay in AsPC-1 cell line have shown that practically all extracts exerted a reduction effect on this cell line mass (Figure 1E), being GuT₁-Di₁ (76% of control), GuT₁-Re₁ (85% of control) and GuT₂-Re₁ (84% of control) extracts more powerful in the highest concentration.

Interestingly, the results of the cell proliferation assays (Figures 1C and 1F) were the most expressive ones. In both cell lines, the anti-proliferative effect seemed to be concentration-dependent to all extract samples and it was possible to observe a reduction greater than 90% (comparing to control) in all extracts in the highest concentrations. These impressive results show that both crude extracts (GuT₁ and GuT₂) and phenolics-enriched fractions could have an important role against cancer, particularly in inhibiting cancer cell proliferation and tumor growth. Most of the observed effects are probably linked to the antioxidant and anti-inflammatory properties of polyphenols [21] especially gallic acid, ellagic acid, catechin, epicatechin [22] by acting on multiple molecular and cellular targets and may influence the three phases of chemoprevention, namely primary prevention (inhibition of cancer initiation), secondary prevention (inhibition of cancer promotion) and therapy (inhibition of cancer progression) [23].

Considering the results of all the previous steps (product extraction yield, enrichment rate, total phenolics and flavonoids contents, and anti-tumoral activities), both GuT₁-Di₁ and GuT₁-Re₁ detached as the best fractions. Due to the similarity between them, we selected one (GuT₁-Di₁) as a reference sample for the following studies, namely, the preparation of lipid-based micro/nano particle protected with natural polymers.

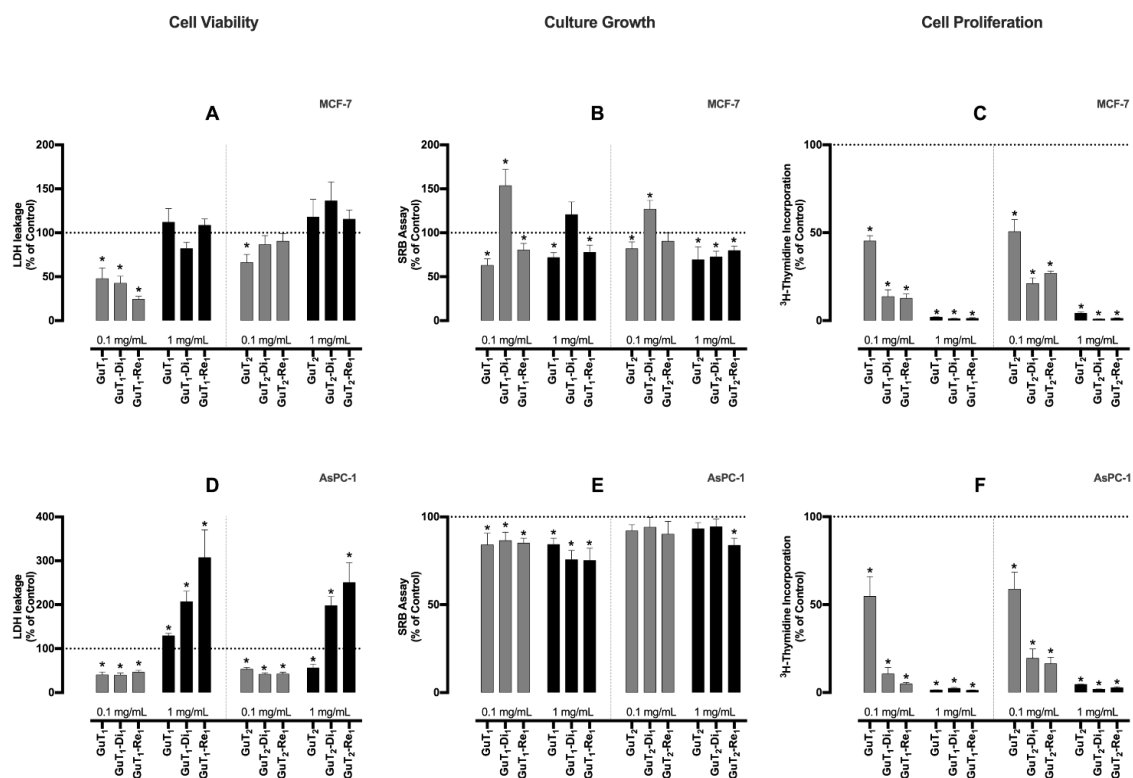


Figure 1. GuT₁, GuT₁-Di₁, GuT₁-Re₁, GuT₂, GuT₂-Di₁, GuT₂-Re₁ extracts activity on MCF-7 breast (A, B and C) and AsPC-1 pancreatic cancer cells (D, E and F). Long-term (24 h) effects of the extracts in two different concentrations (0.1 and 1mg/mL) on cell viability (extracellular LDH activity), culture growth (SRB assay) and proliferation rates (³H-thymidine incorporation) of MCF-7 and AsPc-1 cell lines (*n*=5-7). Data shows arithmetic means ± S.E.M. **p*<0.05 vs control, by Student's t-test.

4.3.2.5. Physicochemical Properties of Nano/micro-Particles

Nano-phytosomes were produced as described in Section 3.7.1. The main objective of this step was to considerably improve the physicochemical properties of the lipid-based nanoparticles leading to the production of nano-phytosomes and phytosome conjugated cholesterol protected by different natural polymer with the best properties to protect the phenolic content (Chart 1). Indeed, the physicochemical properties of a particle such as shape, appearance, flowability, miscibility, and ease of handling play a vital role to achieve the desired performance [24]. For that, different proportion of substrates were tested for coating phytosome layers, and the final particles were compared in terms of production yield, particle size distribution, surface charge, and morphologies. Additionally, the bilayer formation as well as the coating layers variability were monitored regarding the particle's size distribution of inner layers which perform an essential role on functionality. For that, the probable formation of inner layers was discussed and clarified, for first time, in terms of size, surface charge, and particle distribution. Technical analyses like Fourier-Transform Infrared

Spectroscopy (FTIR) and molecular docking (for the first time) were also carried out, to confirm the interaction between phenolic compounds and the lipid bilayer.

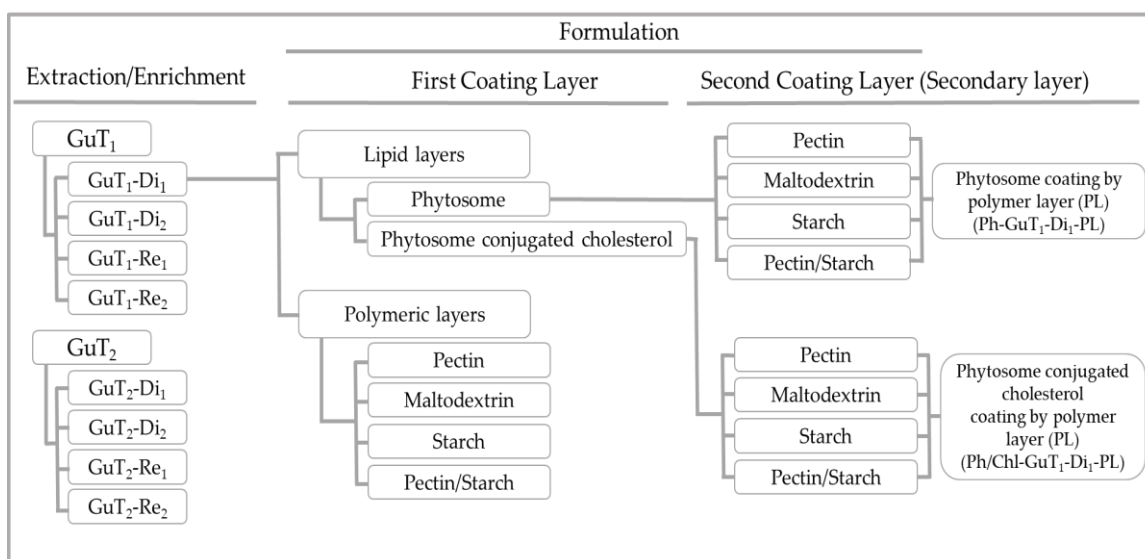


Chart 1. Schematic of sample preparation.

4.3.2.5.1. Formulation Yield

The formulation product yield is determined by subtracting the amount of final powder recovered from the spray dryer collector to the initial amount of feeding solution. This parameter is affected by solution viscosity, volume of feed solution, and feeding temperature, having also in account the type of natural polymer (coating layer) as well as the drying conditions [25]. In the present work, the inlet temperature was set based on the natural polymers features (Section 3.7.4.), ranging between 115-130 °C, in order to reduce the moisture in the final product and enhance the formulation product yield by optimizing the inlet and outlet temperatures [26]. The product yields varied between 32.0 and 68.9%, significantly differing according to the type of natural polymer and drying conditions. The following range is in agreement with different studies mentioned by Tontul and Topuz in their review paper [25] that explained based on literature data that a considerable variability on product yield is entirely influenced by the type of natural polymer [25]. In this work, the highest product yields were obtained with starch, while the lowest ones were observed for maltodextrin. Our results are in accordance with those of Higuera *et al.* [27] that reported high product yield for starch and a low product yield for maltodextrin. It must be taken into consideration that a significant mass loss occurred throughout the drying process due to 100% aspiration by the vacuum system (most of the light and small particles were vacuumed and placed on the outlet filter). Additionally, a certain powder mass was diminished due to adhesion of un-encapsulated particles to the inner surface of the drying cyclone just after

nozzle aspiration (drying chamber). The following fact quite justified the low product yield values obtained for some variables [26].

In the case of the polymeric layer and Ph-GuT₁-Di₁ secondary layer, there were no significant differences between the product yields. The results demonstrate that the secondary layer was following routes like a single polymeric layer, and no considerable changes were observed in terms of product yield compared to polymeric layer. In turn, in the case of Ph/Chl-GuT₁-Di₁ secondary layer series, the product yield values were observed in a higher rank compared to other series (53.0-70.2%). More details are presented in Table 4. It could be concluded that the related factor might depend on the impact of cholesterol to improve the stability of lipid particles, and the affinity of cholesterol to a natural polymer originates a more stable particle during the process. Also, incorporating cholesterol in the phytosome layer might result on a strong adhesion between Ph/Chl-GuT₁-Di₁ colloidal particles, causing a considerable increase on total product yield. The following is certain: the effect of phytosome conjugated cholesterol improved the product yield of secondary layer, in contrast to the reported by a researcher who explained that the agglomeration and adhesion of particle to the walls of the drying chamber caused a considerable decrease on product yield. Indeed, adhesion of particles on the wall of drying chamber can be influenced by variable factors such as type of carriers, inlet temperature, wall properties, and the feature and percentage of first or binary coating layers [28]. But our results are in accordance to those of Wang *et al.* [29] who reported that hydroxypropyl methylcellulose provides a surface coverage of the particles and reduces adhesion during spray drying, resulting on improving the product yield.

Table 4. Particle size distribution regarding the effective diameter in number and volume as well as zeta potential of inner and external layer.

	Sample Name	Yield	ED ¹ In volume	ED ¹ In number	ZP ¹ Inner layer	ZP ¹ External layer
Lipid layer	Ph-GuT ₁ -Di ₁	-	120.0	33.5	-67.50 ± 1.43	
	Ph/Chl-GuT ₁ -Di ₁	-	354.4	140.2	-62.57 ± 2.95	
Polymeric layer	GuT ₁ -Di ₁ -PE	34.55	1196.5	916.5	-29.72 ± 1.32	-16.21 ± 8.44
	GuT ₁ -Di ₁ -Malto	46.00	397.9	327.1	-33.82 ± 0.76	-31.40 ± 10.29
	GuT ₁ -Di ₁ -Stch	68.55	509.1	355.0	-55.01 ± 3.24	-53.47 ± 1.94
	GuT ₁ -Di ₁ -PE/Stch	38.00	824.6	737.2	-43.07 ± 2.17	-28.24 ± 3.06
Secondary layer	Ph-GuT ₁ -Di ₁ -PE	43.54	1364.6	1016.1	-21.26 ± 7.19	-17.21 ± 8.34
	Ph-GuT ₁ -Di ₁ -Malto	37.23	411.5	226.3	-33.82 ± 0.76	-39.14 ± 5.92
	Ph-GuT ₁ -Di ₁ -Stch	47.38	523.1	446.7	-31.38 ± 3.95	-25.37 ± 1.75
	Ph-GuT ₁ -Di ₁ -PE/Stch	41.67	951.2	752.7	-44.76 ± 3.38	-18.87 ± 9.20
Secondary layer	Ph/Chl-GuT ₁ -Di ₁ -PE	68.74	1207.9	1073.0	-31.67 ± 1.69	-14.99 ± 2.79
	Ph/Chl-GuT ₁ -Di ₁ -Malto	66.22	392.7	212.7	-34.18 ± 3.41	-29.31 ± 3.38
	Ph/Chl-GuT ₁ -Di ₁ -Stch	70.22	680.5	555.1	-43.07 ± 2.17	-38.46 ± 1.94
	Ph/Chl-GuT ₁ -Di ₁ -PE/Stch	52.97	707.6	528.1	-34.40 ± 2.84	-30.09 ± 1.99

¹ED; Effective Diameter, ZP; Zeta Potential. PE; Pectin, Malto; Maltodextrin, Stch; Starch, Pectin/Stch; PE/Stch. The effective diameter was reported on nm, and zeta potential was reported on mV.

4.3.2.5.2. Fourier-Transform Infrared Spectroscopy analyses (FTIR)

The recorded FTIR spectra confirmed the interaction between GuT₁-Di₁, lecithin (phosphatidylcholines), and cholesterol in the nano-phytosome structure (Figure 2). The FTIR measurements were performed in the scanning range from 4000 to 400 cm⁻¹. The spectra prove the interaction between the lipid carrier and the core material via significant differences between the pure compounds and the complexes. The suppressing of critical functional groups of the substrates which appear on Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ spectra are marked in Figure 2. Furthermore, the significant changes in the fingerprint area 400-1500 cm⁻¹ (purple box in Figure 2) of formulated samples clearly shows the existence of phytosome interactions. Moreover, regarding (Figure 2); Peaks (b) marked with orange color at 2930 cm⁻¹, appear at the same area of Ph/Chl-GuT₁-Di₁. In contrast, there is a lack of existence of this peak in the same area (c) in Ph-GuT₁-Di₁ spectra which did not contain cholesterol, confirming the interaction of cholesterol with lecithin in Ph/Chl-GuT₁-Di₁. The peaks (d) marked with blue color at 2924 cm⁻¹ appear with low intensity on Ph-GuT₁-Di₁, and with high intensity on Ph/Chl-GuT₁-Di₁, confirming the interaction of lecithin in both structures. Furthermore, the suppressed area (e) at Ph-GuT₁-Di₁, and Ph/Chl-GuT₁-Di₁, compared to GuT₁-Di₁ confirm the interaction between substrates. The physicochemical interaction between the lipid layer and GuT₁-Di₁ as a subsequent phytosome nanoparticle

is then confirmed by observing the differences between pure compounds and phytosome complexes [30]. Moreover, suppressing on the sharp endothermic functional groups of curcumin as an active ingredient as well as phospholipid spectra were reported before which confirmed our outcome [31].

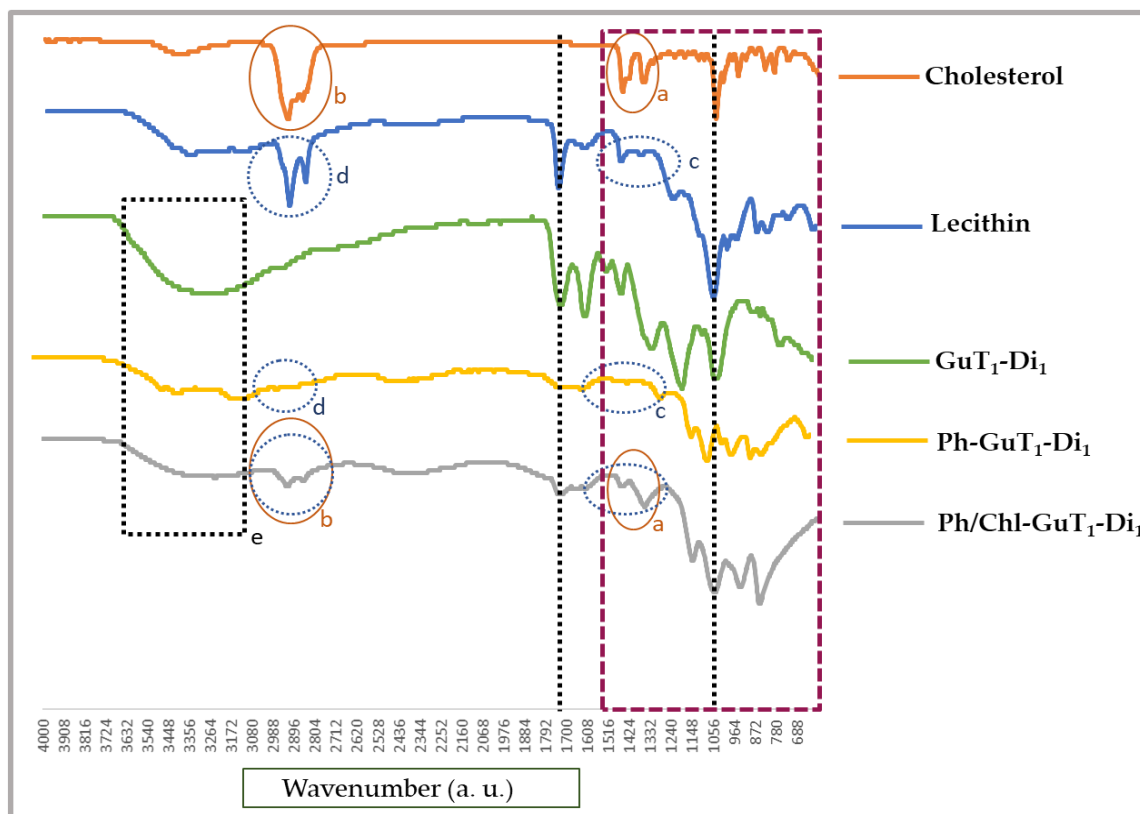


Figure 2. The critical change on functional groups of Ph-GuT₁-Di₁, and Ph/Chl-GuT₁-Di₁ are clearly marked in FTIR spectra.

4.3.2.5.3. Particle Size Distribution and Morphology

Particle size was analyzed regarding effective diameter on number and volume. The particle size presented almost at an inharmonious size distribution in all samples. Three types of particles were monitored in the current study: (i) the lipid layer of nano-phytosome (Ph-GuT₁-Di₁) and cholesterol conjugated phytosome (Ph/Chl-GuT₁-Di₁); (ii) the simple coating of different natural polymers (polymeric layer); and (iii) the polymeric layer coating a both lipid layers individually (secondary layer). For this purpose, the particle sizes of all samples were analyzed regarding the external and internal layers. The particle categories have been listed in Table 4, and more details are schematically explained in Figure 3.

4.3.2.5.3.1. Lipid-based Layers

Particle size distribution was evaluated after 20 min of sonication for Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ due to the preparation method. The Ph-GuT₁-Di₁ particle size observed

was 33.5 and 120 nm regarding number and volume, respectively, being considered a nano-phytosome. Our result is in accordance with those of Nazari *et al.* [32] that reported a nano-phytosome with a particle size of 115 nm. The Ph/Chl-GuT₁-Di₁ particle size was 140.2 and 354.4 nm, regarding number and volume, respectively, being considered a colloidal particle [33] [34]. In comparison, Ph-GuT₁-Di₁ showed to produce a smaller particle and the presence of cholesterol made the phytosome bigger. This is in accordance with Rasaie *et al.* [35], who also reported that a cholesterol conjugated phytosome produced a particle with a size of 393.67 ± 2.89 nm while promoting a significant physical stability on the phytosome layer (particle size stability for more than 20 days).

4.3.2.5.3.2. Polymeric Layers

A 7 min sonication was applied before analyzing the particle size to crush the outer layer and detect the size of inner layers (Figure 3). The particle size of the external layer is discussed in *Section 2.5.4*. The particle size was observed in the range of 327.1-916.5 nm in number and 397.9-1196.5 nm in volume, regarding the inner layer. Pectin, the complex of pectin/starch, starch, and maltodextrin produced the biggest to the smallest particles, respectively, (Table 4). Regarding a considerable protection of pectin as coating layer, finding a strategy to reduce the size is essential [36]. For this purpose, considering the size of starch in simple coating and in the complex of starch with pectin, it could be concluded that starch affected the pectin size and considerably decreased the particle size in consequence of complexation (Table 4). Therefore, based on experimental data, starch acts as a size reducing agent in the complex with pectin. It could be taken into consideration that a carbohydrate like starch link to the surface of polymer layers and prevent the agglomeration due to steric hindrance mechanisms. Therefore, starch could limit the size by affecting the surface of micro/nanoparticles [37], which was proved in the current study, as well.

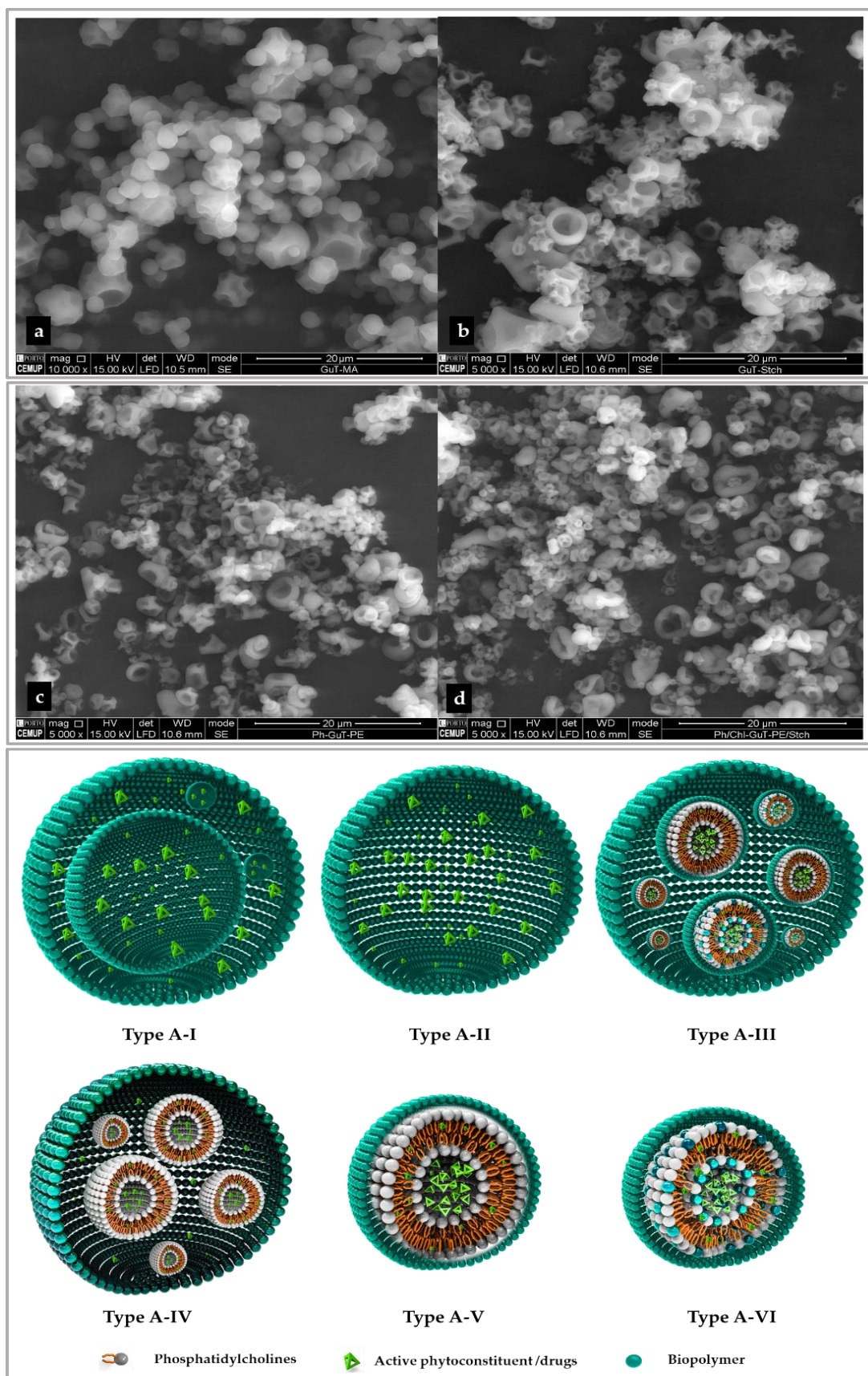


Figure 3. Surface morphology of final products: (a) maltodextrin; (b) starch; (c) pectin; (d) pectin/starch.

4.3.2.5.3.3. Secondary layers

A 5 min sonication was applied before analyzing the particle size to detect the size of inner layers, just like in the previous *Section 2.5.3.2.* (Figure 3). Polymeric secondary layers coating Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ were quite similar in size distribution. The particles size of these two series were quite bigger compared to polymeric layers. The following result might occur due to aggregation of lipid layers inside one another, creating a bigger particle (Figure 3). The particle sizes of the inner layers regarding the secondary coating layers were observed in the range 226.3-1016.1 nm, in number, and 411.5-1364.6 nm, in volume, for the Ph-GuT₁-Di₁ series. In addition, ranges between 212.7 and 1073.0 nm in number, and 392.7-1207.9 nm in volume were observed for Ph/Chl-GuT₁-Di₁ series (Table 4). The biggest and the smallest particles were recognized for pectin at 1016 nm in number and 1364 nm in volume, and maltodextrin (212.7 nm in number and 392.7 nm in volume), respectively. It must be reinforced that a significant reduction on particle size of pectin in complex with starch was observed in these, as well. Therefore, it could be a nice prove to consider starch as a size reducing agent [37].

4.3.2.5.4. Scanning Electron Microscopy (SEM)

SEM images were used to confirm the surface morphology regarding the type of coating layer and particles size, although it could not give any information about the inner layer (Figure 3). Surface morphologies were approximately spherical, crushed sphere, with regular and irregular surface shape for all analysed particles (Figure 3). The most smooth and spherical surface shape was observed for maltodextrin in polymeric layers and secondary layers. A spherical uniform surface with low concavities was also previously observed for maltodextrin [38]. Starch and pectin in simple matrix as well as in complex have a similar surface shape. Both particles have a regular and spherical surface with some cavity and some small particles seem to be crushed spheres (which might be due to the use of high temperature during preparation). For comparison, pectin has more depth concavities and starch was observed more sphere. Our results are in accordance with those of Cortes *et al.* [39]. Considering the particle morphology observed by SEM, it was shown that the particle morphology is directly influenced by the type of coating layer and conditions of the drying process [25] and it was not influenced by the type of inner layer. Since the size of the inner layer for most of particles was demonstrated to be in the nanometer range (except for pectin-based particles, *Section 2.5.3.*), it was obviously proved by SEM images that the secondary layer has a bigger size, almost around 1-20 μm (colloidal system [33]) for all samples without exception (Figure 3). This is in agreement with the results published by Kolan *et al.* [36], regarding maltodextrin and pectin, and Cortes *et al.* [39], regarding starch

and pectin, in terms of particle size and morphology. Furthermore, Figure 3 schematically introduces the probable formation of layers in several particles and demonstrate the multiplate formation of layers which significant influences the particle size. In the case of layer-by-layer encapsulation (coated by natural polymer), if the polymeric layer only encapsulates the active part in normal conditions (polymeric layer) the particle inner layer might get shaped similar to image structure without any internal layer: Type A-I or Type A-II with some internal layers. In the case of phytosome coated with a polymer, the particle inner layers appear as Type A-III, A-IV, A-V, and A-VI or a combination of all. In structure Type A-II, A-III, and A-IV some agglomerations of small particles with big dimensions cause these structures. It must be noticed that a combination of all these structures occurs typically in the total sample (Type A-IV). The type of layer could be approximately estimated based on a gap between volume and number for each particle. When the difference between a volume and number is low the structure Type A-II was formed. When the difference between a volume and number is high, some structures (Type A-III, and A-IV) occur. And, when the particles size in both number and volume are quite near to size of nano phytosomes, it shows that the images Type A-V and A-VI are present. In addition, by optimizing preparation conditions, some of these categories might decrease, although it is not possible to eliminate them. Hence, coated particles are formed into different main structures that directly influence the size, sequence, and shape of particle layers.

4.3.2.5.5. Zeta Potential (Surface Charge)

The particles surface charge/zeta potential (ZP) was measured for all samples regarding the lipid layer, inner layers, polymeric layer, and secondary layer. Commonly, surface charge can directly affect a cellular permeate tendency due to cellular membranes surface charge. A surface charge greater/lower than ± 30 mV is desirable in terms of cationic/anionic stability at aqueous media since it induces particle repulsion and diminishes agglomeration. Also, if surface charge is within ± 10 mV the particle is considered neutral. The range between ± 10 mV to ± 30 mV is adjusted to the nearest charge tendencies. It must be noted that the cationic particle provides more toxicity associated with cell wall membrane [40, 41]. As expected, the phytosome particles were observed at the negative charge of $(-67.50 \pm 1.43 \text{ mV})$, and $(-62.57 \pm 2.95 \text{ mV})$ associated to Ph-GuT₁-Di₁, Ph, and Chl-GuT₁-Di₁, respectively. The anionic charge is related to the layer of phosphatidylcholine (lecithin), and cholesterol. The negative charge of lipid phytosome layer has been also reported previously with slight differences [42] [43]. Ph-GuT₁-Di₁, Ph, and Chl-GuT₁-Di₁ showed a zeta potential greater than 60 in anionic mode, suggesting a high stability of the nanoparticle in solution media, while the results published in literature were lower and consequently represent a lower stability [40, 41].

The surface charge of polymeric and secondary layer was observed in negative charge even in inner and external layer with small differences in surface charge in the ranges from -21.26 ± 7.19 to -55.01 ± 3.24 , associated to inner layer, and from -14.99 ± 2.79 to -53.47 ± 1.94 , associated to external layer, for all samples, in general. The negative ZP of pectin, maltodextrin and starch was confirmed previously [44, 45]. To a large extent, the surface charge of inner layer presented a higher value, which proved that the inner layers are considered desirable in terms of stability due to the negative electric charge that induce particle repulsion and reduce agglomeration in solution media. Typically, a higher surface charge promotes a higher colloidal stability and lower particle agglomeration [46]. Although the stability of inner layer was considered more desirable, the external layer was also in an acceptable range [40]. As a result, particles in all categories were observed in acceptable negative charge mood except pectin in terms of inner layer, both on polymeric and coating layers which showed the lowest values (Table 4). Although the following value are not adjusted on best range, there are suited to negative surface charge tendencies [40]. However, a size reducing tendency of starch in the mixture of pectin/starch was demonstrated in *Sections 2.5.3.1.*, *2.5.3.2.*, and *2.5.3.3.*, showing a relative feature on ZP, as well. The stability of pectin was improved when combined with starch regarding the ZP value, in all related particles (Table 4). Concluding, the complex of pectin with starch can not only be considered a size reducing agent, but also a particle stabilizer agent leading to greater mechanical resistance in aqueous media. A considerable enhance on surface charge of pectin/starch complex was shown in all experimental samples. It must be taken into account that the physiochemical properties of the inner layer such as particle size, zeta potential and, generally, its availability has not been focus of attention in any related research till now. Therefore, in this study, for the first time, the inner layer formation is clarified and discussed, regarding physiochemical properties as particle size and zeta potential.

4.3.2.6. In silico Investigation Based on Molecular Arrangement

Gallic acid, ellagic acid, catechin, epicatechin, and quercetin (Figure 4) have been described as major phenolics of *G. tinctoria* [8, 20], and all of them are widely known for their antioxidant properties which were discussed on (*Section 2.4.*), as well. Considering the high content of phenolic compounds identified in our samples (Table 3), *in silico* investigation and molecular arrangement of phospholipids was evaluated by molecular docking to confirm the incorporation of phenolic compounds in the simulated lipid bilayer. The represented 3D model of lipid bilayers (Figure 5) was constructed using the MemGen webserver. The selected phenolic compounds docked with both constructed bilayer and cholesterol conjugated phytosome according. The low docking score was attributed to have more

hydrogen binding and stronger interactions [47]. Table 5 presents the docking Score and grid energy in detail.

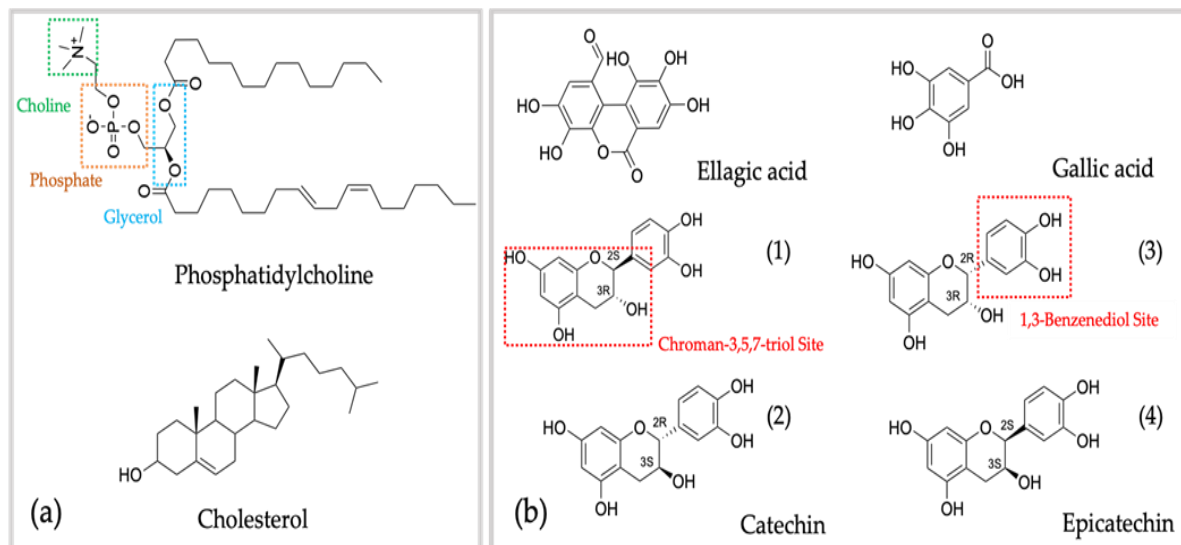


Figure 4. Chemical structure of lipid layer constituents (a) and phenolic compounds previously identified in *G. tinctoria* (b). Regarding catechin (1); (3*R*)-2(*S*)-(3,4-dihydroxyphenyl)chromane-3,5,7-triol, (2); (3*S*)-2(*R*)-(3,4-dihydroxyphenyl)chromane-3,5,7-triol, (3); (3*R*)-2(*R*)-(3,4-dihydroxyphenyl)chromane-3,5,7-triol, (4); (3*S*)-2(*S*)-(3,4-dihydroxyphenyl)chromane-3,5,7-triol.

The docking score result revealed the hydrogen binding between the polar site of phenolic compounds and the choline site of phosphatidylcholine. For example, a significant number of hydrogen bonds between quercetin hydroxy groups with polar bilayer parts revealed a lower docking score and stronger interaction. This phenomenon may be due to two different factors: size and polarity of the docked molecule which was joined and created the same feature with higher intensity. Considering the structural similarity between catechin, epicatechin and quercetin, the interaction mode and binding energy are estimated to be similar. However, the rigid structure of quercetin regarding the lack of chiral center may cause a more potent binding energy. Nonetheless, the predominate glucosides formation of quercetin at natural sources may change the docking score due to their conjugated molecule and functional group (Figure 6, Table 5).

Regarding the cholesterol-conjugated phytosome, cholesterol was adjusted approximately at the same level of phosphatidylcholine (Figure 4). The hydrogen binding might occur between the phosphate site of phosphatidylcholine and the 3-hydroxy substituent of cholesterol, creating the stability on lipid bilayer that was mentioned on Section 2.5.1., regarding product yields. Additionally, a considerable increase on the size of Ph/Chl-GuT₁-Di₁ compared to Ph-GuT₁-Di₁ (Section 2.5.3.1, Table 4) was clarified regarding the specific molecular arrangement of cholesterol on lipid bilayer shown in Figures 4 and 7.

Docking score was recorded in lower value attributed to higher binding energy in all samples belong to phytosome conjugated cholesterol compared to phytosome structure in the same conditions except gallic acid. However, ellagic acid adjusted entirely to the choline site of phosphatidylcholine and to the 3-hydroxy substituent of cholesterol by multiple hydrogen binding (Figure 7).

In contrast, the molecular arrangement of catechin and epicatechin were quite different at the grid of the cholesterol-conjugated phytosome compared to nano-phytosome bilayer. Catechin and epicatechin were entirely adjusted in the opposite molecular arrangement (Figure 5, 6). It was proved that the hydrogen binding between the polar site of phosphatidylcholine and cholesterol with 1,3-benzenediol of epicatechin was strengthening this architecture compared to catechin (Figures 4 and 7). In the case of quercetin, it could be concluded that the hydrogen binding between chroman-3,5,7-triol with the polar site of phosphatidylcholine and the hydroxyl group of cholesterol create the strongest interaction compared to all the sites of action under study. Our results confirm the idea of Ghanbarzadeh *et al.* [48], who demonstrated the occurrence of hydrogen binding between the hydroxyl group of the catechin and the polar site of phosphatidylcholine (phosphate group) in catechin loaded nano-phytosome.

It could be concluded that the polar group is adjusted in the upper head side of phospholipid (polar site) or any type of lipid layer, and the nonpolar structure is placed near the tail. Therefore, a variety of active compounds, in this case phenolic structures, provide a diversity on molecular arrangement of lipid bilayers. Based on the provided information, the polarity of the active compound is a major key factor in the molecular arrangement of lipid bilayer, and the molecule size placed in the second rank. Moreover, taken into consideration the results obtained from molecular docking, the structural arrangement of the nano-phytosome layer was confirmed and it was in accordance with previous research in the area of liposome and phytosome that reported that active structures are placed in core or lipid layer based on the polarity of the active phytoconstituents/drugs, in the case of liposome [49], and adjusted just in polar site in the case of phytosome [48] However, the existence of hydrogen bonding in phytosome layer was confirmed in this study which is in accordance with Ghanbarzadeh *et al.* [48], who reported that the chemical bonding also occurs between the active substrate and phospholipids in the phytosome structure. Additionally, it might be more accurate to confirm the target delivery of lipid layer by molecular docking. Concludingly, the features could be easily adjusted to all type of lipid layers for future research.

Table 5. Binding energy of docked molecule expressed on Kcal/mol.

	Phenolic Structure	Docking Score			Grid Energy		
		Min	Max	Average	Min	Max	Average
Phytosome designed with lecithin	Gallic acid	-4.47	-4.86	-4.73 ± 0.23	-29.98	-31.37	-30.83 ± 0.75
	Ellagic acid	-4.32	-4.47	-4.31 ± 0.15	-31.33	-4.47	-32.88 ± 1.31
	Quercetin	-5.31	-5.41	-5.05 ± 0.50	-29.90	-34.23	-33.04 ± 2.57
	Catechin	-3.50	-5.36	-4.49 ± 0.66	-19.71	-38.93	-29.14 ± 9.18
	Epicatechin	-3.38	-5.23	-4.36 ± 0.66	-22.43	-39.96	-33.05 ± 6.71
Phytosome designed with lecithin conjugated by cholesterol	Gallic acid	-4.06	-4.13	-4.09 ± 0.04	-12.66	-15.75	-14.62 ± 1.70
	Ellagic acid	-3.54	-3.75	-3.62 ± 0.10	-18.79	-19.24	-19.05 ± 0.21
	Quercetin	-5.12	-6.79	-5.69 ± 0.65	-23.47	-26.29	-25.01 ± 1.15
	Catechin	-5.93	-6.95	-6.42 ± 0.44	-21.62	-25.47	-23.51 ± 1.41
	Epicatechin	-4.89	-7.15	-6.50 ± 0.57	-20.84	-25.09	-24.09 ± 1.49

*Docking score exhibited the number of hydrogen bindings occurring between structures. Lower docking scores denote a higher hydrogen binding.

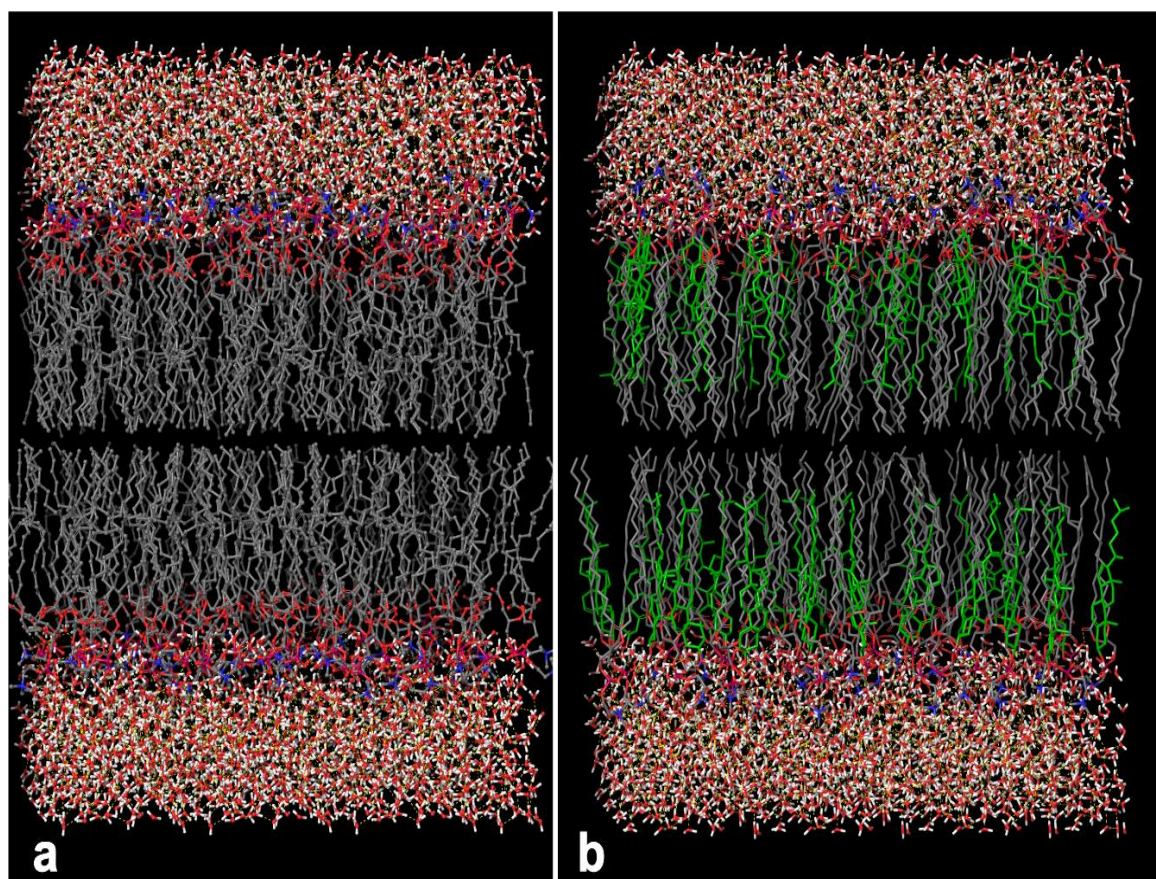


Figure 5. Simulated 3D model of lipid bilayer. (a) nano-phytosome prepared using lecithin; the polar site of phosphatidylcholine is represented with red color and the aggregation of white and red molecules at the top and bottom of the bilayer grid exhibit the water molecules the surround the lipid bilayer. (b) phytosome prepared using lecithin-conjugated by cholesterol; the green structure shows the arrangement of cholesterol within phosphatidylcholine.

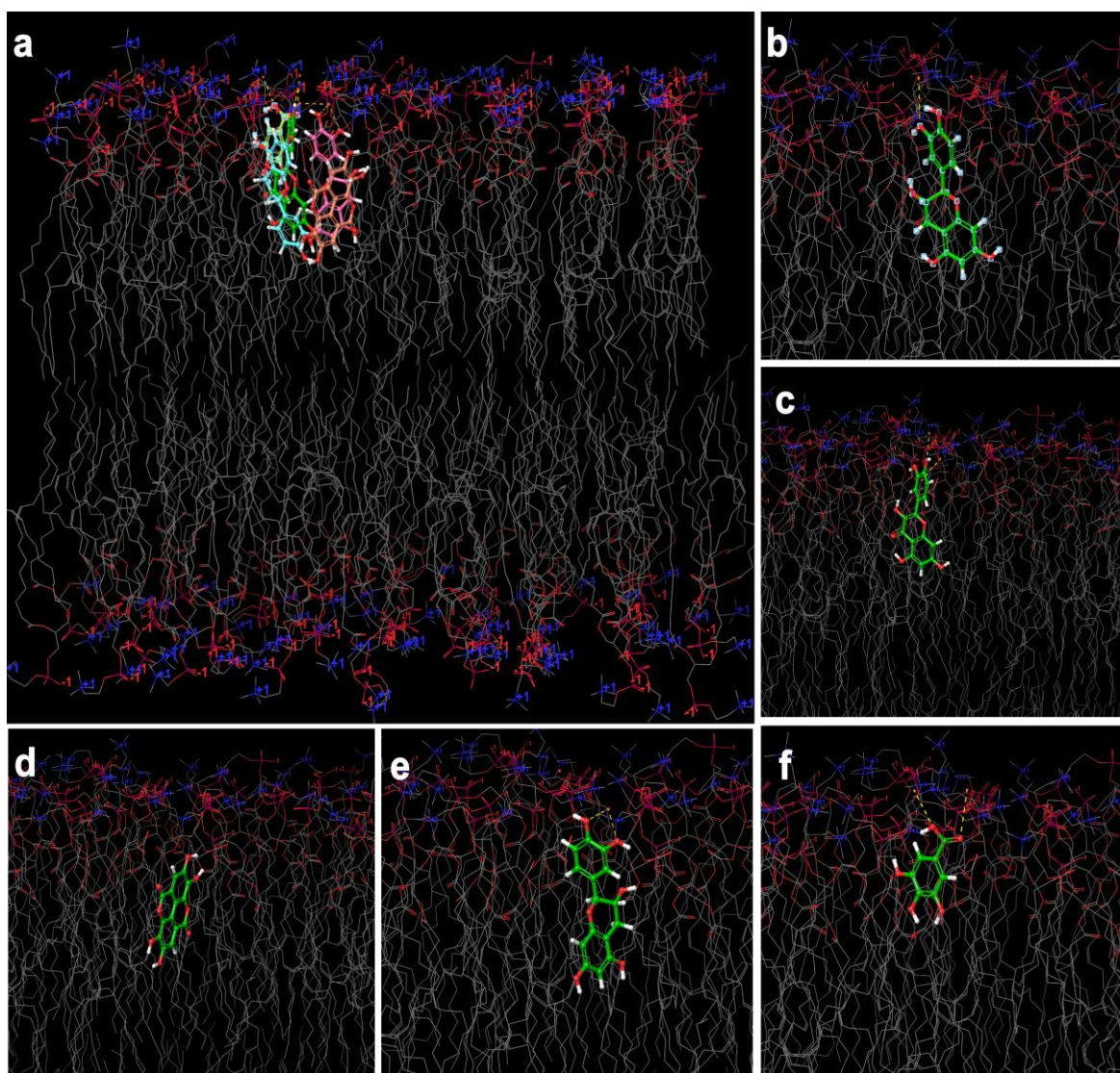


Figure 6. Simulated 3D model of the nano-phytosome bilayer covered by thousands of water molecules (the red structure in the top and bottom). In real media, the bilayer appears as spherical, elliptical, or as a structure between both. The grid section of nano-phytosome bilayer is shown. The molecular docking images present the phenolic compound placed within the nano-phytosome bilayer structure with a number of hydrogen bindings; **(a)** overlayed of all compounds interaction with the bilayer, **(b)** catechin, and **(c)** epicatechin; both interacted through the 1,3-benzenediol site with the polar site of lipids with lower docking score and stronger hydrogen binding. **(d)** ellagic acid, **(e)** quercetin, **(f)** gallic acid.

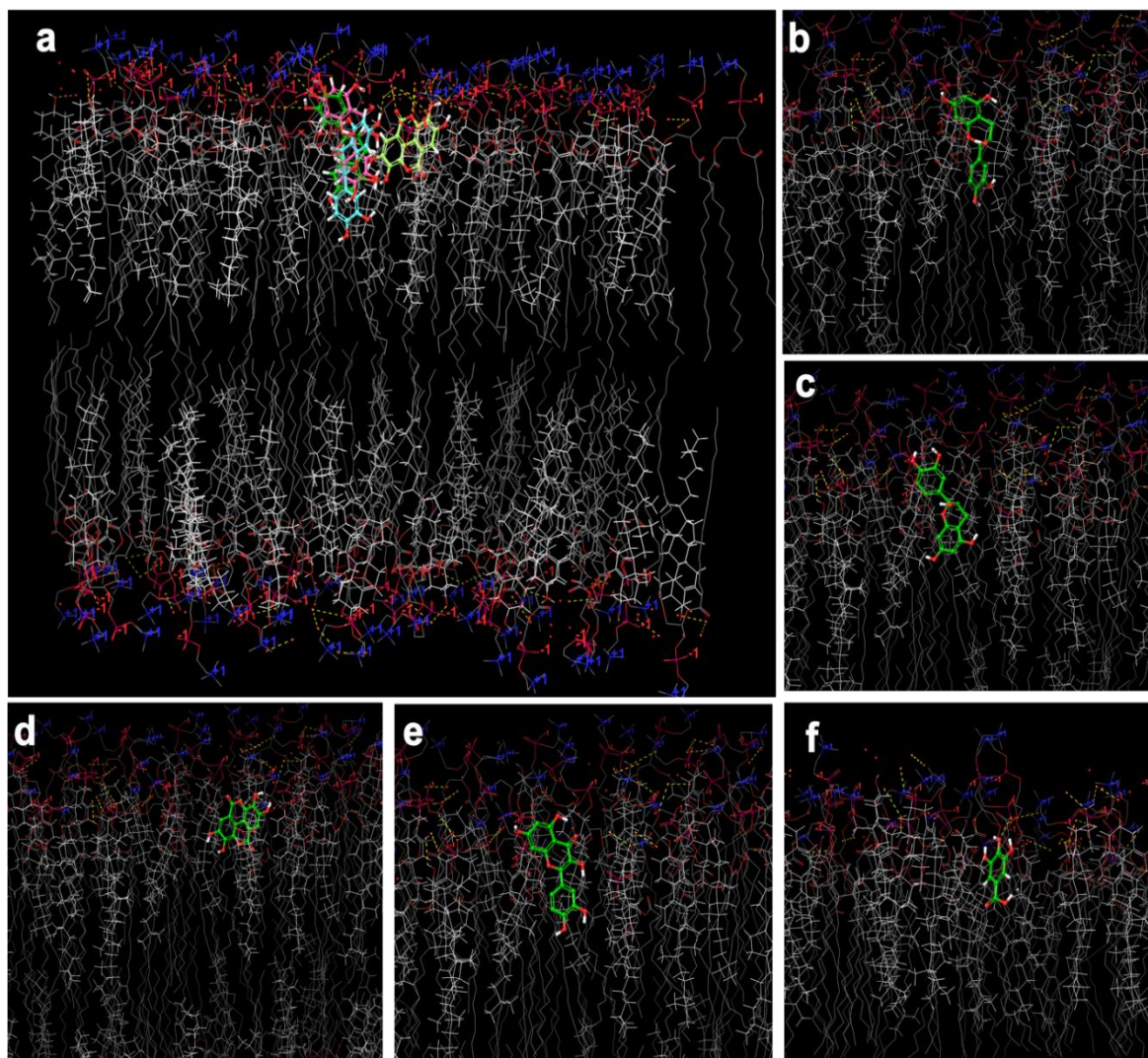


Figure 7. Simulated 3D model of cholesterol-conjugated phytosome docked by phenolic compounds. The phenolic structure is placed within the cholesterol bilayer with number of hydrogen bindings; (a) overlaid of all compounds interaction with bilayer, (b) catechin; the hydrogen binding occurred between the chroman-3,5,7-triol site and the polar site of phosphatidylcholine (phosphate and choline sites) and the hydroxyl group of cholesterol, and the 1,3-benzenediol site of catechin was adjusted approximately near to the tail, (c) epicatechin; the hydrogen binding occurred between the 1,3-benzenediol site of epicatechin and the polar site of phosphatidylcholine and cholesterol. In addition, the chroman-3,5,7-triol site was adjusted near the tail. (d) ellagic acid, (e) quercetin, (f) gallic acid.

4.3.3. Materials and Methods

4.3.3.1. Chemicals and Reagents

Ferrous sulfate heptahydrate, Folin-Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), sodium nitrite, ferric chloride, aluminum chloride, 2,4,6-tripyridyl-s-triazine (TPTZ), sodium acetate, trolox, gallic acid, chlorogenic acid, ($\pm$) catechin hydrate, and an enzyme kit (α -amylase, protease, and amyloglucosidase) were all supplied from Sigma Chemical Co. (St. Louis, MO, USA). Anhydrous sodium carbonate was from VWR International (Leuven, Belgium).

Sodium hydroxide, Kjeldahl catalyst tablets ($\text{Na}_2\text{S}_2\text{O}_8/\text{CuSO}_4$), anhydrous sodium sulfate, and concentrated sulfuric acid 98% were from Merck (Darmstadt, Germany). Petroleum ether and ethanol were from Honeywell (Saint-Germain-en-Laye, France). Boric acid was from Panreac (Barcelona, Spain). Acetone, sodium hydroxide, and hydrochloric acid were from Carlo Erba (Val de Reuil, France).

^3H -thymidine ([methyl- ^3H]-thymidine; specific activity 79 Ci/mmol) (GE Healthcare GmbH, Freiburg, Germany); antibiotic/ antimycotic solution (100 U ml^{-1} penicilin, 100 $\mu\text{g ml}^{-1}$ streptomycin, and 0.25 $\mu\text{g ml}^{-1}$ amphotericin B, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid), NADH (nicotinamide adenine dinucleotide), sulphorhodamine B, trichloroacetic acid (TCA), Tris, triton X-100, and trypsin-EDTA solution (Sigma, St. Louis, MO); fetal bovine serum (Invitrogen Corporation, Waltham, USA).

The ion exchange resin Relite EXA90 (Re), and the spherical porous polystyrene resin Diaion™ HP20LX (Di) were kindly gifted from Resindion group of Mitsubishi Chemical, Binasco, Italy. Ethanol with purity of $\geq 99.8\%$, 78%, and 96% and dichloromethane were supplied from Honeywell, Germany. Absolute ethanol was purchased from (Loughborough, England). Sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) and sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) were purchase from PanReac, Barcelona, Spain. Maltodextrin was purchased from Fargon, Barcelona, Spain. Apple pectin was supplied from LABChem, Portugal. Potato Starch (water soluble) was supplied from PanReac-Applichem, Darmstadt, Germany. Cholesterol was supplied from SIGMA life science, USA. Soybean lecithin was purchased from Alfa Aesar, Thermofisher, Kandel, Germany. The ultra-pure water was obtained from PRO 60 CN and Seradest (water purification system).

4.3.3.2. Plant Material

Roots, inflorescences, baby leaves, and adult leaves of *G. tinctoria* were collected in Sete Cidades, São Miguel Island, Azores, Portugal. The baby leaves were collected when they were 3 to 6 cm long, while adult leaves presented about 50 cm of diameter. Collected roots had approximately 20 cm length, and inflorescences up to 40 cm in length.

The samples were frozen at -80°C and freeze-dried (TELSTAR, Cryodos freeze dryer, Terrassa, Spain). The lyophilized samples were powdered and stored in vials. The ground freeze-dried adult leaves had a texture similar to wheat flour, while the baby leaves presented a fluorescent green texture and smell similar to tea leaf. In turn, ground freeze-dried inflorescence had a fibrous non-uniform texture while the roots presented a fibrous uniform texture.

4.3.3.3. Nutritional Composition

4.3.3.3.1. Moisture Content

The moisture content was determined by KERN infrared lamp, DBS60-3. Briefly, 2-3 g of sample were dried at 105°C in triplicate. The results are expressed in g of moisture per 100 g of sample.

4.3.3.3.2. Ash Content

The ash content was determined by incineration (0.6 g of sample) at a muffle furnace heated gradually from 110°C to 500-550°C (Thermolyne 48000, Electrothermal Engineering Ltd, Essex, UK), till white ashes obtention (AOAC 920.153) [50], The ash content was determined by differences in mass content before and after incineration. Triplicate analyses were conducted, and the results are expressed in g of ash per 100 g of sample.

4.3.3.3.3. Protein Content

The protein content was determined with quantification of total nitrogen using the Kjeldahl method (AOAC 928.08) [50]. Briefly, a small amount of sample (0.5-1.0 g) was weighed on nitrogen-free paper and placed in a Kjeldahl tube, along with 20 ml of concentrated sulfuric acid (H_2SO_4 , 96%) and two catalyst tablets (Kjeldahl tablets). Acid digestion was performed in an automatic digester (K-438, Büchi®, Büchi Labortechnik AG, Flawil, Switzerland). An amount of 90 ml of sodium hydroxide (NaOH, 32%) was added to the digested to release ammonia, which was distilled and collected in 60 ml of boric acid (H_3BO_3 4%) at pH 4.65. The Process were operated according to Büchi Labortechnik AG, 2007. The solution was then titrated with H_2SO_4 (0.2 M) using methyl red as indicator. Total nitrogen content was multiplied by the conversion factor 6.25 [51, 52] to estimate total protein content. The analyses were done in duplicate, and the results expressed in g protein per 100 g of sample.

4.3.3.3.4. Fat Content

The Soxhlet method was used for determination of total fat content [50]. Briefly, 5 g of sample was mixed with anhydrous sodium sulfate (Na_2SO_4) and placed into cellulose cartridges. Extraction was carried out with petroleum ether during 8 h and, afterwards, the solvent was evaporated and collected. Then, the residue was dried at 100°C until a constant mass value was obtained. The analyses were performed in triplicate and the results are presented in g of fat per 100 g of sample.

4.3.3.3.5. Total, Soluble, and Insoluble Fiber Content

Total fiber content was determined using an enzymatic-gravimetric technique (AOAC 991.43) [50]. Briefly, 0.5 g of sample were mixed with 100 μ L of α -amylase and 50 mL of phosphate buffer for 20 minutes at 95°C. After, 10 mL of NaOH (0.275 M) were added, adjusting the pH to 7.5 ± 0.2 , and 100 μ L of protease were added, followed by incubation at 60°C for 35 min, under stirring conditions. Then, an amount of 10 mL HCl (0.325 M) was added, adjusting the pH to 4-4.6. After adding 100 μ L of aminoglucosidase, a new incubation at 60°C for 35 min was performed. At room temperature, 200 ml of ethanol 96% were added to precipitate soluble fiber. The residue was filtered, washed and dried at 105°C overnight. Insoluble fiber was determined by a similar procedure but without adding ethanol 96%. Soluble fiber was determined by difference. The results are expressed in g per 100 g of sample.

4.3.3.3.6. Available Carbohydrates

The content of available carbohydrates was determined according to the Equation 1 [52]. The results were presented in g per 100 g of sample.

$$\text{Available carbohydrates\%} = 100\% - (\text{Protein\%} + \text{Fat \%} + \text{Ash \%} + \text{Total Fiber \%}) \quad (1)$$

4.3.3.4. Antioxidant Profile

Spectrophotometric methods were used for determination of total phenolics and total flavonoids contents and antioxidant activity (by DPPH• inhibition and FRAP assays) [53]. Extractions were performed in triplicate using water:ethanol (1:1) as solvent. The extraction was carried out on a hot plate at 40°C with magnetic stirring for 60 minutes. The obtained extracts were filtered and frozen at -20°C till analysis.

4.3.3.4.1. Total Phenolics Content

Total phenolics content was determined as described by Costa *et al.* [53]. Briefly, 150 μ L of Folin–Ciocalteu reagent (1:10) and 120 μ L of 7.5% m/v sodium carbonate were added to 30 μ L of extract, followed by incubation at 45°C for 15 min. Absorbance was monitored at 765 nm (BioTek, Synergy HT, PMT 49984, US). The calibration curve was calculated based on gallic acid standard, with linearity ranging between 5 and 100 mg/L, ($R^2 = 0.9998$). Analyses were performed in triplicate and the results expressed in mg of gallic acid equivalents (GAE) per g of sample.

4.3.3.4.2. Total Flavonoid Content

Total flavonoids content was determined as described elsewhere [53]. One ml of extract was mixed with 4 ml of distilled water and 300 μ L of 25% sodium nitrite. After 5 min at room

temperature, 300 μ L of 10% aluminum chloride were added, and after another min, 2 ml of 1 M sodium hydroxide solution and 2.5 ml of distilled water were also added. Absorbance was measured at 510 nm (BioTek, Synergy HT, PMT 49984, US). Catechin was used as standard for calibration purposes (2.5-400 mg/L; $R^2 = 0.9996$). Analyses were performed in triplicate and the results reported in mg catechin equivalents (CE) per g of sample.

4.3.3.4.3. Ferric Reducing Antioxidant Power

The ferric reducing antioxidant power (FRAP) was determined According to Costa et al. [53]. The FRAP reagent (0,3 M acetate buffer; 10 mM TPTZ and 20 mM ferric chloride) was added to an extract aliquot and the mixture was incubated for 30 min at 37°C, stored in dark place. Absorbance was measured at 595 nm (BioTek, Synergy HT, PMT 49984, US). The results were calculated using a calibration curve with ferrous sulfate (25-500 mg/L; $R^2 = 0.9998$). Analyses were performed in triplicate and the results expressed in mmol of ferrous sulfate equivalents (FSE) per g of sample.

4.3.3.4.4. DPPH• Scavenging Ability

DPPH• scavenging ability was determined according to method described by Costa *et al.* [53]. An amount of 270 μ L of a DPPH• solution (6×10^{-5} M) was added to 30 μ L of extract and the reaction was monitored by 40 min at 525 nm (BioTek, Synergy HT, PMT 49984, US). Trolox was used to prepare the calibration curve (5-175 mg/L; $R^2 = 0.9997$). Analyzes were performed in triplicate and the results expressed in mg of trolox equivalents (TE) per g of sample.

4.3.3.5. Obtention of Phenolic-Enriched Extracts of *G. tinctoria* leaves

Phenolics extraction was carried out using two different methodologies for comparison: a hydroethanolic maceration (GuT₁) and a ultrasonication extraction (GuT₂).

The hydroethanolic extraction was carried out as follows. The sample (100 g) was macerated, in triplicate, with different and subsequent ethanol: water mixtures (100:0, 50:50, 50:70, and 0:100; 2 L each). Maceration was performed for 24 hours for each solvent. The four extracts were combined and ethanol was removed at 40°C using a rotating vacuum evaporator. The concentrated aqueous extract was stored at 4°C prior to the next step.

Ultrasonic extraction was carried out based on the procedure described by Puga *et al* [54] but with modifications. The extraction was operated, in triplicate, using 100 g of sample macerated for 2 hours with 2 L of deionized water using an ultrasonic probe (BANDELIN

electronic, UW 50, Germany) for 30 min, at 25°C. The solution was then filtered and concentrated at 40°C using a rotating vacuum evaporator. The concentrated aqueous extract was stored at 4°C prior to the next step.

Phenolics enrichment was performed according to the methodology described and scaled up at Medicinal Plants and Drugs Research Institute - Shahid Beheshti University [16] using Diaion™ HP20LX and Releite EXA90 adsorbent resin columns (50 cm x 6 cm), separately. Both columns were activated with ethanol $\geq 99.8\%$ for 12 hours. The solvent was then removed and each column was washed five times with distilled water. A concentrated aqueous extract (GuT, 6 g) was diluted in distilled water (500 ml) and loaded into the resin column at a 5 ml/min flow rate and kept for 30 min till all phenolic structures were adsorbed in the resin porous. The column was washed with 5 L of distilled water to remove undesirable compounds such as sugars and chlorophyll; this solution was discarded. Afterwards, the column was eluted with ethanol $\geq 99.8\%$ (2 L) for phenolics desorption. Ethanol was removed using a rotating vacuum evaporator and the enriched phenolic fraction of *G. tinctoria* leaves was freeze-dried (TELSTAR, Cryodos freeze dryer, Spain) [17, 55].

4.3.3.5.1. Total Phenolic Content

Total phenolic content, in this case, was evaluate regarding GuT_{1,2} and enriched GuT_{1,2} (Table 3). The procedure was done according to *Section 3.4.1*. The extracts were diluted (1:1000) beforehand due to high phenolic content.

4.3.3.6. Bioassays

4.3.3.6.1. Cells and cell cultures

Two types of cell lines were used to perform bioassays. The MCF-7 breast cell line (an estrogen receptor (ER)-positive human breast epithelial adenocarcinoma cell line; ATCC HTB-22; passage numbers 30-38) and the AsPC-1 pancreatic cell line (a human pancreatic cell line with high metastatic rate; ATCC CRL-1682; passage numbers 36-42). MCF-7 and AsPC-1 cells were from ATCC (Manassas, VA, USA).

Cells were maintained in a humidified atmosphere of 5% CO₂-95% air and were grown in RPMI 1640 (catalogue #R6504, Sigma) supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 10% heat-inactivated FBS and 1% antibiotic/antimycotic (MCF-7 and AsPC-1 cells). The culture medium was renewed every 2–3 days, and the culture was split every 8 days. For the determination of viability, proliferation and growth, cells were seeded

on 24-well culture dishes (2 cm; Ø 16 mm; TPP®, Trasadingen, Switzerland) and used at 80–90% confluence.

To test the effects of the extracts on cell viability, culture growth and proliferation, cells were exposed to these compounds for 24 h in serum-free culture medium. The concentration of each extract to be tested was chosen based on literature and previous works [56, 57]. Tested extracts were dissolved in 30% ethanol (ratio v/v: 70% water : 30% ethanol). The final concentration of solvent in culture media was 1% (v/v). The extracts final concentration used were: 0.1 and 1 mg/mL (w/v). Control for the extracts was run in the presence of the solvent.

3.6.2. Cell viability.

Cells were exposed to extracts for 24 h (0.1 and 1mg/mL). After this period, cellular leakage of lactate dehydrogenase (LDH) to the extracellular culture medium was determined, as previously described [58]. LDH activity was expressed as the percentage of extracellular activity in relation to total cellular LDH activity.

4.3.3.6.3. Cell growth

Cells were exposed to extracts for 24 h (0.1 and 1mg/mL). At the end of treatment, culture growth was determined by the sulforhodamine B (SRB) assay, which reports on intracellular protein content, as previously described [58].

3.6.7. Evaluation of cell proliferation

Cells were exposed to extracts (0.1 and 1mg/mL) or vehicle for 24 h, and cell proliferation rates were determined by a 3H-thymidine incorporation assay, as described [56]. DNA synthesis rate was evaluated by quantification of incorporation of 3H-thymidine (mCi/mg total protein) [56]. Intracellular radioactivity was measured by liquid scintillation counting (LKB Wallac 1209 Rackbeta, Turku, Finland). The results were normalized for total protein content (Bradford method) [59].

4.3.3.7. Particles Production

4.3.3.7.1. Preparation of nano-phytosome and cholesterol-conjugated phytosome

The phytosome preparation followed a thin film hydration method with small modifications for nano-phytosome and cholesterol-conjugated phytosome, both loaded by the enriched phenolic extract [60, 61].

In case of the nano-phytosome, lecithin (containing 90% soybean; L- α -Phosphatidylcholine) with an optimum molar ratio of (1:1) relative to GuT₁Di₁ was used as a lipid layer. 50 mg of lecithin were primarily dissolved on 2 mL of dichloromethane and

vortexed (Shaker & Mixers Reax top, Heidolph, Germany) for 5 min, at room temperature, till a transparent yellow solution was obtained. 50 mg of GuT₁-Di₁ were dissolved on 20 ml of ethanol at 55 °C. The solution of lecithin-dichloromethane was added to GuT₁-Di₁ in ethanol and refluxed under stirred conditions, for 2 h, at 55°C, until a transparent solution was obtained. The solution was cooled down to 37°C and a thin layer was formed using a rotary vacuum evaporator (37°C; 60-200 rpm; 55 mbar of vacuum pressure). Then, N₂ was flowed into the thin layer for 1 min and the flask was sealed and kept overnight in a desiccator [42]. The thin layer was then hydrated with phosphate buffer (pH 5.5), at a vacuum pressure of 200 mbar, 200 rpm, and 40°C, for 10 min. The nano-phytosome (Ph-GuT₁-Di₁) was subjected to ultra-sonication for 25 min, at 60% amplitude, in pulsation mood (5:1 s) [60, 62].

In turn, the cholesterol-conjugated phytosome was prepared using lecithin and cholesterol loaded with GuT₁-Di₁ with an optimum molar ratio of 1:0.5:1, and fabricated using the above described procedure for nano-phytosome with some differences. Lecithin was added to GuT₁-Di₁ and stirred for 10 min. Then, the cholesterol solution was prepared just like lecithin solution on dichloromethane and sprayed into the mixture of lecithin/ GuT₁-Di₁, followed by reflux under stirred conditions for 2h, at 55°C. At the end, the solvent was evaporated, and a thin film was obtained using the same above mentioned condition. The cholesterol-conjugated phytosome (Ph/Chl-GuT₁-Di₁) was hydrated and ultra-sonicated using the same referred conditions, as well.

4.3.3.7.2. Polymeric Layer

GuT₁-Di₁ was employed to load a 10% (w/v) natural polymeric layer based on the solution viscosity and efficiency for feeding the spray dryer (mini spray-dryer B-290 from BÜCHI (Flawil, Switzerland). Maltodextrin, pectin, and starch, as simple matrixes, and a pectin/starch (1:1) complex were used for biopolymer coating. Four different polymeric solutions with 1% (w/v) of GuT₁-Di₁ in ultrapure water were prepared and stirred for 2 h at 55°C. The solutions were then injected into the spray dryer under the specific conditions described in *Section 3.7.4*. [63].

4.3.3.7.3. Secondary Layer

The secondary layer (binary coating or biopolymer-coated) was prepared with 10% (w/v) of natural polymer, for Ph-GuT₁-Di₁, and 15% (w/v) of natural polymer, for Ph/Chl-GuT₁-Di₁. For this purpose, the natural polymer or the complex (described in *Section 3.7.2*.) was added to Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ immediately after their preparation according to the described order:

- GuT₁-Di₁, lecithin, biopolymer (individual) (1:1:0.5:10)
- GuT₁-Di₁, lecithin, biopolymer (complex) (1:1:0.5:5:5)
- GuT₁-Di₁, lecithin, cholesterol, biopolymer (individual) (1:1:0.5:15)
- GuT₁-Di₁, lecithin, cholesterol, biopolymer (complex) (1:1:0.5:7.5:7.5).

For that, natural polymers were dissolved on ultrapure water and stirred for 2 h at 55°C. After the solutions were mixed, Ph-GuT₁-Di₁/natural polymer and Ph/Chl-GuT₁-Di₁/natural polymer were subjected to ultra-sonication for 5 min, at pulsation mode, and stirred for 20 min. The solutions were then injected into the spray dryer to obtain particles in the form of powder [63].

4.3.3.7.4. Spray drying conditions

The polymeric layer and the secondary layer were dried using a mini spray-dryer B-290 from BÜCHI (Flawil, Switzerland) with a standard 0.5 mm nozzle. The spray drying conditions were optimized regarding the feature of polymers and solutions viscosity. All solutions were prepared in ultrapure water and injected at a flow rate of 10 ml/min, aspiration 100% (36 m³/h), air pressure of 5.5-6 bar, and the nozzle cleaner was set to 3.

The inlet temperature was differently selected according to the type of the natural polymer used. For starch and pectin/starch, the inlet temperature was 130°C and the outlet temperature was 63 ± 3°C. In the case of pectin, the inlet temperature was 120°C and the outlet temperature 58 ± 2°C. The inlet temperature for maltodextrin was 115°C and the outlet temperature was 55 ± 2°C. Throughout the drying process, the emulsion was continuously agitated with a magnetic stirrer, at room temperature, to avoid aggregation of its solid content. Finally, the powders were recovered from the collector and stored in falcon tubes, sealed, and stored at 4°C. The product yield (%) was calculated by the amount of the powder recovered from the collector and the total mass content of the initial feed solution (Equation 2) [64, 65].

$$Yield = \frac{\text{Total weight of final powder}}{\text{Total weight of initial feed}} \times 100 \quad (2)$$

4.3.3.8. Physicochemical Properties of Nano/Micro-Particles

4.3.3.8.1. Fourier-transform Infrared Spectroscopy Analyses (FTIR)

The formation of phytosome and cholesterol-conjugated phytosome, regarding the existence of interactions between lecithin and cholesterol with GuT₁-Di₁, was evaluated for Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ in the powder form (dried with TELSTAR freeze dryer, Cryodos, Spain) by Fourier transform infrared (FT-IR) apparatus (Frontier, PerkinElmer, Beaconsfield, UK) equipped with an attenuated total reflectance (ATR) accessory (PerkinElmer, Beaconsfield, UK), operated by Spectrum software (PerkinElmer, Beaconsfield, UK).

4.3.3.8.2. Particle Size Distribution

Particle size distribution was evaluated by Particle Size Analyser (Brookhaven Instruments Corporation, operated by particle sizing v.5 Brookhaven instruments software, Holtsville, NY, USA). Particle size determination was performed for Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ in solution, and polymeric layer, as well as secondary layer in powder. In terms of solution, the droplets of Ph-GuT₁-Di₁ and Ph/Chl-GuT₁-Di₁ were dispersed on ethanol 99% and evaluated just after preparation. In terms of dried particles, a small amount of powder was dispersed in ethanol 99% and sonicated by SOLTEC (SONICA 2200MH S360Hz, Milano, Italy) for 7 min to avoid probable agglomeration and discover the inner layer. Particle's size was characterized by mean size in volume and number. The operations were done in 6 runs of 1 min at 21°C.

4.3.3.8.3. Scanning Electron Microscopy (SEM)

The particles surface morphology was characterized by surface structural morphology under image performed by SEM (Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M, Netherlands). Dried particles were put on a brass stub (carbon stub) using a double-sided adhesive tape, dried with N₂ and then coated with an electrically conductive (a thin layer of gold) in vacuum by sputtering in a Jeol JFC 100 apparatus. The operation was performed at Centro de Materiais da Universidade do Porto (CEMUP).

4.3.3.8.4. Zeta Potential (Surface charge)

The surface charge was measures in a ZetaPLAS (Zeta Potential Analyser, Brookhaven Instruments Corporation, operated by PALS Zeta Potential Analyser v.5 Brookhaven Instruments software, Holtsville, NY, USA). The process was evaluated in 6 runs of 30 s at

21°C. All samples regarding the external and inner layer were analysed regarding individual parameters in separated runs, including solution and dried powder.

4.3.3.8.5. Molecular Docking Arrangement

The 3D structure of phospholipids and selected phenolic compounds were downloaded from ChemSpider database. The structures were prepared and refined using Ligprep application in Maestro 12.8 (New York, Schrödinger). The 3D model of bilayers were generated using a MemGen webserver, which is specified for lipid membrane simulation systems [66]. The generated models were downloaded as PDB format and subjected for additional optimization using OPLS3 force field (Maestro 12.8). For the investigation interaction of selected phenolic compounds with the prepared bilayers the grid box (x=8.96, y=28.10, z=30.36, Size of 40, 40, 40 Å) was generated using grid generation. The interaction of ligands with bilayers was carried out using Glide application with extra precision (XP) level in Maestro 12.8. For each ligand, five poses have been used for evaluating docking interactions.

4.3.3.9. Statistical analysis

The results were expressed as mean $\pm$ standard deviation (for chemical assays) or mean $\pm$ standard error of mean (for bioassays), for at least 2 independent experiments. One-way ANOVA test followed by post-hoc comparisons according to Tukey's HSD were used to investigate significant differences between samples. Statistical differences between two groups under consideration were evaluated by Student's t-test. Values were considered statistically significant if $p < 0.05$. IBM SPSS 25 for Windows (IBM Corp., Armonk, NY) and GraphPad Prism 7.0 (San Diego, CA, USA) software were used for data treatment.

4.4. Conclusions

Concludingly, this study shows that the different parts of *G. tinctoria* are rich in nutrients, specifically protein and fiber, and could be an interesting alternative source of such components for human diet. *G. tinctoria* is also a source of antioxidants, namely, phenolic compounds, particularly their baby and adult leaves. Besides, it was possible to significantly enrich (more than 2-fold) the plant phenolic fraction using adsorbent resins (best overall performance for Diaion™ HP20LX), also showing that this enriched fraction presented anti-tumoral properties in breast and pancreas cancer cell lines.

Regarding the nano-phytosome formulation, a highest product yield (53-70%) was obtained for cholesterol-conjugated phytosome (attributed to a considerable stability confirmed by molecular docking). Furthermore, the surface charge of all particles was found

to be in an acceptable negative mood in which the nano-phytosome and cholesterol-conjugated phytosome provided the highest values which considerably enhance stability, flexibility and permeability of the substrate to cell membrane (especially through a brain broad barrier or skin penetration), resulting in improved penetration performance.

In addition, the schematic formation of the polymeric layer was estimated based on the probable formation of the coating layer expected and in accordance with particle size distribution of inner layers. The following outcome may have an important impact on future research regarding the mechanism of drug permeability in human body. Hence, molecular docking computational assay confirmed the accurate arrangement of phenolic compounds within the bilayer in terms of affinity based on polarity and size of phenolic compounds. Those features were confirmed by binding energy and schematic molecular arrangement images reported from molecular docking output. Although molecular docking arrangement was first developed for protein arrangement, it was shown, in the current study, that this methodology could be easily adjusted to all type of lipid-based layer for future research.

As a final remark, this work intends to contribute to highlight the potential of *G. tinctoria* as a source of nutrients and bioactive compounds and present an innovative strategy for its valorization.

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4.5. References

1. Bernardini, S.; Tiezzi, A.; Laghezza, M.V.; Ovidi, E. Natural products for human health: An historical overview of the drug discovery approaches. *Nat. Prod. Res.* **2018**, *32*, 1926–1950.
2. Williams, P.A.; Ogle, C.C.; Timmins, S.M.; La, C.G.D.; Clarkson, J. *Chilean Rhubarb (Gunnera tinctoria): Biology, Ecology and Conservation Impacts in New Zealand*; Department of Conservation: Wellington, New Zealand, 2005; pp. 1–27.
3. Fuller, D.Q.; Hickey, L.J. Systematics and leaf architecture of the Gunneraceae. *Bot. Rev.* **2005**, *71*, 295–353.
4. Gioria, M.; Osborne, B.A. Biological flora of the british Isles: *Gunnera tinctoria*. *Ecology* **2013**, *101*, 243–264.
5. Silva, L.; Tavares, J.; Pena, A. Ecological basis for the control of *Gunnera tinctoria* in São Miguel Island. In Proceedings of the Second International Weed Control Congress Copenhagen, Brown, Copenhagen, Denmark, 25–28 June 1996.
6. Petzold, G.; Catril, G.; Duarte, C. Caracterización fisicoquímica de pecíolos del pangué (*Gunnera tinctoria*). *Rev. Chil. Nutr.* **2006**, *33*, 539–543.
7. Estomba, D.; Ladio, A.; Lozada, M. Medicinal wild plant knowledge and gathering patterns in a Mapuche community from North-western Patagonia. *J. Ethnopharmacol.* **2006**, *103*, 109–119.
8. Sabando, C.; Rodríguez-Díaz, M.; Ide, W.; Pastene, E.; Avello, M.; Simirgiotis, M.; Rojas, S.; Villarroel, E.; Silva-Grecchi, T.; Gutiérrez, C. Improvement of endothelial function by *Gunnera tinctoria* extract with antioxidant properties. *J. Biol. Res.* **2020**, *53*, 1–14.
9. Silva, C.; Correia-Branco, A.; Andrade, N.; Ferreira, A.C.; Soares, M.L.; Sonveaux, P.; Stephenne, J.; Martel, F. Selective pro-apoptotic and antimigratory effects of polyphenol complex catechin: Lysine 1: 2 in breast, pancreatic and colorectal cancer cell lines. *Eur. J. Pharmacol.* **2019**, *859*, 172533.
10. Karakaya, S. Bioavailability of phenolic compounds. *Crit. Rev. Food Sci. Nutr.* **2004**, *44*, 453–464.
11. Grgić, J.; Šelo, G.; Planinić, M.; Tišma, M.; Bucić-Kojić, A. Role of the encapsulation in bioavailability of phenolic compounds. *Antioxidants* **2020**, *9*, 923.
12. Bayati, M.; Mehrbod, P.; Ebrahimi, S.N. Natural products as inhibitors of COVID-19 main protease—A virtual screening by molecular docking. *Res. Sq.* **2020**, 1–13, doi.org/10.21203/rs.3.rs-57351/v1.
13. Ballester, P.J.; Mitchell, J.B. A machine learning approach to predicting protein–ligand binding affinity with applications to molecular docking. *J. Bioinform.* **2010**, *26*, 1169–1175.
14. Commission, E. Commission Directive 2006/141/EC of 22 December 2006 on infant formulae and follow-on formulae and amending Directive 1999/21/EC. *Off. J. Eur. Union* **2006**, *49*, 1–33.

15. Zamorano, P.; Rojano, B.I.; Morales, M.; Magariños, H.; Godoy, P.; Muñoz, O. Biological and antioxidant activity of *Gunnera tinctoria* (Nalca). *J. Med. Plants Res.* **2017**, *11*, 318–330.
16. Davoodi, M.; Rustaiyan, A.; Ebrahimi, S.N. Monoterpene flavonoid from aerial parts of *Satureja khuzistanica*. *Rec. Nat. Prod.* **2018**, *12*, 175–178.
17. Kokabi, M.; Ebrahimi, S.N. Polyphenol enriched extract of pomegranate peel; A novel precursor for the biosynthesis of zinc oxide nanoparticles and application in sunscreens. *Pharm. Sci.* **2020**, *27*, 102–110.
18. Scordino, M.; Di Mauro, A.; Passerini, A.; Maccarone, E. Selective recovery of anthocyanins and hydroxycinnamates from a byproduct of citrus processing. *J. Agric. Food Chem.* **2005**, *53*, 651–658.
19. Mahato, N.; Sinha, M.; Sharma, K.; Koteswararao, R.; Cho, M.H. Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. *Foods* **2019**, *8*, 523.
20. Bridi, R.; Giordano, A.; Peñailillo, M.F.; Montenegro, G. Antioxidant effect of extracts from native Chilean plants on the lipoperoxidation and protein oxidation of bovine muscle. *Molecules* **2019**, *24*, 3264.
21. Hussain, T.; Tan, B.; Yin, Y.; Blachier, F.; Tossou, M.C.; Rahu, N. Oxidative stress and inflammation: What polyphenols can do for us? *Oxid. Med. Cell. Longev.* **2016**, *2016*, 7432797.
22. Yilmaz, Y.; Toledo, R.T. Major flavonoids in grape seeds and skins: Antioxidant capacity of catechin, epicatechin, and gallic acid. *J. Agric. Food Chem.* **2004**, *52*, 255–260.
23. Oyenih, A.; Smith, C. Are polyphenol antioxidants at the root of medicinal plant anti-cancer success? *J. Ethnopharmacol.* **2019**, *229*, 54–72.
24. Teleki, A.; Hitzfeld, A.; Eggersdorfer, M. 100 years of vitamins: The science of formulation is the key to functionality. *Kona* **2013**, *30*, 144–163.
25. Tontul, I.; Topuz, A. Spray-drying of fruit and vegetable juices: Effect of drying conditions on the product yield and physical properties. *Trends Food Sci. Technol.* **2017**, *63*, 91–102.
26. Cano-Higueta, D.M.; Vélez, H.A.V.; Telis, V.R.N. Microencapsulation of turmeric oleoresin in binary and ternary blends of gum Arabic, maltodextrin and modified starch. *Cienc. Agrotecnologia* **2015**, *39*, 173–182.
27. Jovanović, A.A.; Lević, S.M.; Pavlovic, V.B.; Markovic, S.B.; Pjanovic, R.V.; Đorđević, V.B.; Nedović, V.; Bugarski, B.M. Freeze versus spray drying for dry wild thyme (*Thymus serpyllum* L.) extract formulations: The impact of gelatin as a coating material. *Molecules* **2021**, *26*, 3933.
28. Keshani, S.; Daud, W.R.W.; Nourouzi, M.; Namvar, F.; Ghasemi, M. Spray drying: An overview on wall deposition, process and modeling. *J. Food Eng.* **2015**, *146*, 152–162.
29. Wang, Y.; Xie, Y.; Xu, D.; Lin, X.; Feng, Y.; Hong, Y. Hydroxypropyl methylcellulose reduces particle adhesion and improves recovery of herbal extracts during spray drying of Chinese herbal medicines. *Dry. Technol.* **2014**, *32*, 557–566.
30. Hou, Z.; Li, Y.; Huang, Y.; Zhou, C.; Lin, J.; Wang, Y.; Cui, F.; Zhou, S.; Jia, M.; Ye, S. Phytosomes loaded with mitomycin C–soybean phosphatidylcholine complex developed for drug delivery. *Mol. Pharm.* **2013**, *10*, 90–101.

31. Zhang, J.; Tang, Q.; Xu, X.; Li, N. Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. *Int. J. Pharm.* **2013**, *448*, 168–174.
32. Nazari, M.; Ghanbarzadeh, B.; Kafil, H.S.; Zeinali, M.; Hamishehkar, H. Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model. *Colloids Interface Sci. Commun.* **2019**, *30*, 100176.
33. Souza, J.E.D.; Casanova, L.M.; Costa, S.S. Bioavailability of phenolic compounds: A major challenge for drug development? *Rev. Fitos (Rio J.)* **2015**, *9*, 55–67.
34. Khan, I.; Saeed, K.; Khan, I. Nanoparticles: Properties, applications and toxicities. *Arab. J. Chem* **2019**, *12*, 908–931.
35. Rasaei, S.; Ghanbarzadeh, S.; Mohammadi, M.; Hamishehkar, H. Nano phytosomes of quercetin: A promising formulation for fortification of food products with antioxidants. *J. Pharm. Sci.* **2014**, *20*, 96–101.
36. Pieczykolan, E.; Kurek, M.A. Use of guar gum, gum arabic, pectin, beta-glucan and inulin for microencapsulation of anthocyanins from chokeberry. *Int. J. Biol.* **2019**, *129*, 665–671.
37. Ansari, F.; Sobhani, A.; Salavati-Niasari, M. Simple sol-gel synthesis and characterization of new CoTiO₃/CoFe₂O₄ nanocomposite by using liquid glucose, maltose and starch as fuel, capping and reducing agents. *J. Colloid Interface Sci.* **2018**, *514*, 723–732.
38. Papoutsis, K.; Golding, J.B.; Vuong, Q.; Pristijono, P.; Stathopoulos, C.E.; Scarlett, C.J.; Bowyer, M. Encapsulation of citrus by-product extracts by spray-drying and freeze-drying using combinations of maltodextrin with soybean protein and ι-Carrageenan. *Foods* **2018**, *7*, 115.
39. Cortes, U.A.B.; Gutiérrez, M.C.; Mendoza, D.G.; Salitre, L.G.; Vargas, A.S.; Catzim, C.E.A.; Durán, C.C.; Valenzuela, B.E.L. Microencapsulation and antimicrobial activity of extract acetone-methanol of *Hibiscus sabdariffa* L. using a blend modified starch and pectin as a wall material. *Ind. Crops Prod.* **2021**, *170*, 113725.
40. Clogston, J.D.; Patri, A.K. Zeta potential measurement. In *Characterization of Nanoparticles Intended for Drug Delivery*, 1st ed.; McNeil, S., Ed.; Springer: Gewerbesrasse, Switzerland, 2011; Volume 697, pp. 63–70.
41. Campos, J.C.; Ferreira, D.C.; Lima, S.; Reis, S.; Costa, P.J. Swellable polymeric particles for the local delivery of budesonide in oral mucositis. *Int. J. Pharm.* **2019**, *566*, 126–140.
42. Singh, R.P.; Gangadharappa, H.; Mruthunjaya, K. Phytosome complexed with chitosan for gingerol delivery in the treatment of respiratory infection: In vitro and in vivo evaluation. *Eur. J. Pharm. Sci.* **2018**, *122*, 214–229.
43. Hindarto, C.K.; Surini, S.; Saputri, F.C.; Irawan, C. In vivo evaluation of luteolin-loaded phytosome. *J. Pharm. Innov.* **2017**, *6*, 347–349.
44. Nagaraju, P.G.; Sindhu, P.; Dubey, T.; Chinnathambi, S.; Priyadarshini, P.C.G.; Rao, P.J. Influence of sodium caseinate, maltodextrin, pectin and their Maillard conjugate on the stability, in vitro release, anti-oxidant property and cell viability of eugenol-olive oil nanoemulsions. *Int. J. Biol. Macromol.* **2021**, *183*, 158–170.
45. Jeong, O.; Shin, M. Preparation and stability of resistant starch nanoparticles, using acid hydrolysis and cross-linking of waxy rice starch. *Food Chem.* **2018**, *256*, 77–84.

46. Li, X.; Kong, X.; Shi, S.; Zheng, X.; Guo, G.; Wei, Y.; Qian, Z. Preparation of alginate coated chitosan microparticles for vaccine delivery. *B.M.C. Biotechnol.* **2008**, *8*, 1–11.
47. Choudhary, M.I.; Shaikh, M.; tul-Wahab, A.; ur-Rahman, A. In silico identification of potential inhibitors of key SARS-CoV-2 3CL hydrolase (Mpro) via molecular docking, MMGBSA predictive binding energy calculations, and molecular dynamics simulation. *PLoS ONE* **2020**, *15*, 1–15.
48. Bozzuto, G.; Molinari, A. Liposomes as nanomedical devices. *Int. J. Nanomed.* **2015**, *10*, 975.
49. Ghanbarzadeh, B.; Babazadeh, A.; Hamishehkar, H. Nano-phytosome as a potential food-grade delivery system. *Food Biosci.* **2016**, *15*, 126–135.
50. AOAC. Official Methods of Analysis of Association of Analytical Chemistry, 19th ed.; AOAC International: Gaithersburg, MD, USA, 2012.
51. Ayadi, M.; Abdelmaksoud, W.; Ennouri, M.; Attia, H. Cladodes from *Opuntia ficus indica* as a source of dietary fiber: Effect on dough characteristics and cake making. *Ind. Crops Prod.* **2009**, *30*, 40–47.
52. Food, F. Agricultural organization of united nations. Food energy-methods of analysis and conversion factors. *FAO Food Nutr. Pap.* **2003**, 1–77.
53. Costa, A.S.; Alves, R.C.; Vinha, A.F.; Barreira, S.V.; Nunes, M.A.; Cunha, L.M.; Oliveira, M.B.P. Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. *Ind. Crops Prod.* **2014**, *53*, 350–357.
54. Puga, H.; Alves, R.C.; Costa, A.S.; Vinha, A.F.; Oliveira, M.B.P. Multi-frequency multimode modulated technology as a clean, fast, and sustainable process to recover antioxidants from a coffee by-product. *J. Clean. Prod.* **2017**, *168*, 14–21.
55. Shehzad, O.; Jin-Ha, I.; Park, Y.; Wan-Ha, Y.; Shik-Kim, Y. Development of a rapid and convenient method to separate eight ginsenosides from *Panax ginseng* by high-speed counter-current chromatography coupled with evaporative light scattering detection. *J. Sep. Sci.* **2011**, *34*, 1116–1122.
56. Silva, C.; Nunes, C.; Correia-Branco, A.; Araújo, J.R.; Martel, F. Insulin exhibits an antiproliferative and hypertrophic effect in first trimester human extravillous trophoblasts. *Reprod. Sci.* **2017**, *24*, 582–594.
57. Andrade, N.; Araújo, J.R.; Correia-Branco, A.; Carletti, J.V.; Martel, F. Effect of dietary polyphenols on fructose uptake by human intestinal epithelial (Caco-2) cells. *J. Funct. Foods* **2017**, *36*, 429–439.
58. Azevedo, C.; Correia-Branco, A.; Araújo, J.R.; Guimaraes, J.T.; Keating, E.; Martel, F. The chemopreventive effect of the dietary compound kaempferol on the MCF-7 human breast cancer cell line is dependent on inhibition of glucose cellular uptake. *Nutr. Cancer* **2015**, *67*, 504–513.
59. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
60. Fathi, F.; Ebrahimi, S.N. Investigation of physiochemical properties nanophytosome obtained from polyphenolic enrich fraction of *Satureja khuzistanica* by Freeze-drying. *Nashrieh Shimi va Mohandesi Shimi Iran* **2021**, 1–15.
61. Gandhi, A.; Dutta, A.; Pal, A.; Bakshi, P. Recent trends of phytosomes for delivering herbal extract with improved bioavailability. *J. Pharmacogn. Phytochem.* **2012**, *1*, 6–14.

62. Anwar, E.; Farhana, N. Formulation and evaluation of phytosome-loaded maltodextrin-gum Arabic microsphere system for delivery of *Camellia sinensis* extract. *J. Young Pharm.* **2018**, *10*, 56–62.
63. El-Messery, T.M.; Altuntas, U.; Altin, G.; Özçelik, B. The effect of spray-drying and freeze-drying on encapsulation efficiency, in vitro bioaccessibility and oxidative stability of krill oil nanoemulsion system. *Food Hydrocoll.* **2020**, *106*, 105890.
64. Tuyen, C.K.; Nguyen, M.H.; Roach, P.D.; Stathopoulos, C.E. Microencapsulation of gac oil: Optimisation of spray drying conditions using response surface methodology. *J. Powder Technol.* **2014**, *264*, 298–309.
65. Santana, A.A.; Oliveira, R.A.d.; Kurozawa, L.E.; Park, K.J. Microencapsulation of pequi pulp by spray drying: Use of modified starches as encapsulating agent. *Eng. Agric.* **2014**, *34*, 980–991.
66. Knight, C.J.; Hub, J.S. MemGen: A general web server for the setup of lipid membrane simulation systems. *J. Bioinform.* **2015**, *31*, 2897–2899.

Chapter 5

Formulation of Coffee Silverskin Lipid-based Nano-carriers Coating by Natural Polymer: Physiochemical Properties, In Vitro Release Mechanism, and In Silico Molecular Modeling

Formulation of Coffee Silverskin Lipid-based Nano-carriers Coating by Natural Polymer: Physiochemical Properties, *In Vitro* Release Mechanism, and *In Silico* Molecular Modeling

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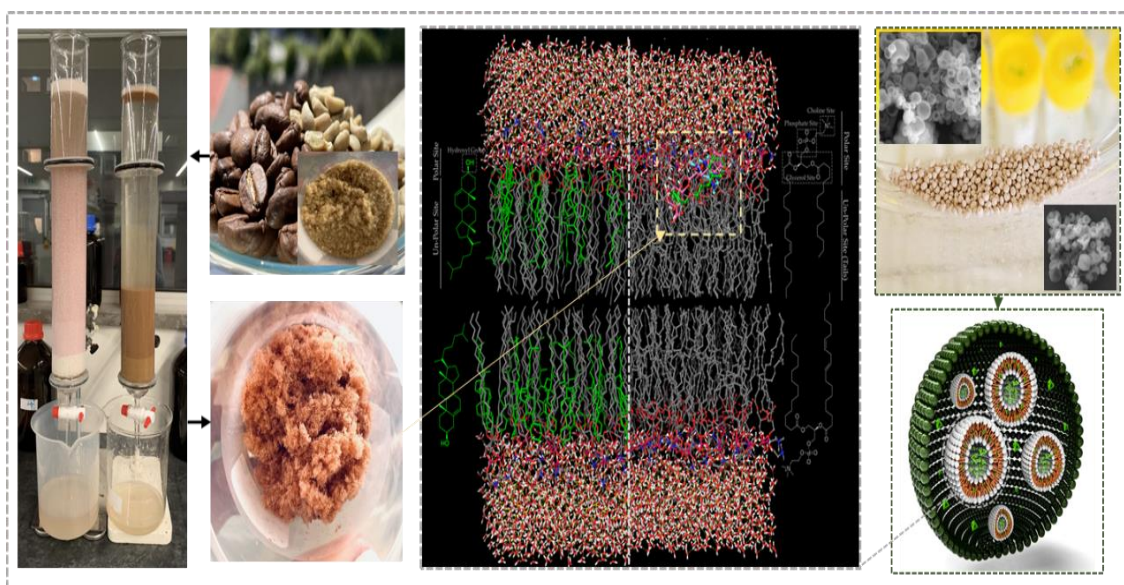
5.1. Abstract

Designing strategies for the effective transformation of food waste into high value products is a priority to address environmental sustainability concerns. Coffee silverskin is the major by-product from coffee roasting industry, rich in compounds with health benefits. Such composition gives it the potential to be transformed into high value products. In this study, silverskin extracts were enriched in caffeine and chlorogenic acid, by adsorbent column chromatography, with content increases of 3.08 and 2.75 times, respectively. The enriched fractions were loaded on nano-phytosomes, and these structures incorporated by cholesterol to improve the permeation rate. Subsequently, these nano-lipid carriers were coated with different natural polymers to improve the protection and stability against degradation. The produced particles were evaluated regarding product yield, encapsulation efficiency, loading capacity, particle size, surface charge, and *in vitro* release assay. All samples exhibited anionic surface charge. FTIR and molecular docking confirmed the

interaction between phytoconstituents and lipid bilayers. The best docking score observed from chlorogenic acid exhibited stronger hydrogen bonding, resulting in stronger interaction. The release mechanism fit well with Korsmeyer-Peppas and Higuchi kinetic models compared with other models. Moreover, most of the formulated particles revealed to follow the diffusion-Fick law and anomalous transport.

Keywords: Coffee Silverskin; By-product; phytosome; cholesterol incorporation; molecular docking; Kinetic release model.

5.2. Graphical abstract



5.3. Main Text

5.3.1. Introduction

Food processing industries produce relevant amounts of by-products that are discarded, despite their content in valuable nutritional and biological constituents. Their valorization can play a vital role in financial prospects [1]. Therefore, a current concern is to implement the concept of sustainable food waste management worldwide in order to use by-products and convert them into new products of high value, while assuring the development and exploitation of eco-friendly techniques to achieve that goal [2].

Coffee silverskin (SI) is a light brownish pellicle integument of the raw coffee bean. These pellicles are detached from the coffee bean during the industrial roasting process. Given the abundance of coffee factories, SI has become a relevant by-product that should be

valorized based on its richness in several phytochemicals, such as caffeine and phenolic compounds [3,4].

Fractionation of plant crude extracts using polymeric adsorbent resin column chromatography promotes an enrichment of bioactive compounds based on functionality and polarity of the phytoconstituents. This enrichment provides an enhancement of the phytoconstituents bioactivity at a low dosage [5].

To overcome limitations related to low stability, absorption rates, bioavailability, or functionality of some phytoconstituents, a wise choice is to focus on natural-based carriers for safe and targeted delivery [6]. Accordingly, phytosomes are targeted delivery carriers, characterized by a specific bonding between phospholipids and the compounds of interest (e.g., drugs, phytochemicals, etc.), with possible distinctive configurations. In other words, phytosomes are non-polar carriers for the efficient delivery of bioactive components through the cell membrane. Indeed, they are considered suitable carriers for medicines, dietary supplements, or cosmetic products due to the improved bioavailability and permeation of compounds through the cell membrane, while protecting natural sensitive structures against environmental conditions [7].

Taking into account the low permeation of polar phytoconstituents through the cell membrane, nano-phytosomes based on soybean lecithin (containing 94% phosphatidylcholine) assure significant similarities with the cell membrane to facilitate permeation, a very important requisite for the development of highly efficient delivery carriers [8]. Additionally, the incorporation of cholesterol into the nano-phytosome lipid layer (cholesterol-incorporated nano-phytosomes) by surface modification provides improved stability of the nano-phytosome in solution media [9].

Natural polymers (NP), which are non-toxic, biodegradable, and biocompatible, can also protect phytoconstituents or drugs, through encapsulation techniques, improving stability during storage and handling, and also allowing a controlled release [10]. Therefore, NP coating of extracts or nano-phytosomes (being, in this last case, a secondary coating) in the form of small capsules obtained by spray-drying can also be applied to enhance protection, solubility, and stability of the compounds in acidic media, and to improve shelf-life during handling and storage [11].

The main aim of this work was to formulate and compare different types of nano/micro-carriers loaded with an SI extract enriched in caffeine and phenolic compounds with antioxidant properties. A potential application, for instance, in the dietary supplement field

is foreseen, more specifically to improve mental and physical performance, competing with other products already existing in the market [12]. A final product is expected that is well absorbed and tolerated, which efficiently delivers the mentioned phytochemicals in targeted body areas, to more effective bioactivity at lower dosages.

For that, lipid carriers (nano-phytosomes or cholesterol-incorporated nano-phytosomes) were applied as first layers to an SI-enriched extract, in order to enhance the phytochemicals permeation rates. In parallel, and for comparison, different NPs were also used as first layers, directly coating the SI-enriched extract (simple encapsulation). Additionally, both lipid-based layers (nano-phytosomes and cholesterol-incorporated nano-phytosomes) were also coated with different NPs as secondary coating layers to increase stability during storage and handling, improve adhesion affinity of particles (which enhance phytochemicals uptake), and provide a controlled release [13–15]. Afterwards, the *in silico* molecular docking was used to confirm the molecular arrangement and interactions among the lipid bilayer and specific phytochemicals [16]. Indeed, this study presents, for the first time, by molecular docking, the interaction between the nano-phytosome bilayer and two major constituents of SI, namely, caffeine and chlorogenic acid (5-caffeoylquinic acid). The arrangement of other phytochemicals within similar lipid bilayers was also recently reported for the first time by our research group [16].

5.3.2. Materials and Methods

5.3.2.1. Chemicals and Standards

Gallic acid (CAS 149-91-7) and chlorogenic acid 95% (CAS 327-97-9) were supplied by Sigma, Life Science, Shanghai, China. Apple pectin (CAS 9000-09-5) was purchased from LABChem, Lisbon, Portugal; maltodextrin (CAS 32671-12) from Fargon, Barcelona, Spain; and gum Arabic from Guinama, Valencia, Spain. Water-soluble starch from potato (CAS 9005-84-9) was supplied from PanReac-Applichem, Darmstadt, Germany. Soybean lecithin (CAS 8002-43-5; purity of 90%) was obtained from Alfa Aesar, Thermofisher, Kandel, Germany. Cholesterol (CAS C8667-25G; purity $\geq 99\%$) was supplied by Sigma, Life Science, MO, USA. Absolute ethanol ($\geq 99.8\%$ and 96% purity), dichloromethane ($\geq 99.9\%$), and HPLC grade analytical solvents were purchased from Honeywell (Hessen, Germany). Relite EXA90 (ion exchange resin) Diaion™ HP20LX (spherical porous polystyrene resin) were both kindly supplied by the Resindion group of Mitsubishi Chemical, Binasco, Italy. Sodium phosphate dibasic heptahydrate ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$) and sodium phosphate monobasic monohydrate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$) were purchased from PanReac, Barcelona,

Spain. Ultra-pure water was obtained from Seralpur PRO 60 CN and Seradest LFM 20 water purification systems.

5.3.2.2. Samples

SI was kindly provided by a Portuguese coffee roaster industry (BICAFÉ). It resulted from the roast of commercial batches composed of both *Coffee arabica* and *Coffea canephora* beans and was representative of the major by-product of the factory.

5.3.2.3. Experimental Design

Chart 1 aims to clearly introduce the flow of the preparation steps, regarding the type of extraction, enrichment, and type of coating layer in terms of first and secondary layers. The final product codes for each category are also presented in Chart 1.

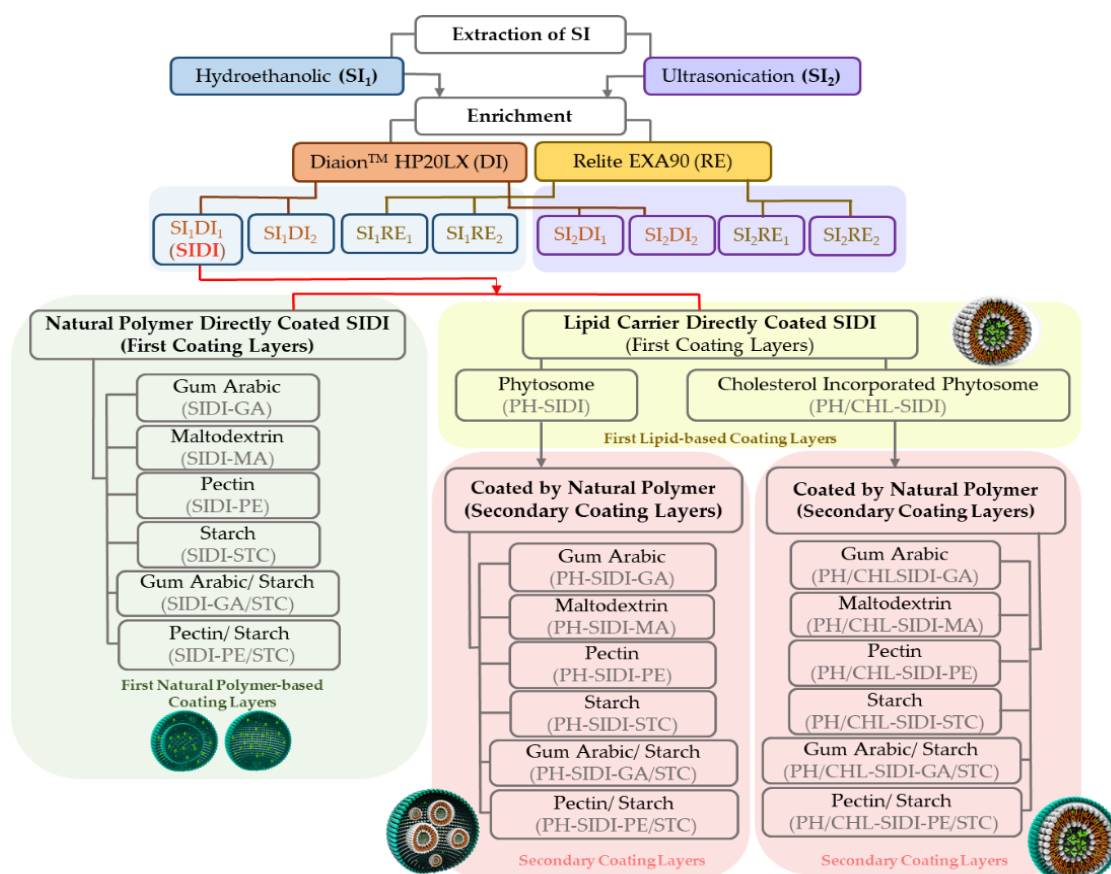


Chart 1. Flow of the preparation steps, regarding the type of extraction, enrichment, and type of coating layer (first or secondary layers). SI, silverskin; DI, Diaion resin; RE, Relite resin; GA, gum Arabic; MA, maltodextrin; PE, pectin; STC, starch; PH, nano-phytosome; PH/CHL, cholesterol incorporated nano-phytosome. Note: DI₁ and RE₁ series are the enriched fractions (regarding caffeine and phenolics contents) obtained with DI and RE, respectively. DI₂ and RE₂ series are the discarded fractions (rich in sugars, chlorophylls, etc.).

SI was extracted using different techniques (maceration with a hydroethanolic solvent (SI_1) or ultrasonication (SI_2)) and each extract was enriched (to increase caffeine and phenolics contents) using one of these resins: Diaion™ HP20LX (DI) or Relite EXA90 (RE). DI_1 and RE_1 series are the enriched fractions obtained with DI and RE, respectively. DI_2 and RE_2 series are the rejected fractions (explained in Section 2.4. and Chart 2)

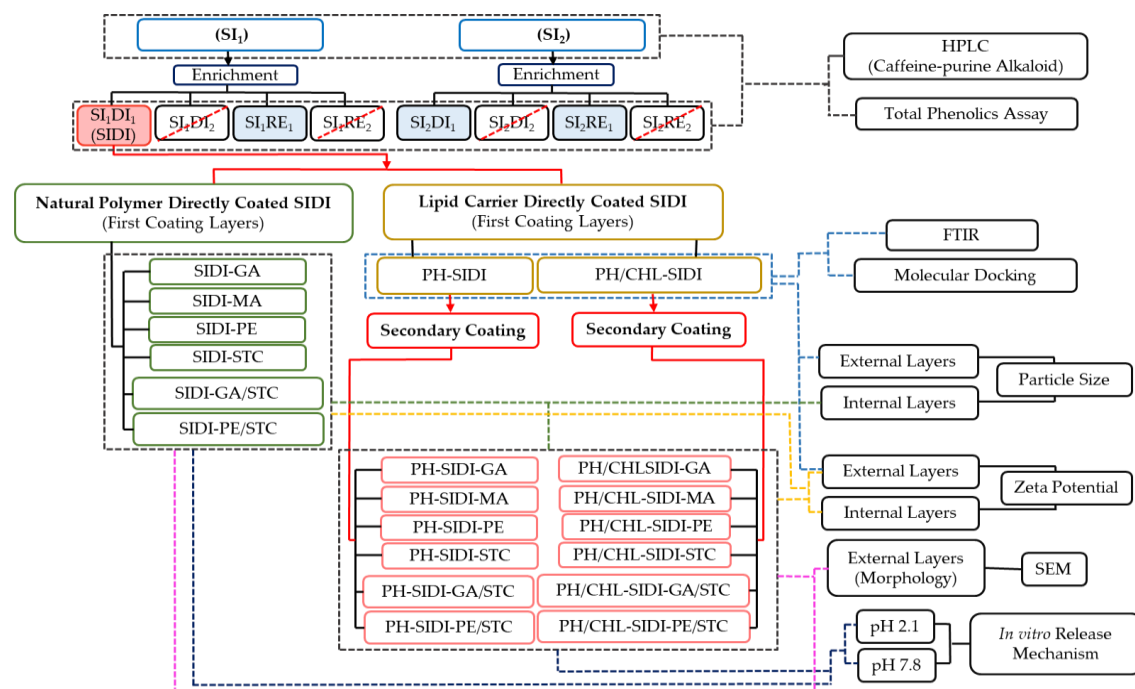


Chart 2. Sample categorization and analyses applied to each category. SI, silverskin; GA, gum Arabic; MA, maltodextrin; PE, pectin; STC, starch; PH, nano-phytosome; PH/CHL, cholesterol incorporated nano-phytosome. DI_1 and RE_1 series are the enriched fractions (regarding caffeine and phenolics contents) obtained with Diaion™ HP20LX (DI) and Relite EXA90 (RE) adsorbent resins, respectively. DI_2 and RE_2 series are the discarded fractions (rich in sugars, chlorophylls, etc.).

The most enriched fraction (SI_1DI_1) was then selected for the following formulation procedures. SI_1DI_1 (from now on named SIDI) was loaded into nano-phytosomes or cholesterol-incorporated nano-phytosomes as first coating layers (Chart 1, in yellow). In parallel, SIDI was also directly coated with NP as the first coating layer (single coating layer) (Chart 1, in green). Meanwhile, to improve the stability and protection of lipid-based carriers, NPs were also used to prepare secondary layers, encapsulating the abovementioned nano-phytosomes and cholesterol-incorporated nano-phytosomes (Chart 1, in pink). In sum, two types of the first coating layers were prepared, namely NP-based and lipid-based. In addition, these lipid-based layers (nano-phytosomes and cholesterol-incorporated nano-phytosomes) were also coated with different NPs as the secondary layer.

5.3.2.4. Preparation of SI Extracts

SI was extracted using two different methodologies: A hydroethanolic maceration (SI₁) and an ultrasonication extraction (SI₂).

The hydroethanolic maceration used 100 g of SI and occurred in a 5 L glass flask during 24 h for each solvent. It was performed in triplicate, with different and subsequent ethanol:water mixtures (100:0, 50:50, 50:70, and 0:100; 2 L each). A filtration step followed each maceration. The four extracts were combined, and ethanol was recovered using a rotating vacuum evaporator at 40 °C. The concentrated aqueous extract (SI₁) was stored at 2 °C prior to the next step.

Ultrasonic extraction was performed, in triplicate, based on the procedure described previously by Puga et al. [17] with some modifications. The sample (100 g) was macerated for 2 h in 2 L of deionized water, and sonicated with an ultrasonic probe (BANDELIN electronic, UW 50, Berlin, Germany) during 30 min, at 25 °C. After, the solutions were filtered and concentrated using a rotating vacuum evaporator at 40 °C. The concentrated aqueous extract (SI₂) was stored at 4 °C prior to the next step.

The enrichment was designed to concentrate SI₁ and SI₂ regarding the contents of caffeine and total phenolics, and was performed according to the methodology described and scaled up at the Medicinal Plants and Drugs Research Institute—Shahid Beheshti University [5]. Diaion™ HP20LX (DI) and Relite EXA90 (RE) adsorbent resin columns (50 cm × 6 cm) were used separately, according to the procedure described in Chart 1. Both columns were activated with ethanol ≥ 99.8% for 12 h, followed by solvent removal and washing with 5 L of distilled water. A 6 g amount of the concentrated aqueous extracts (SI₁ and SI₂) were diluted in distilled water (500 mL) and loaded into the resin columns at a flow rate of 5 mL/min and kept for 30 min for bioactive compounds (caffeine and phenolics) adsorption in resin porous. A new washing of the resin columns, with 5 L of distilled water, was performed to remove residues (sugar, chlorophyll, etc.). These fractions (SI₁DI₂, SI₂DI₂, SI₁RE₂, and SI₂RE₂) were discarded (Chart 2). Afterwards, the columns were eluted with ethanol ≥ 99.8% (2 L) for the desorption of caffeine and phenolics. Ethanol was recovered using a rotating vacuum evaporator and the enriched fractions were frozen at –80 °C, and lyophilized (48 h, –80 °C, 0.022 mbar; TELSTAR, Cryodos freeze dryer, Barcelona, Spain). These lyophilized enriched fractions (SI₁DI₁, SI₁RE₁, SI₂DI₁, SI₂RE₁) were kept in the refrigerator at 2–4 °C for further analysis and to follow the next steps in accordance with Charts 1 and 2) [18,19].

5.3.2.5. Phytochemical Analysis

5.3.2.5.1. Caffeine Analysis by High-Performance Liquid Chromatography (HPLC)

The caffeine content of the extracts was analyzed using an HPLC-DAD system (Jasco, Tokyo, Japan). This system consisted of an LC-NetII/ADC hardware interface, an automatic sampler (Jasco AS-2057 Plus), a pump (Jasco PU-2089 Plus), a multi-wavelength diode array detector (DAD, Jasco MD-2018 Plus), and a column oven (Jasco CO-2060 Plus). The gradient elution used was the following: 0 min, 5% B; 40 min, 25% B; 55 min, 45% B; 60 min, 60% B; 65 min, 5% B (solvent A: 0.5% acetic acid; solvent B: 100% methanol), with a flow rate of 1.1 mL/min. The chromatographic column was a Zorbax-SB-C18 (5 μ m, 250 mm $\times$ 4.6; Agilent Technologies, Santa Clara, CA, USA), at 28 °C. The DAD recorded data from 200 to 600 nm were monitored at 274 nm. For HPLC analyses, the lyophilized extracts were dissolved in H₂O (10 mg/mL) and the injected volume was 20 μ L. Caffeine was used as the standard for HPLC analyses validation. The calibration curve ($y = 36,096x - 227,800$; $R^2 = 0.9996$) was constructed in the linear range of 1.5–800 μ g/mL. The detection limit of the method was 1.24 μ g/mL.

5.3.2.2.2. Total Phenolics Assay

Total phenolics contents were determined as described by Costa et al. [3]. Briefly, 150 μ L of the Folin–Ciocalteu reagent (1:10) and 120 μ L of a sodium carbonate solution (7.5% *m/v*) were added to 30 μ L of the extract, followed by incubation at 45 °C for 15 min and 30 min at room temperature. Absorbance was monitored at 765 nm (BioTek, Synergy HT, PMT 49,984, Winooski, VT, USA). Two calibration curves were prepared using gallic acid (5–100 μ g/mL; $y = 0.0085x - 0.0544$; $R^2 = 0.998$) and chlorogenic acid (5–140 μ g/mL; $y = 0.0056x - 0.0364$; $R^2 = 0.9973$) as standards.

5.3.2.6. Particle Preparation and Characterization

5.3.2.6.1. Preparation of First Coating Layers: Coating Layers Based on Natural Polymers

Gum Arabic (GA), maltodextrin (MA), pectin (PE), and starch (STC), individually, and blends (1:1) of pectin/starch (PE/STC) and gum Arabic/starch (GA/STC) were used as the first NP coating layers (Chart 1, Table 1). The feed solution had 10% (w/v) of NP and 1% (w/v) of SIDI (selected as the best enriched extract, Chart 1). These proportions were used because they do not compromise the layer efficiency and have an adjusted viscosity for feeding the spray dryer. In this context, six different NP-based solutions were prepared in 100 mL of distilled water. The prepared solutions were stirred and homogenized for 2 h at

55 °C and, afterwards, injected into the spray dryer under specific conditions as de-scribed in Section 2.6.5.

Table 1. Encapsulation Design: ratio between ingredients of each formulation

	Categories	Formula	Ingredient Ratio			
			SIDI	LEC	CHL	NP
Lipid-based coating	Phytosome (Lecithin)	PH-SIDI	1	1	0	0
	Phytosome Incorporated by cholesterol	PH/CHL-SIDI	1	1	0.5	0
NP-based coating	Single wall layer	SIDI-GA	1	0	0	10
		SIDI-MA				
		SIDI-PE				
		SIDI-STC				
	Complex wall layer	SIDI-GA/STC	1	0	0	5:5
		SIDI-PE/STC				
NP-Secondary coating I	Single wall layer	PH-SIDI-GA	1	1	0.5	10
		PH-SIDI-MA				
		PH-SIDI-PE				
		PH-SIDI-STC				
	Complex wall layer	PH-SIDI-GA/STC	1	1	0.5	5:5
		PH-SIDI-PE/STC				
NP-Secondary coating II	Single wall layer	PH/CHL-SIDI-GA	1	1	0.5	15
		PH/CHL-SIDI-MA				
		PH/CHL-SIDI-PE				
		PH/CHL-SIDI-STC				
	Complex wall layer	PH/CHL-SIDI-GA/STC	1	1	0.5	7.5:7.5
		PH/CHL-SIDI-PE/STC				

*SIDI - enriched fraction of SI (Silverskin) by DI selected as enriched fraction; LEC - Lecithin (containing 94% phosphatidylcholine); PH – Phytosome; CHL – Cholesterol; NP - Natural Polymer; GA-Gum Arabic; MA-Maltodextrin; PE-Pectin; STC-Starch.

5.3.2.6.2. Preparation of First Coating Layers Based on Lipids: Nano-Phytosomes

Nano-phytosomes were produced by the thin film hydration method described by Fathi and Ebrahimi, with small modifications [5]. To decrease the size of the obtained particles, in the hydration step, a sonication probe was applied. Phytosomes were prepared with lecithin (containing 94% phosphatidylcholine) and loaded with the SIDI enriched fraction, with an optimum molar ratio of (1:1) (Table 1 and Chart 1). This lecithin-based layer was another type of the first coating layer (phytosome coating layer). The experimental procedures were performed as follows: 50 mg of SIDI dissolved in 20 mL of ethanol was placed in a 50 mL round flask and heated up to 55 °C until a transparent solution was achieved. Then, 50 mg of lecithin was dissolved in 2 mL of dichloromethane and vortexed (Shaker & Mixers Reax top, Heidolph, Schwabach, Germany) for 5 min, at room temperature, to obtain a transparent yellow solution. Afterwards, the lecithin solution in

dichloromethane was sprayed on the SIDI ethanolic solution and refluxed under stirring for 2 h at 55 °C. The solution was cooled, and the solvent evaporated (rotary vacuum evaporator) at 37 °C at a medium speed from 40 to 200 rpm and 55 mbar of vacuum pressure until a thin layer was shaped. Afterwards, N₂ was flushed on the thin layer for 1 min, the flask was sealed, and kept overnight on a desiccator [20]. Thereupon, the phytosome mother solution (PH-SIDI) was formed by hydration of the thin film layer with phosphate buffer (pH 5.5), at 40 °C for 10 min (vacuum pressure: 200 mbar; rotation: 200 rpm). Afterwards, ultra-sonication was applied for 25 min, at 60% amplitude in pulsation mode (5:1 s) (total energy: 10,000 ± 100 kJ) [21].

5.3.2.6.3. Preparation of First Coating Layers Based on Lipids: Nano-Phytosomes Incorporated by Cholesterol

Cholesterol-incorporated nano-phytosomes consist of a combination of SIDI, lecithin, and cholesterol in an optimum molar ratio (1:1:0.5) treated according to the description in Section 2.6.2, with some modifications. This was the third type of the first coating layer developed in this study.

The combination of SIDI and lecithin was performed as described in the previous section (Section 2.6.2) and stirred for 10 min (Solution A). Afterwards, 25 mg of cholesterol was dissolved in 2 mL of dichloromethane and vortexed for 5 min. This solution was sprayed on solution A and refluxed under stirring for 2 h at 55 °C. The solvent was evaporated using a rotary vacuum evaporator at 37 °C, at a medium speed of 40 to 200 rpm, and a vacuum pressure of 55 mbar, until a thin layer was shaped. After, N₂ was flushed on the thin layer for 1 min, and the flask was kept overnight in the desiccator. The solution of cholesterol-incorporated nano-phytosomes loaded with SIDI (PH/CHL-SIDI) was hydrated using the same conditions described in the previous section (Section 2.6.2) with phosphate buffer (pH 7.8) (Table 1).

5.3.2.6.4. Preparation of Secondary Coating Layers (Coating of Nano-Phytosomes and Cholesterol-Incorporated Nano-Phytosomes with Natural Polymers as Secondary Layers)

The secondary layer was prepared with 10% (w/v) of NP for PH-SIDI, and 15% (w/v) of NP for PH/CHL-SIDI. For this purpose, immediately after preparation, the lipid coating layers (PH-SIDI or PH/CHL-SIDI), the solutions of NP in the aforementioned concentration, were individually added (Table 1). The NP were first dissolved in ultrapure water and stirred for 2 h at 55 °C. After, the NP solution was mixed and added to PH-SIDI or PH/CHL-SIDI (Table 1, Chart 1) and subjected to ultrasonication with a probe for 5 min, in pulsation mode

(5:1 s) and stirred for 20 min. The solutions were then injected into the spray dryer to obtain the final powder (Table 1 and Chart 1) [16].

5.3.2.6.5. Spray Drying Conditions for Encapsulation

A mini spray-dryer B-290 BÜCHI (Flawil, Switzerland) with a standard nozzle (0.5 mm) was used for the drying of NP coating and secondary NP coating layers. The encapsulation procedure was optimized based on previously reported data with some modifications [16]. The different prepared emulsions (Sections 2.6.1 and 2.6.4) fed the spray dryer under optimized stirring conditions, at a flow rate of 10 mL/min, aspiration of 100% (36 m³/h), air pressure of 5.5–6 bar, and a nozzle cleaner set to 3. Although all solutions were prepared in ultrapure water, the drying process used the same drying conditions with different drying temperatures. The inlet temperature differed according to the type of the NPs. In the case of starch, gum Arabic/starch, and pectin/starch, the drying temperatures were 135 °C as the inlet temperature and 63 ± 3 °C as the outlet temperature. In the case of pectin, the inlet temperature was set at 120 °C, and the outlet temperature almost 58 ± 2 °C. For the other NP, the inlet temperature was set at 115 °C, and the outlet temperature at 55 ± 2 °C. Throughout the injection process, a magnetic stirrer, at room temperature, shook the emulsions continuously to avoid aggregation of its solid content. Finally, the particles were recovered from the collector, sealed in aluminum foil, and stored at 4 °C for further analyses. The product yield (%) was calculated by the amount of the particles recovered from the drying step divided by the total mass content of the initial feeding solution (Equation (1)) [22,23].

$$Yield\% = \frac{\text{Total weight of polymeric or binary layers in powder (mg)}}{\text{Total weight of feed solution (combination of all ingredient) (mg)}} \times 100 \quad (1)$$

5.3.2.7. Physicochemical Properties of Nano/Micro-Particle

5.3.2.7.1. Encapsulation Efficiency and Loading Capacity

The encapsulation efficiency (EE) was defined by the SIDI concentration successfully entrapped in the particles. The EE% is the amount of drug successfully trapped in the particles (the total drug added subtracted by the non-entrapped drug) divided by the total quantity of the drug initially added in preparation steps [24,25]. Therefore, the encapsulation efficiency expressed in a percentage (EE%) and the loading capacity (LC) were calculated using the amount of the remaining free caffeine in the surface of the powder particles quantified by HPLC-DAD immediately after the preparation steps, according to the chromatographic conditions described in Section 2.5.1.

For that, 10 mg of particles were dissolved in 1 mL of solvent (ultrapure water and ethanol 30:70, v/v) under continuous stirring for 10 min, followed by centrifugation (HERAEUS FRESCO 17, Thermo Fisher Scientific, LR56495, Bremen, Germany) for 6 min, at 12,000 rpm. After, a 0.45 µm pore size PTFE syringe filter was used to filter the samples before HPLC injection.

The EE% was calculated via Equation (2). This method was previously described [24,25]. Regarding Equation (2), the amount of encapsulated caffeine in the solution is the $caffeine_{experimental}$ (calculated using HPLC data), and the $caffeine_{theoretical}$ is the SIDI total amount of caffeine hypothetically present in the particles.

$$EE(\%) = \frac{caffeine_{experimental}}{caffeine_{theoretical}} \times 100 \quad 2$$

Thereafter, taking into consideration Equation (3), theoretical LC is the caffeine content if 100% of SIDI caffeine were trapped in the particles. The specific LC% was calculated using Equation (3) [24].

$$LC(\%) = EE \times LC_{theoretical} \times 100 \quad 3$$

5.3.2.7.2. Fourier-transform Infrared Spectroscopy Analyses (FTIR)

The interaction between lecithin and cholesterol with SIDI in the phytosomes (PH-SIDI) and cholesterol-incorporated phytosomes (PH/CHL-SIDI) in a lyophilized form (dried with TELSTAR freeze dryer, Cryodos, Spain) were evaluated using a Fourier transform infrared (FTIR) apparatus (Frontier, PerkinElmer, Beaconsfield, UK) equipped with an attenuated total reflectance (ATR) accessory (PerkinElmer, Beaconsfield, UK), operated by spectrum software (PerkinElmer, Beaconsfield, UK).

5.3.2.7.3. Particle Size Distribution and Zeta Potential (Surface Charge)

Particle size distribution was assessed using a particle size analyzer (Brookhaven Instruments Corporation, operated by particle sizing v.5 Brookhaven instruments software, Holtsville, NY, USA). The qualitative particle properties were determined in PH-SIDI and PH/CHL-SIDI in solution, in external and internal NP coating and NP-secondary coating I and II layers in powder. The dried particles were dispersed in ethanol 99% to avoid probable agglomeration and sonicated (SOLTEC, SONICA 2200MH S360Hz, Milano, Italy) for 7 min to eliminate the agglomeration and deformation of external layer. In the case of PH-SIDI and PH/CHL-SIDI solutions, the small droplets were dispersed in ethanol 99% and

sonicated for more 7 min to avoid aggregation of lipid colloids. The particle size was characterized by mean size in volume and number, obtained in six runs of 1 min at 21 °C. The particles surface charges were obtained with ZetaPLAS (Zeta Potential Analyzer, Brookhaven Instruments Corporation, operated by the PALS Zeta Potential Analyzer v.5 Brookhaven Instruments software, Holtsville, NY, USA), after 6 runs of 30 s at 21 °C. All samples were analyzed regarding individual parameters in separated runs.

5.3.2.7.4. Scanning Electron Microscopy (SEM)

The study of the size and surface morphology of polymeric and secondary layers (external layer) used surface structural analysis under images performed by SEM (Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M). Beforehand, samples were adjusted on a brass stub (carbon stub) using double-sided adhesive tape, dried under a N₂ stream, and then coated by electrical conductivity (a thin layer of gold) in a vacuum by sputtering in a JEOL JFC 100 apparatus at Centro de Materiais da Universidade do Porto (CEMUP).

5.3.2.7.5. *In-Vitro* Drug Release

The in vitro drug release study (dissolution assay) was based on the caffeine release from particles over time, assessed in a gastrointestinal simulator containing 100 mL PBS buffer (pH 7.8 or 2.1), a Spectra/Por® Dialysis membrane standard (RC Tubing, MWCO: 3.5 KD, width: 45 mm, diameter: 29 mm, USA & Canada), with a stirrer speed of 70 rpm and heated at 37 ± 2 °C. The media was sealed and protected to avoid evaporation during the process. For that, 10 mg of powder was dispersed in ethanol 99% and placed inside the dialysis tubing. The end of the dialysis membrane was closed with clamps, placed horizontally in the middle of PBS media, and the top was stuck with wires. A caffeine calibration curve (2.5–120 µg/L; $y = 0.0051 \times +0.1038$) was used to calculate the total amount of drug release. “t” (time) corresponds to the presence of the characteristic peak of caffeine, evaluated by UV-Vis spectrometry at a maximum wavelength of 274 nm (BioTek, Synergy HT, PMT 49984, USA). The caffeine release was monitored from time zero to 72 h, at regular time intervals, based on the release route of each sample, in triplicate [24].

5.3.2.7.6. Mathematical Kinetic Release Models

Mathematical modeling was exploited to adjust the results obtained in Section 2.7.5. In vitro drug release to different kinetic release models was used as a tool to obtain critical parameters that allow one to predict important aspects concerning the release/dissolution profile. The application of the kinetic model to predict the release behavior of phytoconstituents/drugs was previously reported [26–28]. Therefore, caffeine release from

the synthesized particles was evaluated through correlation studies between the caffeine release data into the medium with different pHs and mathematical kinetic models, namely, zero-order (Equation (4)), first order (Equation (5)), Korsmeyer–Peppas (Equation (6)), Higuchi (Equation (7)), and Hixson–Crowell (Equation (8)) [29,30], as described below:

$$F_t = F_0 + K_0 t \quad (4)$$

where “ F_t ” is the cumulative amount of the active ingredient released at the time “ t ”, “ F_0 ” is the initial amount of the active compound in solution (normally $F_0 = 0$), “ K_0 ” is the zero-order release constant, and “ t ” is a time value.

$$F_t = F_{\max} \times (1 - e^{-K_1 t}) \quad (5)$$

where “ F_t ” is the cumulative amount of the active ingredient released at the time “ t ”, “ $F_{\max}$ ” is the maximum cumulative amount, and “ K_1 ” is the first-order release constant.

$$F_t / F_{\infty} = F_{KP} t^n \quad (6)$$

where “ F_t / F_{∞} ” is the amount of active compound released until time “ t ”, “ F_{KP} ” is the Korsmeyer–Peppas constant, “ t ” is a value of time, and n (release exponent) is an estimating different release mechanism. When $n < 0.43$, drug transport (mass transfer) occurs by pure diffusion following the Fick law (case-I transport). In the case of $n > 0.43$, the mass transfer follows a non-Fickian model; when $0.43 < n < 0.85$, drug transport occurs based on anomalous drug transport resulting in a combination of Fickian diffusion and swelling release (case II). If n is equal to 0.85, drug transport occurs based on Case-II (zero-order kinetic controlling swelling and relaxation of polymer matrix). Finally, when $n > 0.85$, drug transport occurs based on super case-II transport [29,31].

$$F_t = F_H \sqrt{t} \quad (7)$$

where “ F_t ” is the cumulative amount of the active ingredient released at the time “ t ”, “ F_H ” is the Higuchi constant, and “ t ” is a value of time.

$$F_t = 100 \times [1 - (1 - K_{HC} \times t)^3] \quad (8)$$

where “ F_t ” is the cumulative amount of the active ingredient released at the time “ t ”, “ K_{HC} ” is the Higuchi constant, and “ t ” is a value of time.

The fitting of a kinetic model to a release profile is dependent on the calculated adjusted correlation coefficient (R^2_{adj}), since the correlation coefficient (R^2) is influenced by the

number of parameters in the equation, increasing with the number of those parameters [32–34].

5.3.2.7.7. Molecular Docking Arrangement

The 3D structures of phosphatidylcholine ($\geq 94\%$ of lecithin), cholesterol, and the two main phytochemicals of SI—caffeine and 5-caffeoylquinic acid (chlorogenic acid)—were downloaded from the ChemSpider database. They were prepared and refined using the Ligprep application (Maestro 12.8, New York, USA, Schrödinger). The 3D models of nano-phytosome and cholesterol-incorporated nano-phytosome bilayers were generated using a MemGen web server defined for lipid membrane simulation systems [35]. The generated models were downloaded in PDB format and subjected to additional optimization using the OPLS3 force field using Maestro 12.8. To investigate the interaction of the phytochemicals with the related bilayers, a grid box ($x = 8.96$, $y = 28.10$, $z = 30.36$, Size of 40, 40, 40 Å) was created by grid generation. The interaction between ligands and bilayers was carried out using a glide application with extra precision (XP) level in Maestro 12.8. For each ligand, five poses have been used to evaluate docking interactions.

5.3.2.7.7. Molecular Docking Arrangement

The results were expressed as mean $\pm$ standard deviation. A One-way ANOVA test followed by post-hoc comparisons with Tukey's HSD was used to identify significant differences between samples at $p < 0.05$ (IBM SPSS 25 for Windows, IBM Corp., Armonk, NY, USA).

5.3.3. Results

5.3.3.1. Phytochemical Profiling and Enrichment Efficiency

SI was extracted with the hydroethanolic solvent (SI₁) and ultrasonication with water (SI₂). The extracts were enriched using Diaion™ HP20LX (DI) and Relite EXA90 (RE) adsorbent resins (Chart 1) regarding the caffeine and total phenolics contents.

The highest extraction yield was achieved with the hydroethanolic extraction (SI₁, 66.58%), being almost twice that of those obtained with ultrasonication extraction. The highest enrichment yield was obtained with the Diaion™ HP20LX resin (SI₁-Di₁, 50.62%) compared to other fractions under study (Table 2).

Table 2. Caffeine and total phenolics content of SI crude and enriched extracts.

Sample	Yield (%)	Caffeine	ECC	Total Phenolics			
				CAE	ECA	GAE	EGA
SI ₁	66.58	432.57 ± 1.64 ^e	-	10.61 ± 0.42 ^d	-	4.87 ± 0.28 ^d	-
SI ₁ DI ₁	50.62	1333.78 ± 5.51 ^a	3.08	29.15 ± 1.54 ^a	2.75	17.09 ± 1.01 ^a	3.51
SI ₁ DI ₂	DC	7.62 ± 0.18 ^g	-	6.54 ± 0.39 ^e	-	2.19 ± 0.26 ^e	-
SI ₁ RE ₁	52.87	951.76 ± 3.87 ^d	2.20	26.27 ± 1.61 ^b	2.48	15.19 ± 1.06 ^b	3.12
SI ₁ RE ₂	DC	6.73 ± 0.19 ^g	-	4.27 ± 0.27 ^{ef}	-	0.70 ± 0.18 ^{ef}	-
SI ₂	36.67	307.34 ± 1.10 ^f	-	9.30 ± 0.24 ^d	-	4.01 ± 0.16 ^d	-
SI ₂ DI ₁	20.00	1278.45 ± 5.28 ^b	4.16	19.13 ± 0.67 ^c	2.06	10.48 ± 0.44 ^c	2.61
SI ₂ DI ₂	DC	6.94 ± 0.19 ^g	-	5.97 ± 1.17 ^{ef}	-	1.82 ± 0.77 ^{ef}	-
SI ₂ RE ₁	18.00	1259.21 ± 5.19 ^c	4.10	18.23 ± 0.86 ^c	1.96	9.89 ± 0.57 ^c	2.47
SI ₂ RE ₂	DC	6.74 ± 0.19 ^g	-	3.80 ± 0.22 ^f	-	0.38 ± 0.15 ^f	-

Values of caffeine, CAE, and GAE expressed in mg/L (mean ± standard deviation). Different letters within the same column represent significant differences between samples at $p < 0.05$. ECC, enhance on caffeine content; ECA, enhance on chlorogenic acid content; EGA, enhance on gallic acid content; CAE, chlorogenic acid equivalents; GAE, gallic acid equivalents; DC, discarded fractions containing sugar and chlorophylls.

5.3.3.1.1. HPLC Analysis Regarding Caffeine Enrichment

HPLC based on caffeine standard calibration curve allows confirming the caffeine content of crude and enriched fractions (Figure 1). The highest caffeine enrichment was observed in SI₂ series (SI₂DI₁, 1282.18 mg/L) and (SI₂RE₁, 1262.88 mg/L) at almost a same enrichment enhancement related to (SI₂DI₁, 4.16 times) and (SI₂RE₁, 4.10 times). However, a significant high caffeine content (SI₁DI₁, 1337.68 mg/L) and (SI₁RE₁, 954.50 mg/L) with high enrichment enhancement around (SI₁DI₁, 3.08 times) and (SI₁RE₁, 2.20 times) were observed from SI₁ series. Meanwhile, regarding the high extraction yield of SI₁ series and high enrichment yield regarding DI₁ series and considerable enhancement on caffeine content, fraction SI₁DI₁ was chosen as an optimized fraction regarding caffeine content.

The chromatograms obtained from the HPLC analysis of crude and enriched fractions are depicted in Figure 1. The highest caffeine enrichment was observed in the SI₂ series (SI₂DI₁: 4.16-fold and SI₂RE₁: 4.10-fold). However, a significantly higher ($p < 0.05$) caffeine content was achieved in SI₁DI₁ (1333.78 mg/L vs. SI₂DI₁ (1278.45 mg/L) and SI₂RE₁ (1259.21 mg/L)).

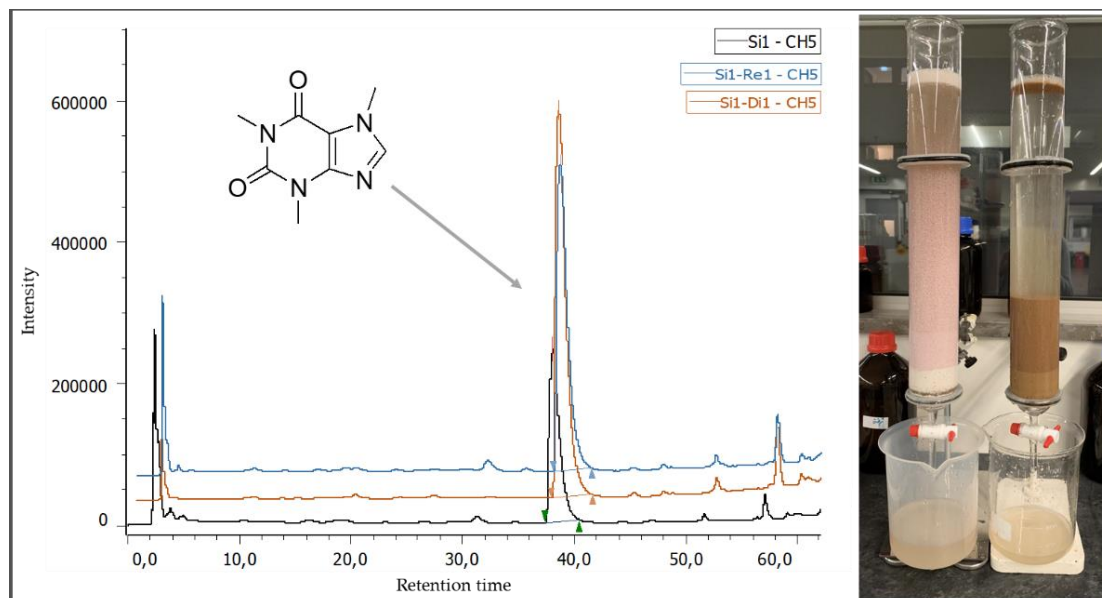


Figure 1. HPLC chromatogram of caffeine before and after enrichment.

In addition, the highest phenolic enrichment was also obtained with the Diaion™ HP20LX (SI₁-DI₁: 29.15 mg CAE/L; SI₁-DI:17.09 mg GAE/L). Total phenolics increased, in relation to the crude extract, almost 2.75- and 3.51-fold for chlorogenic acid and gallic acid equivalents, respectively. Overall, a considerable phenolics enrichment was observed in all samples under study.

Based on the higher extraction yield obtained in the SI₁ series (~50%), the higher phytochemicals enrichment yield regarding the DI₁ series, and the considerable enhancement on caffeine and total phenolics content (Table 2), SI₁DI₁ was selected as the best enriched fraction to proceed with the studies (Charts 1 and 2).

The results obtained herein are in accordance with those already published by Fathi et al. [5] who reported plant extract enrichment in phenolic compounds using a Diaion HP20 resin. The EXA-118 adsorbent resin was also successfully employed in the purification of anthocyanins and hydroxycinnamic acids (phenolic backbone) from a citrus by-product [36]. Moreover, the use of adsorbent resins in chromatography columns for the pre-purification of food ingredients, as a source of economic technology, was previously described in the food area [37]. Additionally, Diaion HP20 was also previously used for the extraction and purification of esculeoside A (steroidal alkaloid glycosides) from tomato [38]. It must be taken into consideration that Relite EXA90 was not reported previously in the extraction and purification of alkaloids from natural sources. Therefore, this study provides relevant information about the specific functionality of Relite EXA90 on the purification of alkaloid-based natural products (caffeine; purine alkaloid) regarding the considerable information obtained for SI₂DI₁ and SI₂RE₁ (Table 2).

Table 3. Product yield, encapsulation efficiency, and loading capacity of formulated samples in powder.

	Formula	Yield (%)	EE (%)	LC (%)
Lipid-based first coating	PH-SIDI	60.22	-	-
	PH/CHL-SIDI	57.44	-	-
	SIDI-GA	50.00	95.67 ± 1.08 ^a	8.70 ± 0.10 ^a
	SIDI-MA	42.55	96.51 ± 1.10 ^a	8.77 ± 0.10 ^a
NP-based first coating	SIDI-PE	42.36	95.02 ± 0.02 ^a	8.64 ± 0.00 ^a
	SIDI-STC	72.18	97.15 ± 2.96 ^a	8.83 ± 0.27 ^a
	SIDI-GA/STC	66.75	94.46 ± 0.20 ^a	8.59 ± 0.02 ^a
	SIDI-PE/STC	37.25	95.43 ± 1.60 ^a	8.68 ± 0.15 ^a
NP-secondary coating I	PH-SIDI-GA	72.92	94.26 ± 0.24 ^a	7.85 ± 0.02 ^a
	PH-SIDI-MA	51.69	94.68 ± 1.24 ^a	7.89 ± 0.10 ^a
	PH-SIDI-PE	46.92	96.62 ± 0.79 ^a	8.05 ± 0.07 ^a
	PH-SIDI-STC	48.31	92.57 ± 1.57 ^a	7.71 ± 0.13 ^a
NP-secondary coating II	PH-SIDI-GA/STC	62.67	94.26 ± 1.46 ^a	7.86 ± 0.12 ^a
	PH-SIDI-PE/STC	35.22	94.29 ± 1.04 ^a	7.86 ± 0.09 ^a
	PH/CHL-SIDI-GA	65.51	92.56 ± 2.63 ^a	5.29 ± 0.15 ^b
	PH/CHL-SIDI-MA	53.62	94.64 ± 1.25 ^a	7.02 ± 2.35 ^{ab}
	PH/CHL-SIDI-PE	50.70	95.11 ± 0.17 ^a	5.43 ± 0.01 ^b
	PH/CHL-SIDI-STC	54.49	91.22 ± 3.59 ^a	5.21 ± 0.21 ^b
	PH/CHL-SIDI-GA/STC	61.73	93.61 ± 0.53 ^a	5.35 ± 0.03 ^b
	PH/CHL-SIDI-PE/STC	42.38	94.44 ± 0.96 ^a	5.40 ± 0.05 ^b

Different letters within the same column represent significant differences between samples at $p < 0.05$. SIDI, enriched fraction of the silverskin extract (SI₁) obtained using the Diaion™ HP20LX resin; PH, nano-phytosome; PH/CHL, cholesterol incorporated nano-phytosome; GA, gum Arabic; MA, maltodextrin; PE, pectin; STC, starch; NP, natural polymer. Note: EE% and LC% were not recorded for PH-SIDI and PH/CHL-SIDI.

However, the product yield showed slight differences, when comparing the NP first coating and NP-secondary coating I and II. A slight increase in the production yield of some of the NP-secondary coatings II (2–15%) occurred, when compared to the single coating and NP-secondary coating I. Furthermore, no practical differences were observed for NP complexes on coating layers in all samples under study. In general, NP-secondary coating II presented slightly higher product yields compared to the other samples under study.

Meanwhile, a certain powder loss occurred in all samples due to the adhesion of particles to the drying cyclone. This phenomenon occurred due to the low glass transition temperature of the initial ingredient (phytoconstituent) at high drying temperature. Therefore, the drying temperature causes surface deformation of the particles to a viscoelastic rubbery state, causing their adhesion to the drying chamber. Although the addition of NP (with a high glass transition temperature) considerably improves this phenomenon, to decrease the mass loss during the process, an optimization of NP concentration is required, as well as adding secondary coating layers (I and II). The referred situation can justify the low product yield values obtained for some variables. However, some mass loss during the drying process is expected and could not be eliminated [37,39].

5.3.3.2. Physicochemical Properties of Nano/Micro-Particle

5.3.3.2.1. Product Yield

The product yield was calculated based on the method described in Section 2.6.5 and Equation (1). It must be taken into consideration that the inlet temperatures were set according to the NP used (Section 2.6.5), ranging among 115 and 135 °C in order to enhance the final product yield and decrease moisture content, resulting in improved storage and stabilization of the particles [39]. The product yields ranged from 35.22 to 72.92%, considering all samples (Table 3). Among the four subgroups under study, no considerable differences were observed. In contrast, the product yield varied from the type of NP and the drying conditions, which might be due to the viscosity, volume, and concentration of the initial feed, drying temperature, features, and ratio of the coating layer, according to Tontul and Topuz [40]. The experimental results showed the high product yield from starch and gum Arabic particles, in both forms, single and complex coating layers. Gum Arabic showed a higher product yield. These results are in accordance with those of Ferreira et al. [41], who reported high product yields ($\geq 50\%$) for 1:1.5 and 1:2 starch/gum Arabic. The authors reported that increasing the ratio of gum Arabic allows a significant increase on the product yield of a complex of starch/gum Arabic. In contrast, a low product yield was observed for pectin. A significant improvement (almost 4–6%) on the product rate was observed in secondary coating layers (I and II) comparatively to NP in the single pectin coating, while, in the case of the pectin/starch complex, the yield decreased in all categories compared with the single coating (Table 3). It could be concluded that the impact of these factors will depend on the lipid layer, in this case, especially in the nano-phytosome incorporated by cholesterol, to improve the stability of lipid particles in solution media [9], provide the adhesion between the lipid layer and NP, and avoid mass loss during drying [16]. Moreover, the complex of pectin/ starch provides surface coverage of the particles and reduces adhesion during spray drying [42]. These two factors lead to a slight improvement from the NP coating to NP-Secondary coatings I and II, and, resulting in a considerable product yield on PH/CHL-SIDI-PE/STC.

5.3.3.2.2. Encapsulation Efficiency, and Loading Capacity

The encapsulation efficacy (EE%) and loading capacity (LC%) of all formulated samples in powder are presented in Table 3. The highest to lowest average EE% was observed as follows: NP first coating (94.46 ± 0.20 – $97.15 \pm 2.96\%$) > NP-secondary coating I (92.57 ± 1.57 – $96.62 \pm 0.79\%$) > NP-secondary coating II (91.22 ± 3.59 – $95.11 \pm 0.17\%$). The highest

EE% was observed for SIDI-STC, and the lowest one was presented by PH/CHL-SIDI-STC (Table 3). The LC followed a similar behavior to the one described for EE%. The highest LC was observed in NP coatings, and the lowest one in NP-secondary coating II (Table 3). In general, the results demonstrated a slight decrease in EE% (although not statistically significant) and a significant decrease in LC% ($p < 0.05$) from the first layers to secondary layers, which may be due to the low encapsulation efficiency of nano-phytosomes and nano-phytosomes incorporated by cholesterol (NP-secondary coatings I and II), as reported by Huang et al. [43]. These authors verified EE% of 35.88% for phytosome structures. However, NPs in the complex form did not significantly influence EE% nor LC% within each group of samples (Table 3).

As shown in this work, EE% is strongly affected by the initial materials used to manufacture the particles, where trial-and-error is usually required to select the best wall materials, as reported by Gharsallaoui et al. [44]. The slight differences found in EE% (not statistically different, $p > 0.05$) may be due to the first coating layer (lipid coating) and the drying and encapsulation conditions, which confirm the results obtained by Roccia et al. [45], which reported an EE% decrease upon increasing the drying temperature. Therefore, it could be concluded that the drying conditions and the lipid carriers might affect the EE%, but not the type of NP used.

5.3.3.2.3. Fourier-transform Infrared Spectroscopy Analyses (FTIR)

The FTIR spectra was recorded in the scanning range from 400 to 4000 cm^{-1} , confirming the interactions among SIDI, lecithin (phosphatidylcholine), and cholesterol in the nano-phytosomes and nano-phytosomes incorporated by cholesterol. The interactions among lipid substrates and SIDI via suppressing a functional group at PH, PH/CHL, PH-SIDI, and PH/CHL-SIDI compared to SIDI, phosphatidylcholine, and cholesterol was proven (Figure 2).

Furthermore, a significant change in the fingerprint area (marked in Figure 2, 400–1500 cm^{-1}) and critical substitution areas clearly show the interaction between the lipid bi-layer and SIDI. Taking into consideration Figure 2, peaks (a) and (b) show, at the specific critical substitution areas, differences in PH-SIDI compared to PH/CHL-SIDI, confirming the lack of a functional group of cholesterol at PH-SIDI, and the existence of phosphatidylcholine on the related area (2924 cm^{-1}). The cholesterol and phosphatidylcholine functional groups at 2924 cm^{-1} in PH/CHL, PH-SIDI, and PH/CHL-SIDI confirmed the interaction of phosphatidylcholine in all related structures.

The following results are in line with those of Hou et al. [46] that confirmed the physicochemical interaction between mitomycin C-soybean phosphatidylcholine by FTIR, paying attention to significant differences between the pure compound and phytosome complex [47]. Moreover, a significant suppression of the sharp endothermic nature of the functional groups of the spectra of curcumin and phospholipid was reported [46], which is in line with the verification in the current FTIR spectra. **Figure 2.** Specific interactions among phosphatidylcholine, cholesterol and SIDI at PH/CHL, PH-SIDI and PH/CHL-SIDI by FTIR spectra.

5.3.3.2.4. Nano/Micro Particle Size Distribution

The particle size was determined via the effective particles diameter, number, and volume of internal layers. In all samples, the particles were heterogeneous, at nanoscale (≤ 800 nm). For particle size evaluation, the initial lipid coatings regarding nano-phytosomes (PH-SIDI) and nano-phytosomes incorporated by cholesterol (PH/CHL-SIDI), initial NP coating (SIDI-NPs), and NP-secondary coating (PH-SIDI-NPs and PH/CHL-SIDI-NPs) were considered (Table 4). Equipment from Brookhaven Instruments (Holtsville, NY, USA) evaluated the particle size (internal layers).

5.3.3.2.4.1. Initial Lipid Coating

The distribution of particle size on lipid layers was evaluated after 20 min of sonication for both nano-phytosomes (PH-SIDI) and cholesterol-incorporated nano-phytosomes (PH/CHL-SIDI), immediately after the hydration to avoid agglomeration. PH-SIDI and PH/CHL-SIDI had less than 120 nm, with nanoparticles in both volume and number (Table 4). The obtained results are in line with those of Minaei et al. [48], in the case of PH-SIDI, and in agreement to Nazari et al. [49], in the case of PH/CHL-SIDI. The nanoparticles size increased in the presence of cholesterol (PH/CHL-SIDI). Therefore, it was demonstrated that incorporating cholesterol in the lipid bilayer, between phospholipids, increases the particles size, but also improves their stability as reported by Rasaie et al. [9].

5.3.3.2.4.2. Initial NP Coating

To crush the NP external coating layer and determine the particle size of the internal layers, a small amount of powder previously dispersed on ethanol 99% was subjected to 7 min of sonication (Table 4). The particles size of the NP external coating is discussed in Section 3.2.5. The particles size of the internal layers was in the range of 187.1–786.8 nm in number and 226.1–808.7 nm in volume. The pectin-based coating produced the biggest particles and the STC and PE/STC complex produced the smallest. Similarities were

observed in the distribution of volume and number. Therefore, the particles followed a harmonious distribution at internal layers regarding the initial NP coating, and followed structural arrangements Type A-I and A-II. The probable molecular arrangement of NP coatings were previously discussed [16].

The size-reducing properties of starch in the PE/STC complex is proven in this study and it is in line with the results reported by Fathi et al. [16]. This behavior may be due to the initial features of some carbohydrates such as starch that affect steric hindrance mechanisms on the particles' surface when placed among the NP molecules. Therefore, this feature could limit the particle size. The impact of starch on gum Arabic was also evaluated. Nevertheless, the observed results with the GA/STC complex showed different outcomes compared to PE/STC. These results are in line with those of Chanamai et al. [50] that reported particles size of the GA/STC complex around 700 nm in emulsion form.

5.3.3.2.4.3. NP-Secondary Coating

The evaluation of PH-SIDI-NPs and PH/CHL-SIDI-NPs was described in Section 3.2.4.**Initial Lipid Coating** The particles size was moderately larger compared to the initial NP coatings. The biggest particles were obtained with maltodextrin and the smallest ones with starch in single form. The biggest and smallest complex particles were from GA/STC and PE/STC, respectively. Distributions in volume and number were also similar (as in Section 3.2.4.**Initial NP Coating**). Considering the PH-SIDI (70.8 nm) and PH/CHL-SIDI (114.1 nm) particle size in the secondary coating layer and considering the formation of the coating layer explained by Fathi et al. [12], NP-secondary coating I and II probably follow A-III and A-IV and A-V and A-VI types. In the way, the small differences between volume and number confirm the structure type A-III and A-IV followed by the majority of particles [16]. Moreover, the size-reducing properties of starch in the PE/STC complex was verified in both series as well (Table 4) [51].

The particle size distribution after four months of preparation was recorded to evaluate their stability against moisture in storage conditions (-4 °C, 95% moisture). A slight increase in particles size in all samples under study was obtained. However, the particles were still in an acceptable size range, and no relevant instability was observed.

Table 4. Particle size (internal layers, nm) and zeta potential (mV) of internal and external layers.

Formula Name	Particle Size (nm)						Zeta Potential (mV) *			
	First Month (Internal Layers)		kcps	PDI	Fourth Month (Internal Layers)		kcps	PDI		
	Volume *	Number			Volume *	Number			Internal Layers	External Layers
PH-SIDI	70.8 ± 0.3	34.3	399.3	0.161	74.1 ± 0.8	36.6	513.3	0.157	-84.22 ± 6.58	
PH/CHL-SIDI	114.1 ± 1.1	39.1	459.6	0.235	179.1 ± 1.0	70.0	536.9	0.206	-75.06 ± 5.86	
SIDI-GA	512.4 ± 62.7	498.6	502.9	0.005	691.3 ± 74.1	672.6	443.3	0.005	-51.02 ± 4.43	-53.54 ± 3.65
SIDI-MA	619.1 ± 147.7	602.3	322.6	0.005	832.3 ± 90.8	602.3	396.9	0.005	-33.83 ± 16.96	-25.42 ± 7.44
SIDI-PE	808.7 ± 102.4	786.8	415.8	0.005	892.3 ± 124.7	868.2	528.2	0.005	-30.07 ± 7.47	-45.28 ± 6.89
SIDI-STC	226.1 ± 28.5	187.1	363.6	0.055	267.3 ± 30.3	260.0	433.2	0.005	-52.79 ± 2.97	-58.17 ± 2.97
SIDI-GA/STC	699.8 ± 98.1	680.0	412.4	0.005	919.8 ± 100.1	894.0	462.3	0.005	-49.22 ± 2.82	-49.22 ± 2.82
SIDI-PE/STC	241.8 ± 26.3	235.2	426.3	0.005	267.7 ± 11.1	260.1	473.7	0.005	-37.69 ± 4.00	-45.41 ± 3.49
PH-SIDI-GA	462.8 ± 71.1	404.2	432.4	0.005	650.0 ± 100.1	632.4	422.4	0.005	-61.76 ± 4.48	-52.25 ± 2.83
PH-SIDI-MA	535.2 ± 194.9	520.7	388.9	0.005	893.3 ± 34.0	869.1	351.2	0.005	-31.49 ± 4.73	-19.31 ± 8.73
PH-SIDI-PE	492.0 ± 52.8	478.7	353.7	0.005	588.0 ± 44.0	572.1	386.3	0.005	-30.66 ± 3.67	-19.08 ± 10.79
PH-SIDI-STC	298.7 ± 28.7	290.6	298.7	0.005	327.0 ± 38.9	318.2	439.7	0.005	-52.88 ± 3.11	-32.61 ± 18.30
PH-SIDI-GA/STC	760.1 ± 132.5	505.1	462.7	0.005	976.8 ± 104.1	950.0	465.0	0.005	-39.41 ± 5.39	-31.80 ± 6.67
PH-SIDI-PE/STC	452.0 ± 86.4	439.8	412.4	0.005	688.5 ± 101.2	669.9	412.4	0.005	-33.01 ± 5.85	-31.28 ± 8.38
PH/CHL-SIDI-GA	429.4 ± 45.6	414.8	388.2	0.005	474.3 ± 49.2	461.4	432.5	0.005	-55.22 ± 1.14	-48.43 ± 5.64
PH/CHL-SIDI-MA	604.5 ± 67.7	588.2	361.1	0.005	691.1 ± 96.4	259.0	450.6	0.215	-24.43 ± 12.00	-26.69 ± 9.68
PH/CHL-SIDI-PE	524.2 ± 52.1	527.5	449.4	0.005	626.4 ± 12.8	609.4	449.4	0.005	-31.61 ± 14.35	-15.25 ± 5.87
PH/CHL-SIDI-STC	259.1 ± 8.8	252.1	523.6	0.005	414.2 ± 19.1	403.0	459.2	0.005	-59.86 ± 4.41	-47.95 ± 5.56
PH/CHL-SIDI-GA/STC	441.1 ± 19.2	429.2	460.1	0.005	526.9 ± 36.2	512.6	460.1	0.005	-51.25 ± 4.25	-45.43 ± 7.81
PH/CHL-SIDI-PE/STC	387.4 ± 35.9	376.9	490.3	0.005	435.9 ± 62.4	424.1	500.9	0.005	-34.69 ± 2.31	-30.68 ± 1.95

* The presented values correspond to the mean ± standard deviation provided by the equipment (Brookhaven Instruments, Holtsville, NY, USA). kcps, average count rate (kilo counts per second) that shows a quality of particle size, in which the kcps range approximately between 300 and 500 (it provides a high precise particle size recording); PDI, polydispersity index that shows the size range index of particles: <0.05, high monodisperse standard in which near to zero shows a perfect uniformed sample; >0.7, very broad particle size distribution in which near to 1.0 shows a super highly polydisperse sample with various particle sizes [15].

5.3.3.2.5. Surface Morphology of External Layers

Scanning electron microscopy (SEM) was used to evaluate the surface morphology and determine the particles size of the external layer for all particles under study. The SEM images cannot provide any information regarding internal layers (Figure 3). The surface morphology was quite different regarding each NP in the single form, and complex forms had an intermediate morphology (mixture of both NPs). In summary, the surface morphology shows spherical shrinkage with regular and irregular concavities related to the differences in the NPs shapes. Regular concavities on the particles' surface were observed in gum Arabic and maltodextrin. An irregular shrinkage, albeit uniform, was observed in starch and pectin particle surfaces in the single matrix (Figure 3). The obtained results concerning maltodextrin were in line with those reported by Papoutsis et al. [52] with slight differences. The authors reported a spherical uniform surface with low concavities in the case of maltodextrin, and the results of this work showed more deep concavities while still spherical and uniform. These differences may be due to differences in the drying temperatures that directly affect the glass transition temperature of NP, resulting in surface deformation. This happened for all samples under study [37,39]. In the case of starch particles, a spherical surface, uniform and regular with some concavities, is in agreement with the uniform irregular surface with a moderate rate of concavities reported by Gangurde et al. [53]. The surface morphology of complex forms followed an intermediate route with a decreasing rate of concavities. The results reported in this study concerning the surface morphology of starch and pectin particles are in accordance with those of Cortes et al. [54].

In terms of the external layer particle size, although no remarkable differences were observed in the particles under study, it ranged 1–5 μm for all samples. These values (the size of external layers) were directly affected by drying conditions, such as inlet and outlet temperatures, the spraying nozzle, and the aspiration rate [40]. Therefore, the similarity between the particle sizes of external coating layers is justified.

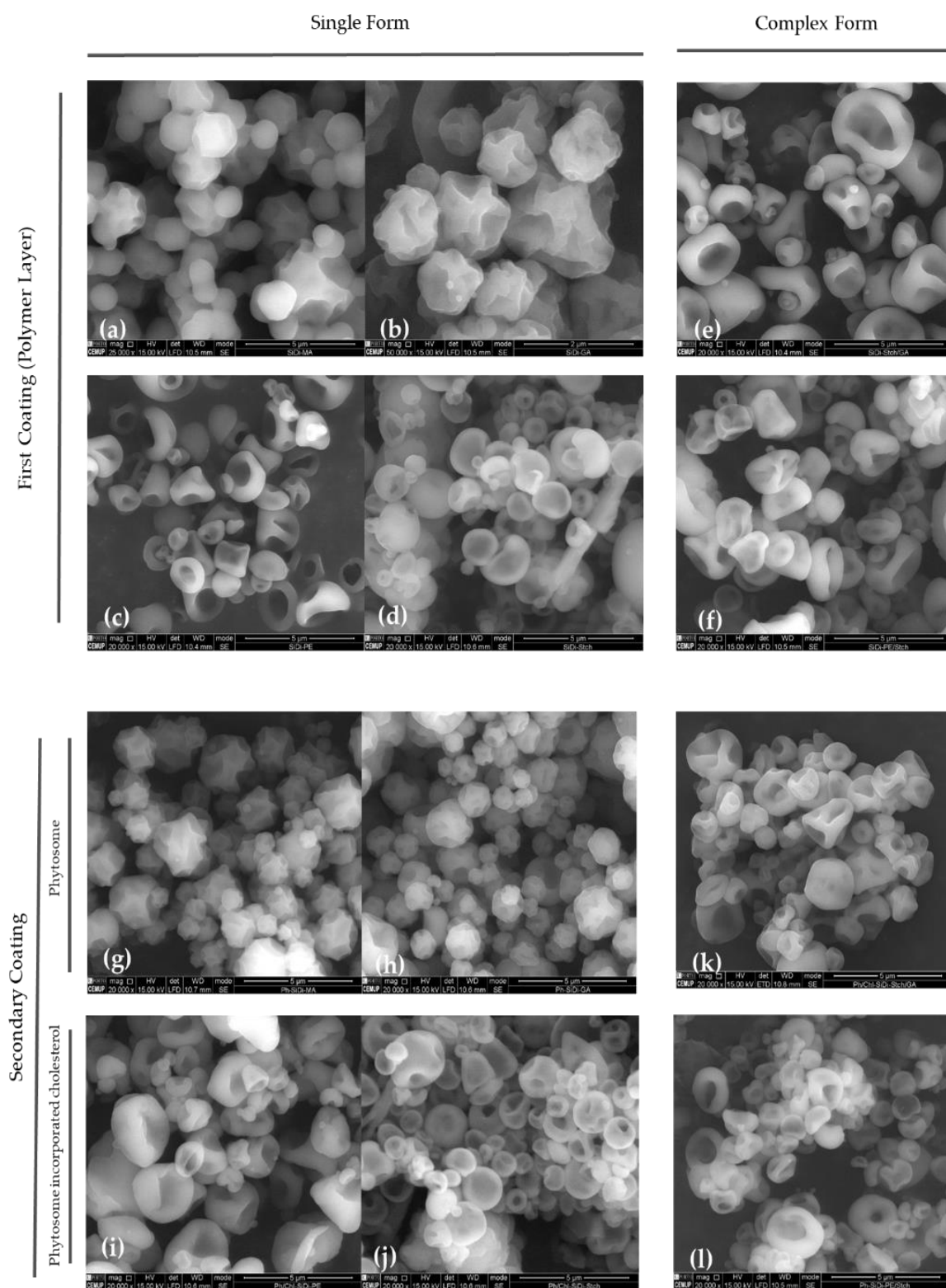


Figure 3. Surface morphology of final products regarding first and secondary coating were listed. In term of first coating: (a) maltodextrin; (b) gum Arabic; (c) pectin; (d) starch; (e) starch/ gum Arabic; (f) pectin/ starch. Regarding the secondary coating: (g) maltodextrin; (h) gum Arabic; (i) pectin; (j) starch; (k) starch/ gum Arabic; (l) pectin/ starch.

5.3.3.2.6. Surface Charge of Internal and External Layers

The surface charge (zeta potential) of all particles (nano-phytosome, nano-phytosome incorporated by cholesterol, NP coatings, and NP-Secondary coatings) were evaluated regarding the internal and external layers. A surface charge lower than -30 mV (anionic surface charge) is desirable in terms of stability at aqueous media, compelling repulsion between particles and avoiding agglomeration. On the other hand, the surface charge of about ± 10 mV produces neutral particles, favoring the agglomeration rate [55,56]. It must be taken into consideration that cationic particles have more toxicity to the cell wall membrane [55], but by contracting, they could interact better with the cell membrane [57]. The lipid bilayer improves the permeability due to its similarity to the cell membrane [58]. Therefore, the anionic lipid-based particles play a vital role in food and pharmaceutical final products, resulting in improved permeability of active ingredients/drugs and avoiding the probable toxicity related to the cationic particles [24]. Keeping this information in mind, all particles presented a desirable anionic charge, ranging from -24.43 to -61.76 in the case of internal layers (Table 4) and from -15.25 to -58.17 in the case of external layers (Table 4). It is to be noted that the surface negative charge of internal layers was moderately higher than the external layers, in the same conditions. This feature can contribute to the stability of particles, avoiding a high agglomeration rate. The lipid layers surface charge ranged between -84.22 ± 6.58 and -75.06 ± 5.86 mV for the phytosome and the cholesterol-incorporated phytosome, respectively. The negative surface charge of lipid layers, in the case of phosphatidylcholine, was already reported by Hindarto et al. [59], and in the case of phosphatidylcholine bilayer incorporated by cholesterol, by Tang et al. [57].

Considering the values presented in Table 4, the zeta potentials decreased significantly from the lipid layer to NP coatings. In general, a slight increase from the NP coating to NP-Secondary coatings I and II was verified. In terms of performance, the zeta potential of gum Arabic and starch considerably decrease in both complex forms (GA/STC, and PE/STC) (Table 4). However, the pectin zeta potential increases in these forms (PE/STC). The mentioned results show the positive impact of starch on stabilizing the PE/STC complex in solution. The results reported are in line with Fathi et al. [16]. Indeed, it could be concluded that the size-reducing properties of starch could improve the zeta potential, increasing colloidal stability.

5.3.3.2.7. *In Vitro* Drug Release Studies

The *in vitro* drug release study (dissolution assay) was performed at pH 2.1 and 7.8, corresponding to gastrointestinal media. The profiles obtained by the dissolution assays

were evaluated regarding kinetic mathematical models and stabilization of the produced particles in an in vitro environment. Table 5 lists the results obtained at pH 2.1 and Table 6 at pH 7.8. Zero-order (ZO), first-order (FO), Higuchi (HG), Hixson–Crowell (HC), and Korsmeyer–Peppas (KrP) were the theoretical mathematical models used to evaluate the capacity for the prediction of release profiles. The criteria for selecting the most appropriate model were based on the adjusted correlation coefficient in which $R^2_{Adj} \geq 0.800$ was considered as an acceptable fit indicator of the model, and the best-fitting model was the one with the R^2_{Adj} closest to 1 [32–34].

Zero-order kinetics were applied to determine if the caffeine release from the particles followed a Fickian or non-Fickian mechanism. A Fickian mechanism occurs when the bioactive/drug release happens at a constant and linear rate, during a specific time, and independently of the concentration at any time [24]. The zero-order kinetics model supports a sustained-release mechanism, that is, the active agent is released at a constant rate during a period of time. This assures that the active agent is continuously released at a constant pharmacological concentration, but on the other hand, it implies a frequent repetitive dosing, considering the limited amount of the active agent per formulation, which rapidly causes the drug concentration to be outside of the therapeutic range [25]. In relation to the tested formulations at pH 2.1, and according to the R^2_{Adj} value, it was concluded that the zero-order kinetic model did not fit well to most of the release results, indicating an overall non-Fickian release mechanism with the exception of five formulae: SIDI-MA (R^2_{Adj} : 0.927), PH/CHL-SIDI-MA (R^2_{Adj} : 0.954), PH-SIDI-PE (R^2_{Adj} : 0.964), SIDI-STC (R^2_{Adj} : 0.824), PH/CHL-SIDI-STC (R^2_{Adj} : 0.975). In contrast, regarding pH 7.8, most of the drug release assays of the particles fitted well to the zero-order kinetic model (indicating a Fickian release mechanism), except for the formulations PH/CHL-SIDI-MA (R^2_{Adj} : 0.642), SIDI-GA (R^2_{Adj} : 0.451), PH-SIDI-GA/STC (R^2_{Adj} : 0.697), and PH/CHL-SIDI-GA/STC (R^2_{Adj} : 0.489). For zero-order kinetics models, the release mechanism relies on the fact that the amount of the drug dissolved only depends on time. Thus, the synthesized formulations that were revealed to be compatible with a zero-order kinetic model allow the drug to be dissolved at a constant rate independently of the active agent concentration.

The first-order kinetic model is normally used to mathematically describe the release, absorption, and dissolution phenomena of a drug from a solid particle in a liquid media, thus being dependent on the surface area of the solid in contact with the solution medium [29]. In this process, the drug release rate is concentration dependent [15], resulting in a super slow release profile since the concentration of the drug diminishes with the release process, which in turn slows down due to the lower drug concentration inside the particles [33,60].

By analyzing the calculated statistical parameters, in Tables 5 and 6, derived from the application of the first-order model to the dissolution assays' results, it was concluded that the first kinetic model fitted well to the release data associated with pH 2.1 ($R^2_{Adj} \geq 0.877$) and associated with pH 7.8 ($R^2_{Adj} \geq 0.797$) except in the particles SIDI-MA (R^2_{Adj} : 0.470) and PH-SIDI-PE (R^2_{Adj} : 0.569). Therefore, the release of the formulated particles conceptualizes a constant compound release over specific times (super slow release) regarding a good fit of the first-order kinetic model.

The Higuchi model is based on a premier mathematical kinetic equation that describes drug release. Even though this model presented an overall good fit to the in vitro release results, some reservations about its suitability were still assumed. As general knowledge, the Higuchi models are sustained by numerous assumptions such as a scarce or non-existing swelling rate and the erosion of the coating matrix, with the drug release only being based on a Fickian diffusion for water-soluble and low-soluble drugs in the polymer matrix. The root-based Higuchi equation was used when the polymer matrices were swellable and biodegradable, giving rise to a *pseudo-steady-state* as described by Higuchi [33,61]. Most of the calculated results for the Higuchi model (Tables 5 and 6) revealed a good fit at both the tested pH ($R^2_{Adj} \geq 0.800$), indicating that the release of caffeine from most of the produced particles was dependent on the erosion of the coating matrix. Moreover, it was proved that the produced particles were swellable in both tested pH values, especially at pH 7.8 with the exception of SIDI-GA (R^2_{Adj} : 0.699) and PH/CHL-SIDI-GA/STC (R^2_{Adj} : 0.700), (Table 6).

The Hixson–Crowell model tries to mathematically justify the dissolution rate of uniform particles in a powder by describing the release profile based on the cube root of cumulative concentration of drugs released at time “t”. This equation was adjusted to numerous pharmaceutical products, namely tablets and drug particles [33,60]. Furthermore, this model assumes that the drug's release velocity is limited by its dissolution rate and not by its diffusion across the polymeric matrix. The Hixson–Crowell model justifies a drug release based on constant sustainability of the geometrical form of particles over time. In other words, the dissolution rate should be constant if the surface morphology of particles (whether cubic or spherical) is also constant. Hence, the samples that fit well with the Hixson–Crowell model have a constant geometrical based dissolution profile [29]. The R^2_{Adj} value of the Hixson–Crowell model fits better to the result obtained from pH 7.8, except PH/CHL-SIDI-GA (R^2_{Adj} : 0.791) and PH-SIDI-PE (R^2_{Adj} : 0.779), compared to results obtained from pH 2.1 in which particles PH-SIDI-MA (R^2_{Adj} : 0.670), SIDI-PE (R^2_{Adj} : 0.755), SIDI-GA/STC (R^2_{Adj} : 0.687), PH/CHL-SIDI-GA/STC (R^2_{Adj} : 0.670), SIDI-PE/STC (R^2_{Adj} :

0.557) showed the lowest fitting correlation determination. Therefore, the R^2_{Adj} obtained by fitting the Hixson–Crowell model to the dissolution results indicated that the caffeine release rate occurred by a dissolution process and not by the diffusion on the surface of particles. The prevalence of the dissolution over diffusion probably occurs because when the solution is agitated deeply it prevents the accumulation of particles at the bottom of the solution, resulting in a slow diffusion rate [33]. This phenomenon justified the slow-release time (stabilization time) obtained from most of particles.

The Korsmeyer–Peppas model is most often used to justify the release profile of substances from polymeric matrixes and additionally allow one to classify the release mechanism based on the interpretation of its release exponent (“ n ” in equation (6)). Korsmeyer–Peppas is a semi-empirical power law equation assuming the particles geometry spherical (6) [15], and when the n value corresponds to 0.43, the release is due to pure Fickian diffusion (Case I) (resulting in low polymer degradation); when n values reach 0.85 then the system release is a Case II transport following a zero-order kinetic, which is dependent on the swelling and relaxation of the polymer chains; when n values are between 0.43 and 0.85, the transport is anomalous, meaning that the mechanism of release involves both Fickian diffusion and Case II transport [29,31,62].

Considering the experimental results in the present work, the Korsmeyer–Peppas model was revealed to have a satisfactory fitting based on R^2_{Adj} at pH 7.8 ($R^2_{Adj} \geq 0.819$), except SIDI-MA (R^2_{Adj} : 0.477), and moderate satisfaction at pH 2.1 regarding that $R^2_{Adj} \geq 0.690$. The obtained results for this model (Tables 5 and 6) might be due to the non-linear release data (microcapsules/particles or microspheres), which was the main aim of the application of Korsmeyer–Peppas kinetic models [60].

By observing the calculated release factors, “ n ” values of the Korsmeyer–Peppas model (Tables 5 and 6), significant differences based on transport mechanisms were observed for all the synthesized carrier-particles under characterization, and additionally, these differences could not be categorized accordingly with the groups of the coating walls exploited in this study. Nevertheless, the drug releases could be divided in just two routes: Case-I drug transport and anomalous drug transport for both pH, with the exception of PH/CHL-SIDI-MA at pH 2.1, which followed super case-II drug transport ($n = 0.932$). When $n < 0.43$, the drug release follows case-I drug transport, justified by the low degradation of the polymers matrix, and as the n value increases, it indicates that the degradation of polymers also increases, giving rise to anomalous super case-II drug transport [15]. A low rate of polymer degradation results in a slow release profile. Therefore, the slow release of

most of the particles was justified [15]. However, overall, and considering the magnitude of the tabulated data (Tables 5 and 6), it was not possible to elaborate profiles of calculated release factors and relate these to the different groups of coating polymers exploited in the study. This way, the specific classification of the particle's dissolution assay at both pH levels could not be determined. This fact may be related to the diversity of the chemical composition of the NP used for producing the polymer coating walls.

In vitro drug release in terms of stabilization time was observed to be discordant in all samples under study with a slow-release time except in some particles (explained below in the same section). The super slow release may be justified by the concentration of the NP (1:10 and 1:15), which can make the drug's diffusion through a particle coating difficult (Tables 5 and 6). Our result is in accordance with the work of Paarakh et al. [33] who reported that the concentration of NP influences the media viscosity and, consequently, the drug's diffusion, significantly increasing the stabilization time. Furthermore, regarding pH 2.1, no significant differences were observed between the NP coating and NP-Secondary coating II, contrary to almost all particles of the NP-Secondary coating I (phytosome-based) in which the stabilization times were enhanced at current pH (Tables 5 and 6). Regarding pH 7.8, the stabilization release time varied between (216.1 and 1080.1 min) except for the gum Arabic coating in which the release time was enhanced from the NP coating to NP-Secondary coating II at both pH (ascending order; increase in release stabilization time by adding the coating layer resulting in the improvement of the particle stability and increase in the release time), which can be justified by findings in the work of Paarakh et al. [33] regarding the polymer concentration (explained in beginning of this paragraph). However, in contrast, maltodextrin stabilization times were observed in a descending order in which the release time decreased from the first coating layer (NP-Coating) to NP-Secondary coating II at pH 7.8, as seen in Table 6.

Considering there are numerous kinetic models already established to predict a great variety of release behaviors of different types of micro/nanoparticles, in the present work, different mathematical kinetic models were used to assess their adjustment to the drug release profiles from the several silverskin-based formulations produced [31]. The Korsmeyer–Peppas provided a better understanding about the release behavior of the generally developed carrier' particles [31,63]. The Korsmeyer–Peppas and first-order kinetic models were able to justify the slow release of most of the as-synthesized particles [15]. In vitro release assays curves of PE/STC series as an example is shown in Figure 4. The other curves are presented in the Supplementary Materials.

Table 5. In Vitro Release Assays at pH 2.1

Variable	MA			GA			PE			STC			GA/STC			PE/STC			
	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	
ST (min)	2670.1	540.1	1620.1	210.1	450.1	630.1	270.1	1688.1	522.1	1880.1	1800.1	1860	340.1	900.1	210.1	520.1	1230.1	1298.1	
ZO	F ₀	13.554	55.964	2.3204	31.877	49.773	29.398	57.423	15.517	53.611	49.120	44.165	7.946	71.305	54.364	49.044	69.368	48.045	45.833
	K ₀	0.027	0.013	0.052	0.162	0.072	0.045	0.023	0.044	0.029	0.024	0.032	0.046	0.019	0.0427	0.018	0.020	0.30	0.033
	R ²	0.938	0.321	0.956	0.679	0.697	0.766	0.469	0.965	0.442	0.829	0.735	0.975	0.285	0.619	0.361	0.2618	0.708	0.699
	R ² _a	0.927	0.297	0.954	0.639	0.673	0.746	0.453	0.964	0.420	0.824	0.726	0.975	0.254	0.600	0.31	0.2230	0.699	0.689
FO	K ₁	0.0004	0.0072	0.0002	0.006	0.0074	0.0065	0.006	0.001	0.0067	0.004	0.003	0.0003	0.009	0.008	0.012	0.018	0.007	0.0051
	K _{max}	135.9	75.82	346.5	106.68	95.485	58.959	87.742	123.62	91.992	85.267	90.691	219.803	93.698	86.740	63.917	92.398	85.443	88.626
	R ²	0.980	0.908	0.960	0.884	0.945	0.897	0.928	0.951	0.953	0.881	0.916	0.978	0.953	0.891	0.895	0.925	0.868	0.941
	R ² _a	0.977	0.905	0.959	0.869	0.941	0.889	0.926	0.949	0.951	0.877	0.913	0.977	0.951	0.886	0.888	0.921	0.863	0.939
HG	K _H	1.686	2.136	2.009	4.890	4.049	2.439	2.625	2.075	2.722	2.0283	2.457	1.933	3.008	3.390	2.515	3.073	2.574	2.629
	R ²	0.944	0.520	0.907	0.808	0.868	0.874	0.649	0.955	0.648	0.942	0.830	0.933	0.530	0.802	0.556	0.499	0.846	0.858
	R ² _a	0.944	0.520	0.907	0.808	0.868	0.874	0.649	0.955	0.648	0.942	0.830	0.933	0.530	0.802	0.556	0.499	0.846	0.858
HC	K _{HC}	0.0002	0.0006	0.0003	0.002	0.002	0.0005	0.0008	0.0003	0.0008	0.0005	0.0006	0.0003	0.001	0.0013	0.0005	0.001	0.0007	0.0007
	R ²	0.976	0.670	0.941	0.909	0.960	0.938	0.755	0.946	0.839	0.923	0.880	0.959	0.687	0.842	0.471	0.557	0.848	0.926
	R ² _a	0.976	0.670	0.941	0.909	0.960	0.838	0.755	0.946	0.839	0.923	0.880	0.959	0.687	0.842	0.471	0.557	0.848	0.926
KrP	K _{kp}	0.465	27.871	0.089	7.084	19.556	10.643	24.663	0.929	19.555	15.812	10.120	0.159	33.097	16.898	23.889	40.193	16.236	13.418
	n	0.664	0.139	0.932	0.435	0.244	0.260	0.181	0.613	0.218	0.231	0.301	0.849	0.149	0.249	0.149	0.123	0.236	0.266
	R ²	0.965	0.700	0.957	0.807	0.892	0.828	0.790	0.965	0.759	0.946	0.890	0.979	0.758	0.830	0.737	0.706	0.894	0.905
	R ² _a	0.960	0.690	0.955	0.783	0.883	0.814	0.783	0.964	0.750	0.944	0.886	0.978	0.748	0.822	0.721	0.691	0.890	0.902
DTM	ANM	C-I	C-II	ANM	C-I	C-I	C-I	ANM	C-I	C-I	C-I	C-I	ANM	C-I	C-I	C-I	C-I	C-I	C-I

***SIDI**; enriched fraction of SI used on all formulated samples, **PH**; phytosome, **PH/CHL**; cholesterol incorporated phytosome, **GA**; gum Arabic, **MA**; maltodextrin, **PE**; pectin, **STC**; Starch. **Min**; minutes, **ZO**; Zero-order, **FO**; First-Order, **KrP**; Korsmeyer-pepps, **HG**; Higuchi, **HC**; Hixson-Crowell, **ST**; Stabilization time (approximately the time when the release start to be stable and the curve change to be linear), **R²_a**; **R²_{adjusted}**, **DTM**; Drug transport mechanism. **C-I**; Case-I, **ANM**; anomalous, **C-II**; Case-II transport, **SC-II**; Super case-II.

Table 6. *In Vitro* Release Assays at pH 7.8

Variable	MA			GA			PE			STC			GA/STC			PE/STC			
	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	SIDI	PH	PH/CHL	
ST (min)	500.1	100.1	50.1	54.1	239.1	1229.1	1634.1	1514.1	1594.1	2160.1	2107.1	2160.1	1080.1	1080.1	1080.1	1900.1	1260	1944.1	
ZO	F ₀	76.984	28.249	38.031	66.060	43.196	38.309	39.624	52.759	40.692	14.169	16.400	7.122	27.114	44.0878	56.337	26.737	28.941	12.727
	K ₀	0.031	0.412	0.461	0.250	0.190	0.178	0.036	0.023	0.037	0.034	0.037	0.036	0.059	0.046	0.035	0.033	0.035	0.046
	R ²	0.827	0.956	0.663	0.475	0.835	0.980	0.910	0.838	0.880	0.961	0.910	0.947	0.913	0.709	0.508	0.952	0.887	0.930
	R ² _a	0.816	0.952	0.642	0.451	0.826	0.979	0.907	0.834	0.975	0.959	0.907	0.945	0.910	0.697	0.489	0.951	0.882	0.927
FO	K ₁	0.010	0.023	0.028	0.097	0.012	0.0009	0.003	0.030	0.003	0.001	0.001	0.0004	0.003	0.007	0.009	0.001	0.003	0.0008
	K _{max}	97.320	77.731	99.845	94.204	97.688	371.43	87.869	77.297	93.081	126.21	120.03	156.62	90.655	86.158	89.574	94.589	71.232	124.290
	R ²	0.502	0.813	0.962	0.874	0.935	0.982	0.887	0.580	0.940	0.986	0.964	0.982	0.957	0.936	0.965	0.961	0.850	0.984
	R ² _a	0.470	0.797	0.959	0.869	0.931	0.982	0.882	0.569	0.937	0.986	0.963	0.982	0.956	0.933	0.964	0.960	0.844	0.983
HG	K _H	4.141	7.076	8.859	10.234	6.069	6.759	2.521	2.475	2.564	1.799	1.922	1.740	2.762	3.101	3.201	2.022	2.078	2.108
	R ²	0.865	0.912	0.837	0.699	0.944	0.967	0.989	0.903	0.977	0.963	0.934	0.950	0.977	0.862	0.700	0.978	0.933	0.956
	R ² _a	0.865	0.912	0.837	0.699	0.944	0.967	0.989	0.903	0.977	0.963	0.934	0.950	0.977	0.862	0.700	0.978	0.933	0.956
HC	K _{HC}	0.0017	0.0036	0.0076	0.0093	0.003	0.0016	0.0005	0.0007	0.0006	0.0002	0.0003	0.0002	0.0006	0.001	0.012	0.0003	0.0003	0.0003
	R ²	0.802	0.919	0.969	0.875	0.971	0.791	0.9562	0.779	0.976	0.986	0.968	0.984	0.958	0.919	0.877	0.982	0.904	0.987
	R ² _a	0.802	0.919	0.969	0.875	0.971	0.791	0.9562	0.779	0.976	0.986	0.968	0.984	0.958	0.919	0.877	0.982	0.904	0.987
KrP	K _{kp}	71.286	14.051	16.452	46.261	15.323	2.331	11.684	30.156	9.538	0.485	0.605	0.211	4.119	14.235	23.560	2.416	5.371	0.613
	n	0.043	0.343	0.367	0.157	0.323	0.657	0.279	0.141	0.312	0.676	0.658	0.781	0.440	0.267	0.198	0.475	0.361	0.671
	R ²	0.508	0.857	0.868	0.827	0.912	0.985	0.976	0.899	0.964	0.980	0.944	0.966	0.978	0.910	0.809	0.975	0.919	0.964
	R ² _a	0.477	0.845	0.860	0.819	0.907	0.984	0.975	0.897	0.962	0.979	0.942	0.965	0.977	0.906	0.809	0.974	0.915	0.962
DTM	C-I	C-I	C-I	C-I	C-I	ANM	C-I	C-I	C-I	ANM	ANM	ANM	ANM	C-I	C-I	ANM	C-I	ANM	

* **SIDI**; enriched fraction of SI used on all formulated samples, **PH**; phytosome, **PH/CHL**; cholesterol incorporated phytosome, **GA**; gum Arabic, **MA**; maltodextrin, **PE**; pectin, **STC**; Starch. **Min**; minutes, **ZO**; Zero-order, **FO**; First-Order, **KrP**; Korsmeyer-pepps, **HG**; Higuchi, **HC**; Hixson-Crowell, **ST**; Stabilization time (the release start to be stable and the curve change to linear). **R²_a**; **R²_{adjusted}**, **DTM**; Drug transport mechanism. **C-I**; Case-I, **ANM**; anomalous, **C-II**; Case-II transport, **SC-II**; Super case-II.

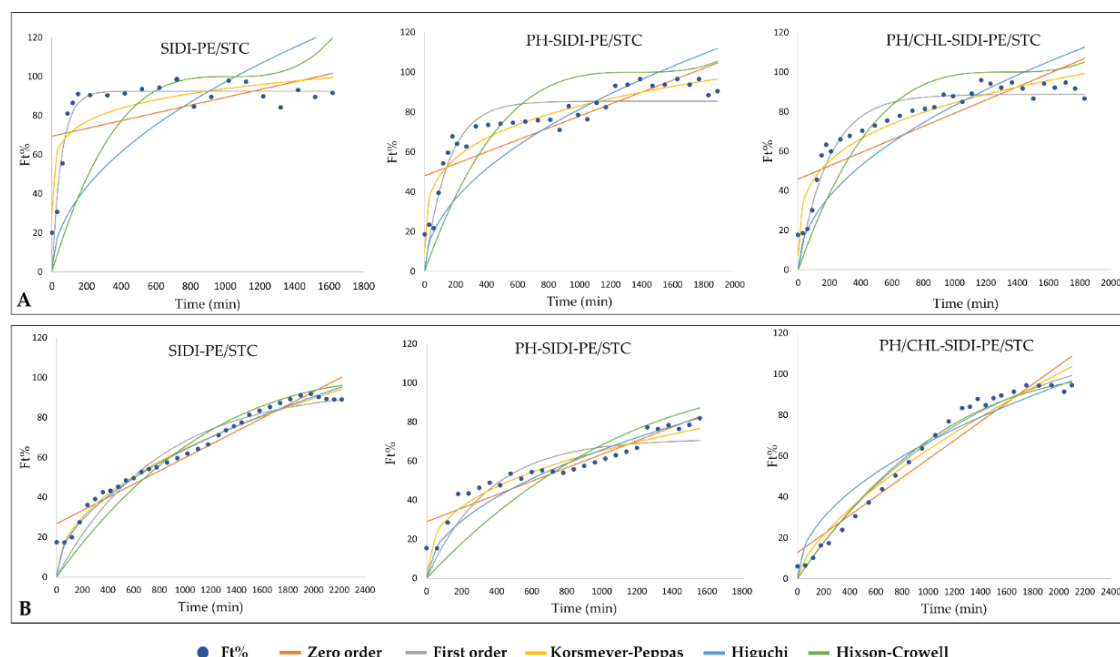


Figure 4. In vitro release assays curves of PE/STC series at two different pH (**A**: pH 2.1; **B**: pH 7.6). The other curves are presented in the Supplementary Materials.

5.3.3.2.8. *In silico* Investigation Based on Molecular Docking

In silico investigation of molecular arrangement was based on caffeine (the main alkaloid structure) and 5-caffeoylquinic acid (also known as chlorogenic acid), which is the main phenolic of SI (Figure 5 and Table 7). The molecular docking confirmed a good interaction between the phytochemicals and the phospholipid bilayer. The molecular arrangement of phospholipid in the nano-phytosome bilayer and cholesterol-incorporated nano-phytosome was designed using the MemGen webserver. The phytoconstituents were docked on both lipid bilayers and evaluated based on a 3D model (Figures 4–7), docking score, and grid energy (Table 7), taking into account that a low docking score justifies a high number of hydrogen binding and stronger interactions [64].

The data elicited by molecular docking showed lower and higher docking scores for 5-caffeoylquinic acid and caffeine, respectively, in both lipid bilayers (nano-phytosome and nano-phytosome incorporated by cholesterol) (Table 7). Note that lower docking scores denote higher hydrogen binding. 5-Caffeoylquinic acid exhibited a lower absolute docking score in both structures, although was better suited for the phytosome bilayer (Table 7 and Figures 5 and 6). It was shown that the phytoconstituents were positioned within the lipid bilayer, exhibiting high hydrogen binding between the polar site of the lipid bilayer and the hydroxyl groups of 5-caffeoylquinic acid. Pruchnik et al. [65] also confirmed the interaction between chlorogenic acid and biological membranes, as verified in this work. The referenced authors evaluated the chlorogenic acid permeation

mechanism by differential scanning calorimetry and fluorescence spectroscopy, showing the interaction between chlorogenic acid and the polar site of the biological membrane. Therefore, the lower docking score obtained for 5-caffeoylquinic acid (Table 7) is justified.

The interaction between nitrogen and the choline site of phospholipids was confirmed previously by molecular dynamics simulation (MD) regarding the peroxynitrous acid (ONOOH) and phospholipid bilayers [66]. Therefore, the higher docking score suggests the weaker hydrogen binding of caffeine (Table 7). This behavior may be due to its small size, not allowing a good incorporation in the lipid bilayer. However, based on the 3D model (Figures 5 and 6), caffeine was adjusted on the polar site of phosphatidylcholine, just like the chlorogenic acid, but with a low docking score. Phenolics arrangement within a lipid bilayer was reported, recently, for the first time [16]. Based on information reported by Pruchnik et al. [65] and Cordeiro et al. [66] (already discussed in the current section) and the results obtained in this study regarding the *in silico* molecular arrangement, the interaction between active phytoconstituents/drugs with biological cell membranes could be properly predicted by molecular docking and molecular dynamic simulations.

Table 7. Binding energy of docked molecule expressed on kcal/mol.

Lipid Structure	Categories	Main Compounds	Docking Score	Glide evdw	Glide Energy
<i>Phytosome</i>	Alkaloid	Caffeine	-2.518	-20.202	-21.949
	Phenolic	Chlorogenic acid	-9.337	-31.584	-42.286
		5-caffeoylquinic acid	-9.008	-26.590	-39.743
<i>Cholesterol incorporated phytosome</i>	Alkaloid	Caffeine	-2.347	-17.696	-21.247
	Phenolic	Chlorogenic acid	-8.199	-30.543	-43.936
		5-caffeoylquinic acid	-7.120	-25.621	-39.665

* Docking score exhibited the number of hydrogen bindings occurring between structures. Lower docking scores denote a higher hydrogen binding.

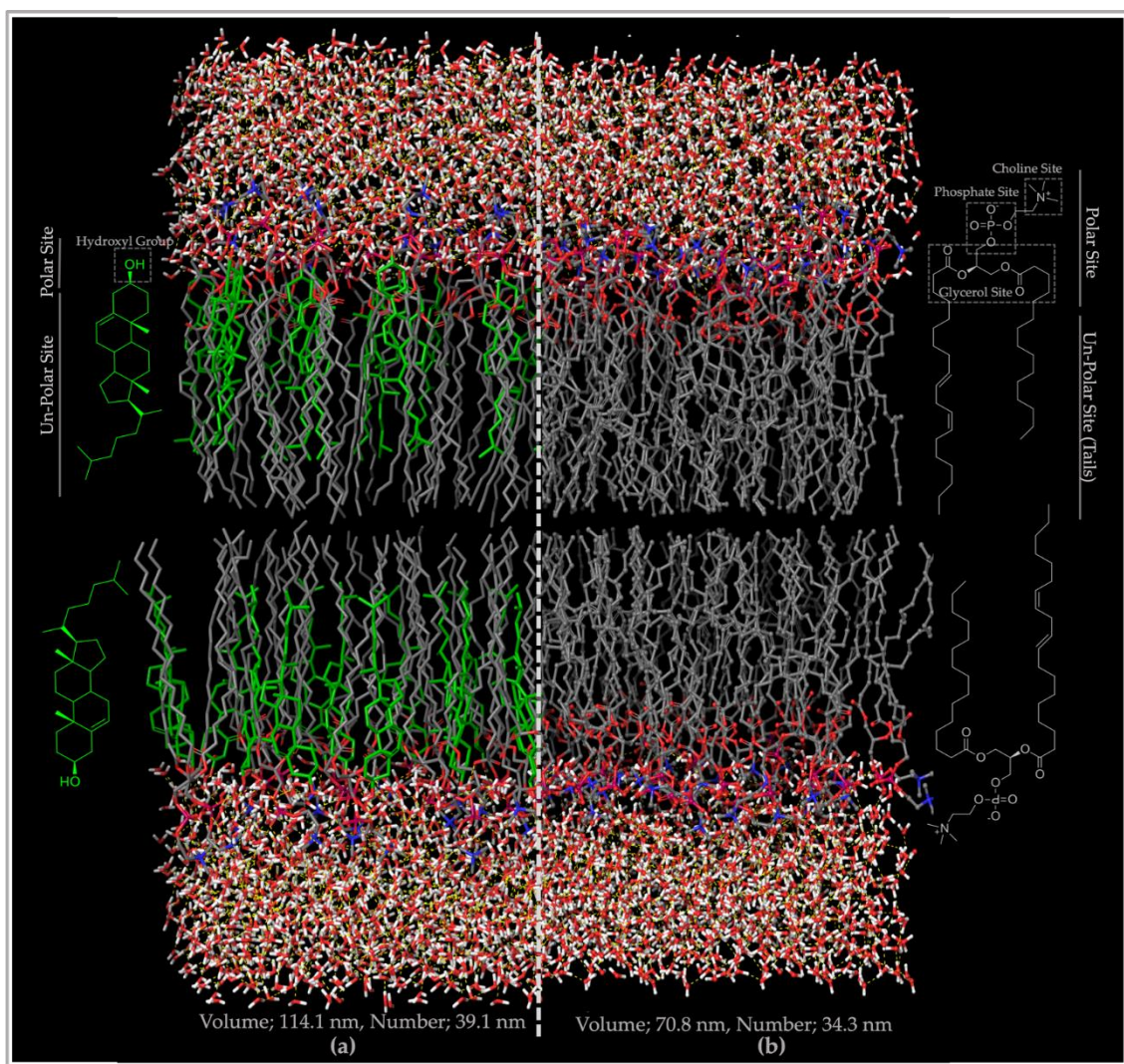


Figure 4. 3D model of lipid bilayers. (a) Bilayer incorporated by phosphatidylcholine and cholesterol; (b) the simulated model for bilayer designed by phosphatidylcholine. The polar site of the bilayer is fitted to the outer layer and covered with hundreds of water molecules (the white and red site on top and bottom of bilayer). The difference on diameter of bilayer between structure (a) and (b) was discussed on *Section 3.2.4.*, and Table 4.

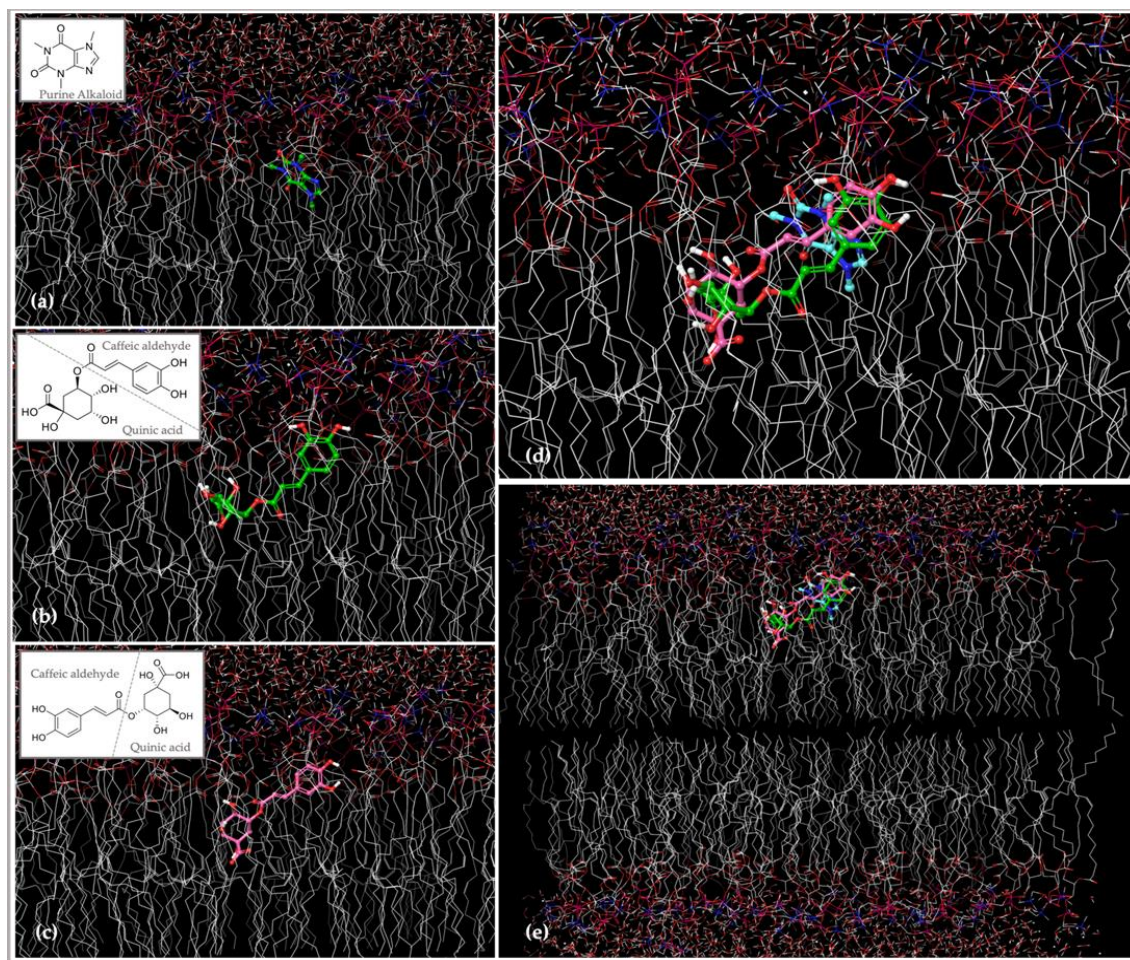


Figure 5. Simulated 3D model of grid of nano-phytosome bilayer docked with main phytoconstituents of SI (Caffeine, chlorogenic acid and 5-caffeoylquinic acid). In the model, for better visualization, only polar hydrogens are represented. The lipid bilayer appears spherical (see on Figure 4). (a) Caffeine (purine alkaloid) adjusted near a polar size with minimum rate of hydrogen binding. (b) Chlorogenic acid. (c) 5-caffeoylquinic acid. The majority of hydrogen binding occurred by caffeic aldehyde site on image (b) and (c). (d) and (e) show an overlap of the compound's interaction with the lipid bilayer.

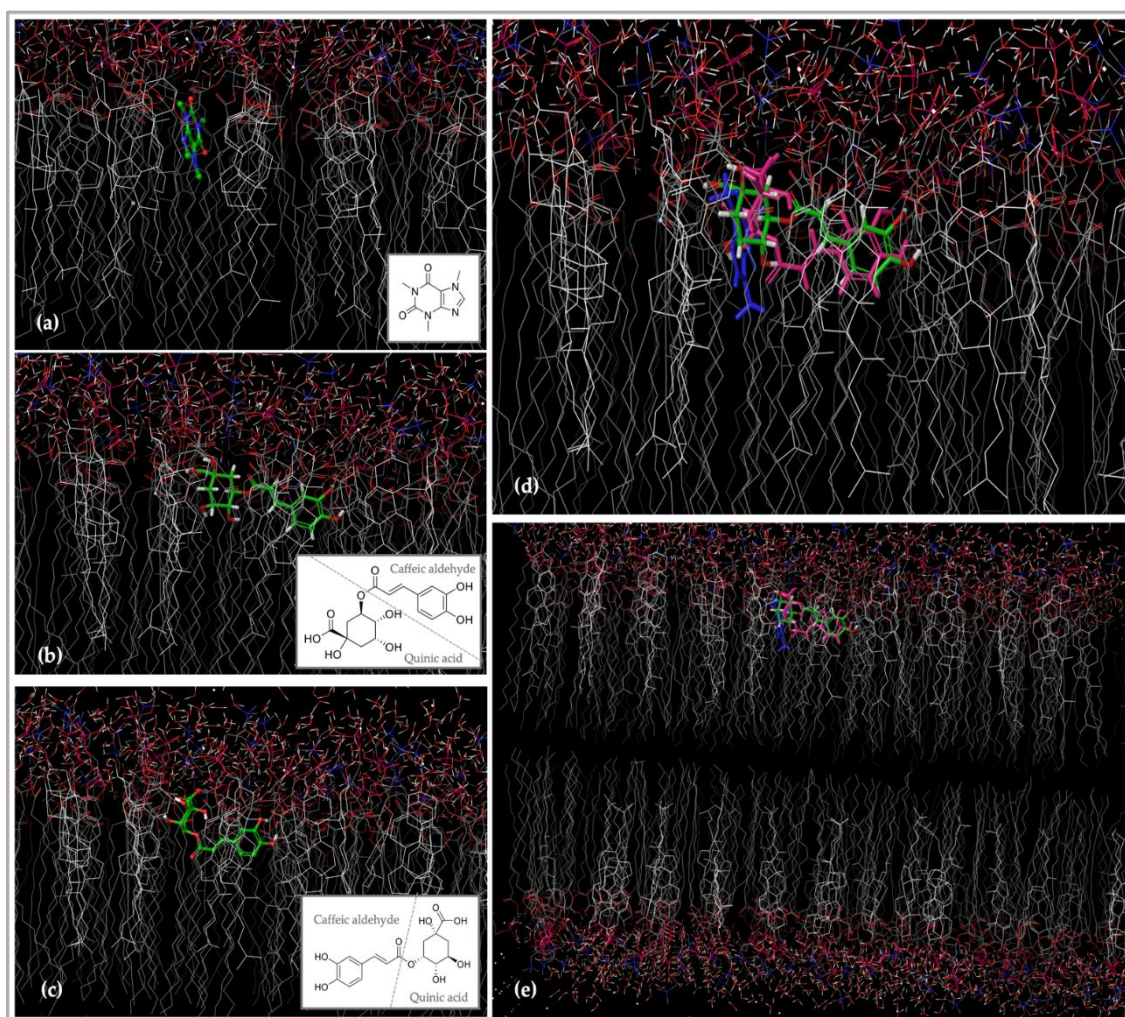


Figure 6. Simulated 3D model of the nano-phytosome incorporated by cholesterol bilayer docked with main phytoconstituents of SI (silverskin by-product, caffeine, chlorogenic acid, and 5-caffeoylquinic acid). The lipid bilayer appears spherical (see on Figure 4). (a) caffeine (purine alkaloid); adjusted near a polar size with minimum rate of hydrogen binding. (b) chlorogenic acid, (c) 5-caffeoylquinic acid. The majority of hydrogen binding occurred by both Caffeic aldehyde and Quinic acid site on image (b), and (c). (d) and (e) show an overlap of the compound's interaction with the lipid bilayer.

5.4. Conclusions

The objective of this work was to obtain the highest enrichment of a coffee roasting by-product (SI) extract, followed by its incorporation in nanoparticles. Nano-phytosomes were formulated with the enriched fraction to improve the phytochemicals' permeation through the cell membrane. The nano-phytosome surface was modified using cholesterol to improve the permeation rate and stability during the formulation process. The structural arrangements were evaluated by *in silico* molecular docking. To enhance the stability of the formulated structures and natural phytoconstituents, the first lipid-based layer was also coated with NPs.

The high yield of SI enrichment and the enrichment rate associated with caffeine and total phenolics as main constituents was obtained in the fraction SI₁DI₁ and, because of that, it was selected to load the micro/nanoparticles.

Different formulation techniques were used, and the nanoparticles and nano-sized colloids were evaluated regarding physicochemical properties and in vitro drug release.

The experimental data revealed different formulation groups and significant similarities within related subgroups. In conclusion, higher and lower product yields were observed at $\geq 70.00\%$ (SIDI-STC and PH-SIDI-GA) and $\leq 40.00\%$ (SIDI-PE/STC and PH-SIDI-PE/STC), respectively. No significant differences ($p > 0.05$) were found between the EE% of the different groups of particles, while significantly lower ($p < 0.05$) LC% values were observed in NP-secondary coating II (PH/CHL-SIDI-NPs).

Both lipid-based layer structures were confirmed as nanoparticles, and the external layer (NP-coating) varied between 1 and 5 μm for all samples under study. Concerning the inner layer size, it formed various subgroups and varied mainly between 226.1 and 808.7 nm in volume, and from 187 to 786.8 nm, in number.

All formulated particles exhibited anionic surface charges and high stability in aqueous media. FTIR and molecular docking confirmed the interaction of the layers with the purine alkaloid (caffeine) and the phenolic compound (5-caffeoylquinic acid). The best docking score was recorded for 5-caffeoylquinic acid, which exhibited stronger hydrogen bonding, resulting in a better/stronger interaction.

The in vitro release assays conceptualize the dissolution rate of a specific drug encapsulated in the formulated particles. Considering that the natural polymers' layers used in this study have good solubility at neutral pH, a higher number of samples with a good fitting of drug release models at pH 7.8 was confirmed. The obtained results allow us to hypothesize that the drug release profile from the particles was pH dependent, and that the coating layers in this work allowed a sustained and controlled drug release at pH 7.8.

Moreover, the majority of the particles followed a super slow release time confirmed by all kinetic models under study following both erosion and diffusion release due to the type of coating layers. Korsmeyer–Peppas, First-order, and Higuchi fitted well to the experimental data regarding the R^2_{adj} parameter. Furthermore, the majority of formulated particles followed the case-I and anomalous transport mechanism.

To conclude, independently of the type of the coating layer used (first or secondary), the final products can have high efficiency at lower dosages, making them suitable for oral delivery. Based on their enriched content in caffeine and phenolic compounds (antioxidants), they have potential to be used, for instance, in dietary supplements to improve mental and physical performance, competing with other products already existing in the market.

Supplementary Materials: The supporting information contain the in vitro release assays curves of all samples can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: In vitro release assays curves associated to MA series at pH 2.1; Figure S2: In vitro release assays curves associated to MA series at pH 7.8; Figure S3: In vitro release assays curves associated to GA series at pH 2.1; Figure S4: In vitro release assays curves associated to GA series at pH 7.8; Figure S5: In vitro release assays curves associated to STC series at pH 2.1; Figure S6: In vitro release assays curves associated to STC series at pH 7.8; Figure S7: In vitro release assays curves associated to GA/STC series at pH 2.1; Figure S8: In vitro release assays curves associated to GA/STC series at pH 7.8; Figure S9: In vitro release assays curves associated to PE series at pH 2.1; Figure S10: In vitro release assays curves associated to PE series at pH 7.8.

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5.5. References

1. Rashid, N.; Ashraf, I.; Ramzan, S. Impacts of Food Industrial Wastes on Soil and Its Utilization as Novel Approach for Value Addition. In *Research Anthology on Food Waste Reduction and Alternative Diets for Food and Nutrition Security*, Bhat, R., Qadri, H., Wani, K., Dar, G., Mehmood, M., Eds.; IGI Global: Hershey, PA, USA, 2021; pp. 652–669.
2. Comunian, T.A.; Silva, M.P.; Souza, C.J.F. The use of food by-products as a novel for functional foods: Their use as ingredients and for the encapsulation process. *Trends Food Sci. Technol.* **2021**, *108*, 269–280.
3. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Barreira, S.V.P.; Nunes, M.A.; Cunha, L.M.; Oliveira, M.B.P.P. Optimization of antioxidants extraction from coffee silverskin, a roasting by-product, having in view a sustainable process. *Ind. Crop. Prod.* **2014**, *53*, 350–357.
4. Costa, A.S.G.; Alves, R.C.; Vinha, A.F.; Costa, E.; Costa, C.S.G.; Nunes, M.A.; Almeida, A.A.; Santos-Silva, A.; Oliveira, M.B.P.P. Nutritional, chemical and antioxidant/pro-oxidant profiles of silverskin, a coffee roasting by-product. *Food Chem.* **2018**, *267*, 28–35.
5. Fathi, F.; Ebrahimi, S.N. Investigation of physiochemical properties nanophytosome obtained from of polyphenolic enrich fraction of *Satureja khuzistanica* by freeze-

-
- drying. Nashrieh Shimi va Mohandesi Shimi Iran. In Press. Available online: https://www.nsmsi.ir/article_242399.html?lang=en
6. Grgić, J.; Šelo, G.; Planinić, M.; Tišma, M.; Bucić-Kojić, A. Role of the encapsulation in bioavailability of phenolic compounds. *Antioxidants* **2020**, *9*, 923–958.
 7. Amin, T.; Bhat, S.V. A review on phytosome technology as a novel approach to improve the bioavailability of nutraceuticals. *Int. J. Adv. Res. Technol.* **2012**, *1*, 1–5.
 8. Ozkan, G.; Kostka, T.; Esatbeyoglu, T.; Capanoglu, E. Effects of Lipid-Based Encapsulation on the Bioaccessibility and Bioavailability of Phenolic Compounds. *Molecules* **2020**, *25*, 5545.
 9. Rasaee, S.; Ghanbarzadeh, S.; Mohammadi, M.; Hamishehkar, H. Nano phytosomes of quercetin: A promising formulation for fortification of food products with antioxidants. *Pharm. Sci.* **2014**, *20*, 96–101.
 10. Liu, N.; Park, H.-J. Chitosan-coated nanoliposome as vitamin E carrier. *J. Microencapsul.* **2009**, *26*, 235–242.
 11. Hosseini, H.; Tajiani, Z.; Jafari, S.M. Improving the shelf-life of food products by nano/micro-encapsulated ingredients. In *Food Quality and Shelf Life*; Galanakis, C.M., Ed.; Elsevier: Amsterdam, The Netherlands, 2019; pp. 159–200.
 12. Bessada, S.M.; Alves, R.C.; Oliveira, M.B.P. Caffeine-based food supplements and beverages: Trends of consumption for performance purposes and safety concerns. *Int. Food Res.* **2018**, *109*, 310–319.
 13. Plapied, L.; Duhem, N.; des Rieux, A.; Préat, V. Fate of polymeric nanocarriers for oral drug delivery. *Curr. Opin. Colloid Interface Sci.* **2011**, *16*, 228–237.
 14. Wu, W.; Wang, J.; Lin, Z.; Li, X.; Li, J. Tumor-Acidity Activated Surface Charge-Conversion of Polymeric Nanocarriers for Enhanced Cell Adhesion and Targeted Drug Release. *Macromol. Rapid Commun.* **2014**, *35*, 1679–1684.
 15. Danaei, M.; Dehghankhold, M.; Ataei, S.; Hasanzadeh Davarani, F.; Javanmard, R.; Dokhani, A.; Khorasani, S.; Mozafari, M. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems. *Pharmaceutics* **2018**, *10*, 57.
 16. Fathi, F.; Ebrahimi, S.N.; Valadão, A.I.G.; Andrade, N.; Costa, A.S.G.; Silva, C.; Fathi, A.; Salehi, P.; Martel, F.; Alves, R.C.; et al. Exploring *Gunnera tinctoria*: From Nutritional and Anti-Tumoral Properties to Phytosome Development Following Structural Arrangement Based on Molecular Docking. *Molecules* **2021**, *26*, 5935–5962.
 17. Puga, H.; Alves, R.C.; Costa, A.S.G.; Vinha, A.F.; Oliveira, M.B.P. Multi-frequency multimode modulated technology as a clean, fast, and sustainable process to recover antioxidants from a coffee by-product. *J. Clean. Prod.* **2017**, *168*, 14–21.
 18. Ebrahimi, S.N.; Gafner, F.; Dell'Acqua, G.; Schweikert, K.; Hamburger, M. Flavone 8-C-Glycosides from *Haberlea rhodopensis* Friv.(Gesneriaceae). *Helv. Chim. Acta.* **2011**, *94*, 38–45.
 19. Shehzad, O.; Jin Ha, I.; Park, Y.; Wan Ha, Y.; Shik Kim, Y. Development of a rapid and convenient method to separate eight ginsenosides from *Panax ginseng* by high-speed counter-current chromatography coupled with evaporative light scattering detection. *J. Sep. Sci.* **2011**, *34*, 1116–1122.

20. Singh, R.P.; Gangadharappa, H.V.; Mruthunjaya, K. Phytosome complexed with chitosan for gingerol delivery in the treatment of respiratory infection: In vitro and in vivo evaluation. *Eur. J. Pharm. Sci.* **2018**, *122*, 214–229.
21. Anwar, E.; Farhana, N. Formulation and Evaluation of Phytosome-Loaded Maltodextrin-Gum Arabic Microsphere System for Delivery of *Camellia sinensis* Extract. *J. Young Pharm.* **2018**, *10*, 56–62.
22. Tuyen, C.K.; Nguyen, M.H.; Roach, P.D.; Stathopoulos, C.E. Microencapsulation of Gac oil: Optimisation of spray drying conditions using response surface methodology. *Powder Technol.* **2014**, *264*, 298–309.
23. Santana, A.A.; Oliveira, R.A.d.; Kurozawa, L.E.; Park, K.J. Microencapsulation of pequi pulp by spray drying: Use of modified starches as encapsulating agent. *Eng. Agricola* **2014**, *34*, 980–991.
24. Campos, J.C.; Ferreira, D.C.; Lima, S.; Reis, S.; Costa, P.J. Swellable polymeric particles for the local delivery of budesonide in oral mucositis. *Int. J. Pharm.* **2019**, *566*, 126–140.
25. Nagarwal, R.C.; Kumar, R.; Pandit, J.K. Chitosan coated sodium alginate–chitosan nanoparticles loaded with 5-FU for ocular delivery: In vitro characterization and in vivo study in rabbit eye. *Eur. J. Pharm. Sci.* **2012**, *47*, 678–685.
26. Boidya, J.; Hasan, I.; Reza, M.S. Carvedilol Matrix Tablet: Formulation and In Vitro Assessment. *Bangladesh Pharm. J.* **2020**, *23*, 26–31.
27. Arisoy, S.; Comoglu, T. Kinetic evaluation of L-Dopa loaded WGA-grafted nanoparticles. *Med. Sci.* **2020**, *9*, 385–388.
28. Minisy, I.M.; Salahuddin, N.A.; Ayad, M.M. In vitro release study of ketoprofen-loaded chitosan/polyaniline nanofibers. *Polym. Bull.* **2020**, *78*, 5609–5622.
29. Costa, P.; Lobo, J.M.S. Modeling and comparison of dissolution profiles. *Eur. J. Pharm. Sci.* **2001**, *13*, 123–133.
30. Costa, A.L.d.O.; Enéas, P.C.R.; Miranda, T.A.; Mingoti, S.A.; Soares, C.D.V.; Pianetti, G.A. In vitro dissolution kinetic for mycophenolic acid derivatives tablets. *Braz. J. Pharm. Sci.* **2013**, *49*, 311–319.
31. Permanadewi, I.; Kumoro, A.; Wardhani, D.; Aryanti, N. Modelling of controlled drug release in gastrointestinal tract simulation. *J. Phys. Conf. Ser.* **2019**, *1295*, 012063, <https://doi.org/10.1088/1742-6596/1295/1/012063>.
32. Rehman, Q.; Akash, M.S.H.; Rasool, M.F.; Rehman, K. Role of Kinetic Models in Drug Stability. In *Drug Stability and Chemical Kinetics*; Akash, M.S.H., Rehman, K., Eds.; Springer: Singapore, 2020; pp. 155–165.
33. Paarakh, M.P.; Jose, P.A.; Setty, C.; Christoper, G.P. Release kinetics–concepts and applications. *Int. J. Pharm. Res. Technol* **2018**, *8*, 12–20.
34. Cojocaru, V.; Ranetti, A.E.; Hinescu, L.G.; Ionescu, M.; Cosmescu, C.; Poștoarcă, A.G.; Cintează, L.O. Formulation and evaluation of in vitro release kinetics of Na₃CaDTPA decorporation agent embedded in microemulsion-based gel formulation for topical delivery. *Farmacologia* **2015**, *63*, 656–664.
35. Knight, C.J.; Hub, J.S. MemGen: A general web server for the setup of lipid membrane simulation systems. *Bioinformatics* **2015**, *31*, 2897–2899.

36. Scordino, M.; Di Mauro, A.; Passerini, A.; Maccarone, E. Selective recovery of anthocyanins and hydroxycinnamates from a byproduct of citrus processing. *J. Agric. Food Chem.* **2005**, *53*, 651–658.
37. Mahato, N.; Sinha, M.; Sharma, K.; Koteswararao, R.; Cho, M.H. Modern extraction and purification techniques for obtaining high purity food-grade bioactive compounds and value-added co-products from citrus wastes. *Foods* **2019**, *8*, 523–604.
38. Fujiwara, Y.; Takaki, A.; Uehara, Y.; Ikeda, T.; Okawa, M.; Yamauchi, K.; Ono, M.; Yoshimitsu, H.; Nohara, T. Tomato steroidal alkaloid glycosides, esculeosides A and B, from ripe fruits. *Tetrahedron* **2004**, *60*, 4915–4920.
39. Jovanović, A.A.; Lević, S.M.; Pavlovic, V.B.; Markovic, S.B.; Pjanovic, R.V.; Đorđević, V.B.; Nedović, V.; Bugarski, B.M. Freeze versus Spray Drying for Dry Wild Thyme (*Thymus serpyllum* L.) Extract Formulations: The Impact of Gelatin as a Coating Material. *Biomaterials* **2021**, *26*, 3933–3951.
40. Tontul, I.; Topuz, A. Spray-drying of fruit and vegetable juices: Effect of drying conditions on the product yield and physical properties. *Trends Food Sci Technol.* **2017**, *63*, 91–102.
41. Ferreira, N.G.; Pereira Martin, L.G.; Matta Fakhouri, F.; Oliveira, A.d.R. Microencapsulation of blackberry pulp with arrowroot starch and gum arabic mixture by spray drying. *J. Microencapsul.* **2018**, *35*, 482–493.
42. Wang, Y.; Xie, Y.; Xu, D.; Lin, X.; Feng, Y.; Hong, Y. Hydroxypropyl methylcellulose reduces particle adhesion and improves recovery of herbal extracts during spray drying of Chinese herbal medicines. *Dry. Technol.* **2014**, *32*, 557–566.
43. Huang, Z.; Brennan, C.S.; Zhao, H.; Liu, J.; Guan, W.; Mohan, M.S.; Stipkovits, L.; Zheng, H.; Kulasiri, D. Fabrication and assessment of milk phospholipid-complexed antioxidant phytosomes with vitamin C and E: A comparison with liposomes. *Food Chem.* **2020**, *324*, 126837.
44. Gharsallaoui, A.; Roudaut, G.; Chambin, O.; Voilley, A.; Saurel, R. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Res. Int.* **2007**, *40*, 1107–1121.
45. Roccia, P.; Martínez, M.L.; Llabot, J.M.; Ribotta, P.D. Influence of spray-drying operating conditions on sunflower oil powder qualities. *Powder Technol.* **2014**, *254*, 307–313.
46. Hou, Z.; Li, Y.; Huang, Y.; Zhou, C.; Lin, J.; Wang, Y.; Cui, F.; Zhou, S.; Jia, M.; Ye, S. Phytosomes loaded with mitomycin C-soybean phosphatidylcholine complex developed for drug delivery. *Mol. Pharm.* **2013**, *10*, 90–101.
47. Zhang, J.; Tang, Q.; Xu, X.; Li, N. Development and evaluation of a novel phytosome-loaded chitosan microsphere system for curcumin delivery. *Int. J. Pharm.* **2013**, *448*, 168–174.
48. Minaei, A.; Sabzichi, M.; Ramezani, F.; Hamishehkar, H.; Samadi, N. Co-delivery with nano-quercetin enhances doxorubicin-mediated cytotoxicity against MCF-7 cells. *Mol. Biol. Rep.* **2016**, *43*, 99–105.
49. Nazari, M.; Ghanbarzadeh, B.; Kafil, H.S.; Zeinali, M.; Hamishehkar, H. Garlic essential oil nanophytosomes as a natural food preservative: Its application in yogurt as food model. *Colloids Interface Sci. Commun.* **2019**, *30*, 100176.

50. Chanamai, R.; McClements, D. Comparison of gum arabic, modified starch, and whey protein isolate as emulsifiers: Influence of pH, CaCl₂ and temperature. *J. Food Sci.* **2002**, *67*, 120–125.
51. Ansari, F.; Sobhani, A.; Salavati-Niasari, M. Simple sol-gel synthesis and characterization of new CoTiO₃/CoFe₂O₄ nanocomposite by using liquid glucose, maltose and starch as fuel, capping and reducing agents. *J. Colloid Interface Sci.* **2018**, *514*, 723–732.
52. Papoutsis, K.; Golding, J.B.; Vuong, Q.; Pristijono, P.; Stathopoulos, C.E.; Scarlett, C.J.; Bowyer, M. Encapsulation of citrus by-product extracts by spray-drying and freeze-drying using combinations of maltodextrin with soybean protein and ι-Carrageenan. *Foods* **2018**, *7*, 115–127.
53. Gangurde, A.B.; Ali, M.T.; Pawar, J.N.; Amin, P.D. Encapsulation of vitamin E acetate to convert oil to powder microcapsule using different starch derivatives. *J. Pharm. Investig.* **2017**, *47*, 559–574.
54. Cortes, U.A.B.; Gutiérrez, M.C.; Mendoza, D.G.; Salitre, L.G.; Vargas, A.S.; Catzim, C.E.A.; Durán, C.C.; Valenzuela, B.E.L. Microencapsulation and antimicrobial activity of extract acetone-methanol of *Hibiscus sabdariffa* L. using a blend modified starch and pectin as a wall material. *Ind. Crops Prod.* **2021**, *170*, 113725.
55. Clogston, J.D.; Patri, A.K. Zeta potential measurement. In *Characterization of Nanoparticles Intended for Drug Delivery*; Scott, E.M., Ed.; Humana Press: Totowa, NJ, USA, 2011; Volume 697, pp. 63–70.
56. Bhattacharjee, S. DLS and zeta potential-what they are and what they are not? *J. Control. Release* **2016**, *235*, 337–351.
57. Tang, Q.; Cao, B.; Wu, H.; Cheng, G. Cholesterol-peptide hybrids to form liposome-like vesicles for gene delivery. *PLoS ONE* **2013**, *8*, e54460.
58. Amit, P.; Tanwar, Y.; Rakesh, S.; Poojan, P. Phytosome: Phytolipid drug delivery system for improving bioavailability of herbal drug. *J. Pharm. Sci. Biosci. Res.* **2013**, *3*, 51–57.
59. Hindarto, C.K.; Surini, S.; Saputri, F.C.; Irawan, C. In vivo evaluation of luteolin-loaded phytosome. *J. Pharm. Innov.* **2017**, *6*, 347–349.
60. Ramteke, K.H.; Dighe, P.A.; Kharat, A.R.; Patil, S.V. Mathematical models of drug dissolution: A review. *Sch. Acad. J. Pharm.* **2014**, *3*, 388–396.
61. Siepmann, J.; Peppas, N.A. Higuchi equation: Derivation, applications, use and misuse. *Int. J. Pharm.* **2011**, *418*, 6–12.
62. Varga, N.; Turcsányi, Á.; Hornok, V.; Csapó, E. Vitamin E-loaded PLA-and PLGA-based core-shell nanoparticles: Synthesis, structure optimization and controlled drug release. *Pharmaceutics* **2019**, *11*, 357.
63. Hamed, S.; Koosha, M. Designing a pH-responsive drug delivery system for the release of black-carrot anthocyanins loaded in halloysite nanotubes for cancer treatment. *Appl. Clay Sci.* **2020**, *197*, 105770.
64. Choudhary, M.I.; Shaikh, M.; tul-Wahab, A.; ur-Rahman, A. In silico identification of potential inhibitors of key SARS-CoV-2 3CL hydrolase (Mpro) via molecular docking, MMGBSA predictive binding energy calculations, and molecular dynamics simulation. *PLoS ONE* **2020**, *15*, e0235030.

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65. Pruchnik, H.; Bonarska-Kujawa, D.; Kleszczyńska, H. Effect of chlorogenic acid on the phase transition in phospholipid and phospholipid/cholesterol membranes. *J. Therm. Anal. Calorim.* **2014**, *118*, 943–950.
 66. Cordeiro, R.M. Reactive oxygen and nitrogen species at phospholipid bilayers: Peroxynitrous acid and its homolysis products. *J. Phys. Chem. B.* **2018**, *122*, 8211–8219.

Chapter 6

Final Remarks and Future Perspectives

The current thesis focus firstly on the importance of lipid-based nano carriers, focusing specially on phytosomes and liposomes and surface modifications to enhance their physiochemical properties, permeability and *in vivo* and *in vitro* properties. Different liposome modifications were discussed based on their functionalities. Complete information about preparation techniques and functionalities was provided, as well as future perspectives, especially regarding surface modification of phytosomes. It was shown that modifications on liposome surface allow more flexible structures comparatively to other delivery carriers.

Secondly, novel drying techniques with special attention on food products were discussed. It was concluded that a good drying technique can considerably improve the overall properties of food and pharmaceutical products. Moreover, it was defined that the use of a drying agent directly affects the protection and preservation of the active components and provide a product with a higher shelf-life. Spray-drying was considered the most efficient technique due to its low energy consumption, the existence of safe and readily available equipments, obtention of a high-quality product, fast production, as well as particle production abilities. Meanwhile, these technologies offer a controlled/sustained release that considerably improves storage stability in the lack of general preservatives. On the other side, optimizing a drying technique and using an appropriate drying agent should provide a promising candidate to preserve, for example, probiotic bacteria or antimicrobial preservatives, being either effective on phytoconstituents bioavailability or storage stability.

A natural-based preservative containing a minimum dosage of emulsifier (5%) and co-emulsifier (1% castor oil) was developed based on ultrasonication of multi-component antibacterial agents (*E. globulus* and *M. piperita* essential oil). The stable formulation shown a particle size of less than 95 nm and exhibited a high shelf-life and 1.002 SMD/VMD criteria. It must be taken into consideration that the natural-based preservative contained a combination of *E. globulus* and *M. piperita* EOs with a significant reduction in MIC values (32-, 250-, 15-times fold against *E. coli*, *S. aureus* and *S. enteritidis*, respectively) compared to an original mixture of both EOs. The synergic effect of a small amount of castor oil and ultrasonic homogenization, even in the presence of a minimum amount of emulsifier, significantly improved the stability of the multi-component nanoemulsion for almost two years.

In term of *Gunnera tinctoria*, it was concluded that, fractions enrichment could be promising methodology to improve food and food by-product features for food industries. A phenolic-enriched fraction of *G. tinctoria* presented anti-tumoral properties in breast

and pancreas cancer cell lines which highlights its potential use as a food supplement for cancer prevention. To improve physiochemical properties, cholesterol-conjugated phytosomes were developed, showing a high product yield. Moreover, a molecular docking computational assay confirmed an accurate arrangement of phenolic compounds within the bilayer in terms of affinity based on polarity and size of phenolic compounds. Those features were confirmed by binding energy and schematic molecular arrangement images. The surface charge of all particles was found to be in an acceptable negative mood in which the nano-phytosome and cholesterol-conjugated phytosome provided the highest values which considerably enhance stability, flexibility, and product yield. As a final remark, this work intended to contribute to show the potential of *G. tinctoria* as a source of nutrients and bioactive compounds, presenting an innovative strategy for its valorization, and a novel formulation having in view a future food supplement.

In addition, fraction enrichment of a coffee silverskin extract, followed by incorporation in nanoparticles, was carried out to improve absorption rate through the cell membrane. The surface of nano-phytosomes was improved by incorporating cholesterol into the lipid layer. The structural arrangements were evaluated by *in silico* molecular docking. To enhance the stability of the formulated structures and natural phytoconstituents, coating of NPs was performed. All formulated samples exhibited anionic surface charges and high stability in aqueous media. FTIR and molecular docking confirmed the interaction between a purine alkaloid (caffeine) and the phenolic compounds. The best docking score was recorded from chlorogenic acid that exhibited stronger hydrogen bonding, resulting in better/stronger interaction. The majority of particles followed a super slow release time. Korsmeyer-Peppas and Higuchi fit well to experimental data. Moreover, the majority of formulated particles followed the case-I and anomalous transport mechanism.

In future studies, it will be advisable to occupy the free space in the modified phytosome surface structure in cationic charge (e.g. PEGylated phytosome, temperature responsive, thermo-sensitive phytosome, pseudo-ceramide phytosome, biopolymer coating phytosome, marked phytosome (with virus, bacteria, RNA, proteins, aptamer or antibodies conjugated with the phytosome layer)). Furthermore, a new investigation on drug incompatibilities could be a good challenge in the field of L/P to apply in intensive care units or clinical trials, taking into account the limited scientific investigation in this sensible area.
