Development of the microencapsulation of soluble vitamins with different biopolymers by a spray drying process for medical, pharmaceutical and food applications

A dissertation presented to the Faculty of Engineering of University of Porto for the degree of Doctor of Philosophy in Chemical and Biological Engineering

by

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Conducted in: LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy

Department of Chemical Engineering, Faculty of Engineering, University of Porto

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Declaration

I hereby declare that this thesis and the work presented herein are original, except where explicitly stated otherwise in the text and that all non-original contributions were appropriately referenced.

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(Ioana Cristina Cârlan)

Legal precepts

According to the Decree-Law No. 115/2013, published in *Diário da República*, 1st series no. 151 from 7th of August 2013, the results of the following publications and communications were used in this thesis.

- Publications:

"Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12", **Ioana C. Carlan**, Berta N. Estevinho, Fernando Rocha, Powder Technology, 318, 162–169, 2017. DOI: 10.1016/j.powtec.2017.05.041.

"Study of different encapsulating agents for the microencapsulation of vitamin B12", **Ioana C. Carlan**, Berta N. Estevinho, Fernando Rocha, Environmental Engineering and Management Journal (EEMJ), 17(4), 855-864, 2018. DOI: 10.30638/eemj.2018.086.

"Production of vitamin B1 microparticles by a spray drying process using different biopolymers as wall materials", **Ioana C. Carlan**, Berta N. Estevinho, Fernando Rocha, The Canadian Journal of Chemical Engineering, 8, 1682-1695, 2020. DOI:10.1002/cjce.23735.

"Innovation and improvement in food fortification: Microencapsulation of vitamin B2 and B3 by a spray-drying method and evaluation of the simulated release profiles", **Ioana C. Carlan**, Berta N. Estevinho, Fernando Rocha, Journal of Dispersion Science and Technology, accepted for publication.

- Communications:

"Vitamin B12 microencapsulation with modified chitosan and controlled release studies", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. Congress of Microbiology and Biotechnology 2015 (MicroBiotec'15), Évora, Portugal, 10-12 December 2015 (Poster).

"Microencapsulation of vitamin B12 using different encapsulating agents and controlled release studies", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. The Food Factor I Barcelona Conference, Barcelona, Spain, 2-4 November 2016 (Oral Communication).

"Design and characterization of vitamin B12 microcapsules produced by a spray-drying technique", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. ICCE 2016 - 3rd International Conference on Chemical Engineering, Iasi, Romania, 9-11 November 2016 (Poster).

"Microencapsulation of vitamin B1 by spray-drying: preparation, characterisation and controlled release studies", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. 16th International Conference and Exhibition on Pharmaceutics & Novel Drug Delivery Systems, Berlin, Germany, 19-21 March 2018 (Poster).

"Vitamin B2 loaded microparticles produced by spray-drying process", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. Food Technology and Probiotics, Prague, Czech Republic, 25-26 October 2018 (Oral Communication).

"Microencapsulation of vitamin B3 by the means of spray-drying method", **Ioana C. Carlan**, Berta N. Estevinho, Fernando A. Rocha. ANQUE – ICCE – CIBIQ 2019, Santander, Spain, 19-21 June 2019 (Oral Communication).

In compliance with the Decree-Law, the author of this thesis states that intervened in the development and performance of the experimental work as well as in the interpretation and writing of the results published under the name **Ioana C. Carlan**.

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Dedication page

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Abstract

Vitamins are organic compounds required daily in minute amounts to sustain life. It is recommended to provide these essential micronutrients from natural food products as part of a balanced diet. As the human body needs vitamins for normal growth, reproduction and general state of health, their absence or deficit will lead to nutritional disorders, and medical treatment emerges.

An alternative method to ensure the dietary allowance of the vitamins is to appeal to fortified food products, nutraceutical supplements, or medicines. However, the manufacture of these products is challenging because vitamins are sensitive to external factors. For this reason, incorporation into products must be done in particular conditions, so the vitamin does not degrade.

Microencapsulation is a versatile delivery system used in many industrial sectors and the excessive use from the last years proved to be a promising method for incorporating sensitive bioactive into products as microparticles. In this process, solid, liquid or gas compounds, called core materials, are coated with an encapsulating agent composed of one or a combination of polymers. Among all microencapsulation techniques, spray-drying is preferred because of reasons like simplicity, flexibility, and low cost. Using spray-drying to produce microparticles with vitamin content can be a valuable tool for the manufacture of food, pharmaceutical and cosmetic products.

This research aims to microencapsulate different water-soluble vitamins using the spray-drying process and biopolymers as encapsulating agents. Vitamins B1, B2, B3 and B12 are the vitamins tested during this work. These compounds are part of the B-complex, help the body obtain energy from food and are required for different essential physiological functions. The following biopolymers were used as encapsulating agents: gum arabic, chitosan, modified chitosan, carrageenan, maltodextrin, modified starch, sodium alginate, pectin and xanthan. These materials have been selected due to their properties: biocompatibility, bioavailability, biodegradability, no toxicity, and excellent stability.

These four vitamins were microencapsulated with a Mini Spray-Dryer BÜCHI B-290 equipment and for the evaluation of the process were determined the product yield and the encapsulation efficiency.

The product yield varied between 27 and 50% for the microparticles with 2% of vitamin B12, 17 and 52% for the microparticles with 0.25% of vitamin B1, 45 and 55% for the microparticles with 0.50% of vitamin B2, 45 and 58% for the microparticles with 1% of vitamin B3

The encapsulation efficiency proved to be very good as the results obtained were near 100%, excepting very few cases.

The external morphology of the microparticles was determined by scanning electron microscopy (SEM), and their size was evaluated using laser granulometry method. The microparticles produced with the same encapsulating agent proved to look alike in all the studies. Three categories of morphologies were identified: a regular spherical shape with a smooth surface (modified chitosan), spherical shape with a rough surface or with shallows aspect (carrageenan, chitosan, gum arabic, maltodextrin, sodium alginate, pectin) and with irregular shape (modified starch and xanthan).

The size of each type of microparticle seemed to be different, and the results of differential volume distribution are, for vitamin B12 microparticles: 4 – 8 μm, vitamin B1 microparticles: 4 – 34 μm, vitamin B2 microparticles: 4 – 7 μm and vitamin B3 microparticles: 4 – 10 μm.

Release studies of the vitamins from the microparticles were performed to prove the success of the entrapment of the through the microencapsulation process. The evaluation was done by an analytical method using a spectrophotometer. Different behaviours were observed comparing the release of the vitamins in deionised water (22°C) or simulated gastric fluid (37 °C). All four studies concluded that these vitamins tend to be released from the microparticles very fast, only some minutes is necessary, or very slow, requiring several hours.

The stability over time of the microparticles was evaluated with samples stored for four months protected from external factors like light, humidity, and temperature. Calculations revealed a mass loss less than 20% for all studies, proving good stability of the microparticles produced by the spray-drying method.

Zero order, first order, Higuchi, Korsmeyer-Peppas and Weibull are the specific kinetic models applied to the experimental data to determine the fitting degree. All the models showed good values for R^2, the correlation coefficient, besides the first order model. Best

results were observed for the Weibull model, and this fact can be explained by the applicability of this model: the release from matrix microparticles, very common for spray-drying method.

Differences between formulations in terms of product yield, encapsulation efficiency, size, morphology, release profiles, kinetic models, and stability over time, were observed, but overall results are considered very good and useful for future research. This work should be continued with studies for the incorporation of the microparticles into real products.

Keywords: biopolymers, controlled release, kinetic models, food application, microencapsulation, pharmaceutical application, spray-drying, vitamin B1, vitamin B2, vitamin B3, vitamin B12.

Resumo

As vitaminas são compostos orgânicos imprescindíveis à vida, sendo requerido diariamente um aporte mínimo. Recomenda-se que estes tipos de micronutrientes essenciais sejam fornecidos a partir de produtos alimentares naturais como parte de uma dieta equilibrada. Dado que o corpo humano necessita de vitaminas para o crescimento normal, reprodução, e vida saudável, a sua ausência ou déficit resultará em um distúrbio alimentar e tratamento médico emerge.

Um método alternativo para garantir a dose diária recomendada de vitaminas é recorrer a produtos alimentícios fortificados, suplementos nutracêuticos ou medicamentos. No entanto, a produção desses produtos é desafiante porque as vitaminas são sensíveis a fatores externos, e por este motivo a incorporação das vitaminas deve ser feita em condições particulares para que estas não se degradem.

A microencapsulação é um sistema de libertação versátil que é utilizado em diversos setores da indústria, que com o seu maior uso nos últimos anos se mostrou um método promissor para incorporação de bioativos sensíveis como micropartículas. Neste processo um composto de material sólido, líquido ou gasoso, constitui o núcleo, que é revestido com um agente encapsulante composto por um ou uma combinação de polímeros. Entre todas as técnicas de microencapsulação, a secagem por pulverização ou *spray-drying* é a preferida por diversas razões, como simplicidade, flexibilidade e baixo custo. Usar a secagem por pulverização para produzir micropartículas com conteúdo vitamínico pode ser uma ferramenta valiosa para a fabricação de produtos alimentícios, farmacêuticos e cosméticos.

Esta trabalho tem como objetivo estudar a microencapsulação de diferentes vitaminas hidrossolúveis, utilizando o processo de *spray-drying* e biopolímeros como agentes encapsulantes. As vitaminas B1, B2, B3 e B12 foram as vitaminas testadas neste trabalho. Estas são parte do complexo B, ajudam o corpo a obter energia dos alimentos e são necessárias para diferentes funções fisiológicas essenciais. Os seguintes biopolímeros foram usados como agentes encapsulantes: goma arábica, quitosano, quitosano modificado, carragenina, maltodextrina, amido modificado, alginato de sódio, pectina e xantano. Esses materiais foram selecionados devido às suas propriedades: biocompatibilidade, biodisponibilidade, biodegradabilidade, ausência de toxicidade e excelente estabilidade.

As quatro vitaminas descritas foram microencapsuladas através do equipamento Mini Spray-Dryer BÜCHI B-290 e para avaliação do processo foram determinados o rendimento do produto e a eficiência de encapsulação.

O rendimento do produto determinado variou entre 27 a 50% para as micropartículas com 2% de vitamina B12, 17 e 52% para as micropartículas com 0,25% de vitamina B1, 45 e 55% para as micropartículas com 0,50% de vitamina B2, 45 e 58 % para as micropartículas com 1% de vitamina B3.

A eficiência de encapsulação apresentou bons resultados, pois os resultados obtidos foram próximos de 100%, exceto em muito poucos casos.

A morfologia externa das micropartículas foi determinada através de microscópio eletrónico de varrimento, e seu tamanho foi avaliado pelo método de granulometria a laser. As micropartículas produzidas com o mesmo agente encapsulante mostraram-se semelhantes em todos os estudos. Foram identificadas três categorias de morfologias: forma esférica regular com superfície lisa (quitosano modificado), forma esférica com superfície rugosa ou de aspeto raso (carragenina, quitosano, goma arábica, maltodextrina, alginato de sódio, pectina) e com forma irregular (amido modificado e xantano).

O tamanho de cada tipo de micropartícula ostenta ser diferente, e os resultados da distribuição de volume diferencial são, para micropartículas de vitamina B12: 4 - 8 µm, micropartículas de vitamina B1: 4 - 34 µm, micropartículas de vitamina B2: 4 - 7 µm e micropartículas de vitamina B3: 4 - 10 µm.

Estudos que avaliam a libertação das vitaminas das micropartículas foram realizados e comprovaram o sucesso da retenção do material do núcleo através do processo de microencapsulação.

A avaliação foi feita por método analítico em espectrofotómetro. Diferentes comportamentos foram observados comparando a libertação das vitaminas em água desionizada (22 °C) ou fluido gástrico simulado (37 °C). Em todos os estudos se verificou que as vitaminas tendem a ser libertadas das micropartículas quer muito rapidamente, em que apenas alguns minutos são necessários, ou muito lentamente, exigindo várias horas.

A estabilidade ao longo do tempo das micropartículas foi avaliada com amostras armazenadas por quatro meses protegidas de fatores externos como luz, humidade e temperatura. Os cálculos revelaram uma perda de massa de aproximadamente 20% para

todos os estudos, comprovando boa estabilidade das micropartículas produzidas pelo método de spray-drying.

Ordem zero, primeira ordem, Higuchi, Korsmeyer-Peppas e Weibull são os modelos cinéticos específicos aplicados aos dados experimentais para determinar o grau de ajuste.

Todos os modelos apresentaram bons valores para R^2, o coeficiente de correlação, exceto o modelo de primeira ordem. Os melhores resultados foram observados para o modelo de Weibull, e este facto pode ser explicado pela aplicabilidade deste modelo: a libertação de micropartículas tipo matriz, muito comum no método de *spray-drying*.

Diferenças entre as formulações em termos de rendimento do produto, eficiência de encapsulação, tamanho, morfologia, perfis de liberação, modelos cinéticos e estabilidade ao longo do tempo, foram observadas, mas os resultados gerais são considerados muito bons e úteis para pesquisas futuras. Este trabalho deve ser continuado com estudos para a incorporação das micropartículas em produtos reais.

Palavras – chave: biopolímeros, aplicação em alimentos, aplicação farmacêutica, libertação controlada, modelos cinéticos, microencapsulação, secagem por pulverização, vitamina B1, vitamina B2, vitamina B3, vitamina B12.

Abstract

Vitaminele sunt compuși organici necesari în cantități foarte mici zilnic pentru a susține viața. Se recomandă ca acești micronutrienți esențiali să fie asigurați din produsele alimentare naturale, ca parte a unei diete echilibrate. Deoarece corpul uman are nevoie de vitamine pentru creșterea normală, reproducerea și starea generală de sănătate, absența sau deficitul lor determină tulburări nutriționale ce necesita tratament medical.

O metodă alternativă pentru a asigura aportul de vitamine este consumul de alimente fortificate, suplimente nutraceutice sau medicamente. Cu toate acestea, fabricarea acestor produse este dificilă, deoarece vitaminele sunt sensibile la factorii externi. Prin urmare, încorporarea în produse trebuie făcută în condiții speciale, astfel încât vitaminele să nu se degradeze.

Microîncapsularea este un sistem versatil de livrare folosit în multe sectoare industriale, iar utilizarea excesivă din ultimii ani a dovedit că este o metodă promițătoare pentru încorporarea compușilor bioactivi sensibili în produse precum microparticulele. În acest proces, compuși solizi, lichizi sau gazoși, denumiți și materiale nucleu, sunt acoperiți cu un agent de încapsulare format dintr-un singur polimer sau o combinație de polimeri. Dintre toate tehnicile de microîncapsulare, uscarea prin pulverizare este preferată datorită următoarelor caracteristici: simplitate, flexibilitate și cost redus. Utilizarea uscării prin pulverizare pentru a produce microparticule ce conțin vitamine, poate fi un instrument valoros pentru fabricarea produselor alimentare, farmaceutice și cosmetice.

Această lucrare de cercetare își propune să microîncapsuleze diferite vitamine hidrosolubile folosind procesul de uscare prin pulverizare și biopolimeri ca agenți de încapsulare. Vitaminele B1, B2, B3 și B12 sunt vitaminele testate în timpul acestei lucrări. Acești compuși fac parte din complexul B, ajută corpul să obțină energie din alimente și sunt necesari pentru diferite funcții fiziologice esențiale. Următorii biopolimeri au fost folosiți ca agenți de încapsulare: gumă arabică, chitosan, chitosan modificat, caragenan, maltodextrină, amidon modificat, alginat de sodiu, pectină și xantan. Aceste materiale au fost selectate datorită proprietăților lor: biocompatibilitate, biodisponibilitate, biodegradabilitate, non-toxicitate și stabilitate excelentă.

Aceste patru vitamine au fost microîncapsulate cu un echipament de tip Mini Spray-Dryer BÜCHI B-290, iar pentru evaluarea procesului au fost determinate randamentul și eficiența încapsulării.

Randamentul produsului a variat între 27 și 50% pentru microparticulele cu 2% din vitamina B12, 17 și 52% pentru microparticulele cu 0,25% din vitamina B1, 45 și 55% pentru microparticulele cu 0,50% din vitamina B2, 45 și 58 % pentru microparticulele cu 1% vitamina B3.

Eficiența încapsulării s-a dovedit a fi foarte bună, întru cât rezultatele obținute au fost mai mari de 99%, cu excepția cazurilor în care s-au folosit maltodextrina și amidonul modificat.

Morfologia externă a microparticulelor a fost determinată prin intermediul microscopiei electronice cu scanare (SEM), iar dimensiunea lor a fost evaluată folosind metoda de granulometrie cu laser. Microparticulele produse cu același agent de încapsulare s-au dovedit a fi asemănătoare în toate studiile. Au fost identificate trei categorii de morfologii: formă sferică regulată cu suprafață netedă (chitosan modificat), formă sferică cu suprafață rugoasă sau cu aspect denivelat (caragenan, chitosan, gumă arabică, maltodextrină, alginat de sodiu, pectină) și cu formă neregulată (amidon modificat și xantan).

Mărimea fiecărui tip de microparticule a fost diferită, iar rezultatele distribuției diferențiale a volumului sunt pentru microparticule cu vitamina B12: 4 - 8 µm, vitamina B1: 4 - 34 µm, vitamina B2: 4 - 7 µm și vitamina B3: 4 - 10 µm.

Studiile de eliberare a vitaminelor din microparticule au fost efectuate și pentru a dovedi succesul procesului de microîncapsulare. Evaluarea a fost realizată printr-o metodă analitică folosind un spectrofotometru. S-au observat diferite comportamente pentru eliberarea vitaminelor în apă deionizată (22 °C) și în lichid gastric simulat (37 °C). Cu ajutorul acestor patru studii s-a demonstrat că aceste vitamine tind să fie eliberate din microparticule repede, fiind necesare doar câteva minute sau lent, necesitând câteva ore.

Stabilitatea microparticulelor de-a lungul timpului a fost evaluată prin probe stocate patru luni de zile, protejate de factori externi precum lumina, umiditatea și temperatura. Calculele au arătat o pierdere de aproximativ 20% pentru toate studiile, ceea ce dovedește o bună stabilitate a microparticulelor produse prin metoda de uscăre prin pulverizare.

Modelele cinetice de ordinul zero, ordinul întâi, Higuchi, Korsmeyer-Peppas și Weibull au fost aplicate datelor experimentale pentru a determina gradul de ajustare. Toate modelele au prezentat valori bune pentru coeficientul de corelație, R^2, cu excepția modelului de ordinul întâi. Însă cele mai bune rezultate au fost observate pentru modelul Weibull și acest fapt poate fi explicat prin funcționalitatea acestui model: eliberarea din microparticule de tip matrice, ce este specifică pentru metoda uscării prin pulverizare.

Chiar dacă au fost observate diferențe între formulări în ceea ce privește randamentul produsului, eficiența încapsulării, dimensiunea, morfologia, profilurile de eliberare, modelele cinetice și stabilitatea în timp, rezultatele obținute sunt considerate foarte bune și utile pentru viitoare studii. Această lucrare de cercetare ar trebui continuată cu studii pentru încorporarea microparticulelor în produse reale.

Cuvinte cheie: aplicație alimentară, aplicație farmaceutică, biopolimeri, eliberare controlată, modele cinetice, microîncapsulare, uscare prin pulverizare, vitamina B1, vitamina B2, vitamina B3, vitamina B12.

Notation and glossary

° C	Degree Celsius
T_{in}	Inlet temperature
T_{out}	Outlet temperature
h	Hour
min	Minute
sec	Second
rpm	Rotations per minute
R^2	Correlation coefficient

List of acronyms

Abs	Absorbance
CNCbl	Cyanocobalamin
CV	Coefficient of variation
DW/dW/dH_2O	Deionised water
EE	Encapsulation Efficiency
FAD	Flavin adenine dinucleotide
FAO	Food and Agriculture Organization
FDA	Food and Drugs Administration
FMN	Flavin mononucleotide
GRAS	Generally Recognized As Safe
LOD	Limit of detection
LOQ	Limit of quantification
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
PA	Pernicious Anaemia
PY	Product Yield
RDA	Recommended dietary allowance
SD	Standard deviation
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
TMP	Thiamin monophosphate
TPP	Thiamin diphosphate (thiamin pyrophosphate)
TTP	Thiamin triphosphate
UNICEF	United Nations International Children's Emergency Fund
UV-Vis	Ultraviolet-visible Light
VB1	Vitamin B1
VB2	Vitamin B2
VB3	Vitamin B3
VB12	Vitamin B12
WHO	World Health Organization

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Chapter 1

Introduction

The first chapter, the introduction, starts with the context, the relevance and motivation behind the subject of the current thesis. Next is revealed the aim, together with the related objectives that were pursued. At the end of the chapter is presented the structure of the work briefly.

1.1. Context and relevance

Vitamins and minerals are dietary compounds described as essential micronutrients required to perform specific physiological functions. Both must be supplied in minute amounts from food products since the body cannot synthesise them. Therefore, these micronutrients are considered very important for optimal health status since their activity contributes to normal growth, good immunity system, brain development and reproduction of the human body [1–3].

The easiest and the most recommended way to ensure the adequate intake of micronutrients is through a varied and balanced diet. This diet should consist of generally fresh natural products stored under optimal conditions until consumption or cooking. For the meal preparation should be avoided using high temperatures, significant amounts of water or cooking for a long time [2–4].

However, several populations groups are at risk of developing micronutrient deficiency due to their incapacity to reach nutritional requirements. Most patients, in this situation, suffer from undernourishment, and more rarely of micronutrient malabsorption syndrome [1–3,5].

Mainly it affects developing countries, conflict regions, places overpassing natural disasters or climate changes. Furthermore, the most common determining factors are poverty, low access or even incapacity to provide nutritious food products. It may also occur in developed countries under certain circumstances like lack of nutritional knowledge of what it means to eat healthily or even the personal choice to follow restrictive food diets that exclude vital sources of essential nutrients. In all cases, most predisposed to suffer from micronutrient deficiency are infants, children, pregnant women or under lactation and the elderly [6–8].

Statistics show that more than two billion people are currently affected by this health impairment, and because of the scale of this problem, micronutrient deficiency became a global concern [9–11].

"Hidden hunger" is the second name assigned for micronutrient deficiency because in the early stages of illness the symptoms are not visible; therefore, it is difficult to diagnose without medical investigations that must include blood tests [2,9,12,13].

If left untreated, micronutrient deficiency favours the occurrence of chronic diseases and in worst cases, can even lead to mortality. Hence it affects the quality of life of individuals by decreasing learning and working capacity. With overall reduced productivity, the development will hold back and cause a decline in the socio-economic sector [1–3,5].

The most critical consequences of micronutrient deficiencies during the life cycle and the leading causes are presented schematically in Figure 1.1 [14].

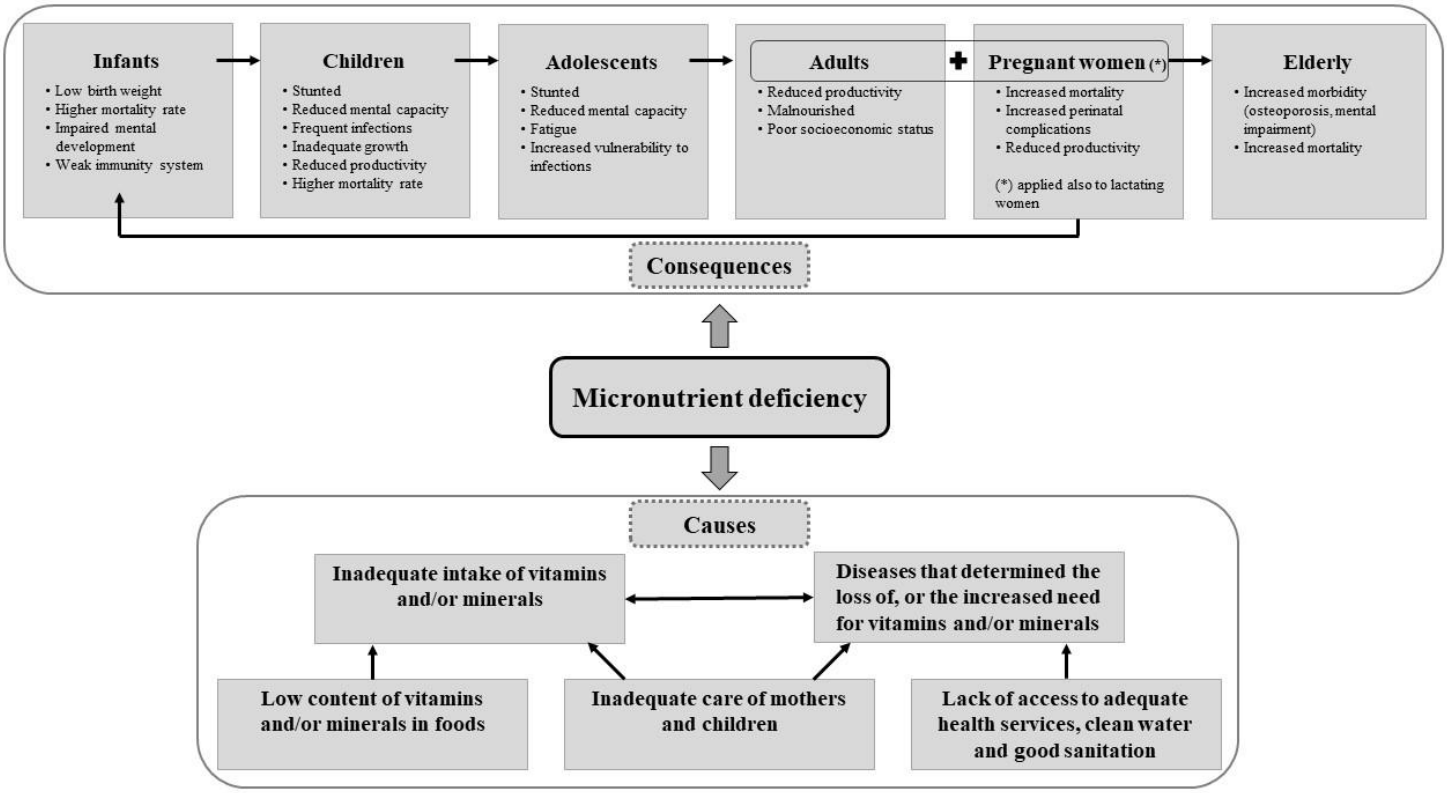

Figure 1. 1. Micronutrient deficiency: causes and consequences during the life cycle.

Diagnosis of micronutrient deficiency can be established only after a medical examination, and the doctor must choose the treatment plan according to the severity and the type of deficiency. Up to three recommendations are usually prescribed: dietary changes, supplements with micronutrient content, and medication [2,10,15,16].

The addition of products rich in minerals and vitamins to the basic diet, accompanied by pharmaceutical supplements, are suitable for treating minor deficiencies and as prevention measures. For severe deficiencies, the first two recommendations are not efficient enough, so, the administration of pharmaceutical products to the patients should be mandatory [3,10,13,14].

The dietary supplements and the medication are both produced by the pharmaceutical industry and are known to be highly effective in treating illnesses. Their price may vary, then in general, such products are affordable for as many segments of the population as possible. However, this industry involves very high costs necessary for research and development, followed by approval trials and safety regulation procedures. Besides the cost, another inconvenience comes from the complexity of the whole process and the time required [3,12,17].

An alternative solution must be found because healthcare services are not accessible worldwide, and by default receiving proper care, treatment and medication is a real problem. To help combat the health crisis provoked by micronutrient deficiency, governments worldwide work with humanitarian organisations like WHO, FAO, UNICEF. They militate to spread awareness of the consequences of micronutrient deficiencies, and they are implementing several programs for the public health sector [5,9,18].

Moreover, from the desire to prevent and treat micronutrient deficiencies differently, the food industry developed useful products enriched with different essential nutrients. Food products are more accessible to people due to their lower price and availability than pharmaceuticals. These foods are known under other names like fortified food products, functional food products or nutraceuticals [5,9,12,14].

The production concept of these products is simple: addition of essential nutrients to the composition of the food products. Their aim is to contribute to daily mandatory requirements or to correct nutritional imbalances. Compared to the pharmaceutical industry, the food industry can produce these enriched foods efficiently, without involving a significant financial effort from both parts, producers, and consumers [1,15,16].

The nutritional value of products increases after the enrichment process, so consuming these food products can act as a preventive measure against nutritional deficiencies or as a treatment for correcting low levels of micronutrients. The amount of nutrients added follows the specific needs of consumers, and the procedure is regulated by law [5,19].

Over time it was proved that regular consumption of these types of foods is a feasible solution, which led to a considerable decrease in the number of people affected by micronutrient deficiency and most important is applicable worldwide [12,20,21].

Recently, food producers started to extend the range of enriched products because there is an increased demand for highly nutritious foods also from people who have no illnesses related to micronutrient deficiencies. These consumers usually follow a healthy lifestyle and believe that such products can improve their diet and keep their health in good parameters. Therefore, it was registered an economic growth and a considerable profit from selling fortified, functional or nutraceutical products.

The research work entitled "***Development of the microencapsulation of soluble vitamins with different biopolymers by a spray drying process for medical, pharmaceutical and food applications"*** proposes a facilitating delivery system via biopolymeric microparticles, for the following four water-soluble vitamins from B-complex: B1, B2, B3 and B12.

These vitamins are valuable ingredients for a significant diversity of food products and pharmaceuticals due to their health benefits. Nevertheless, regardless of the final application, it is difficult to work with vitamins because they are classified as sensitive compounds to temperature, pH, light or humidity, so they are prone to lose stability during processing or even storage time [2,17,22–24].

After reviewing different methods to deliver sensitive bioactive compounds, microencapsulation was assigned as a feasible solution for the current work.

Microencapsulation is defined as a process in which particles or droplets of a solid, liquid or even gaseous product (core material) are coated with a thin polymeric material (encapsulating agent) to form micro size capsules. One of the most important advantage of obtained microparticles is the capacity to release the core material, triggered by the specific action of a stimulus, after a certain time and in a specific target location [25–28].

The microencapsulation technology is very versatile in terms of core and encapsulating agents materials, therefore, it is used worldwide for pharmaceutical, food, cosmetics, agriculture, and many other applications. This work proposes microencapsulation as a packaging delivery system for vitamins that can overcome the low stability inconvenience, by creating a biopolymeric protective system [22,28–31].

1.2. Motivation

The problem of micronutrient deficiencies is far from being eradicated and it is crucial to continue fighting against it. Therefore, the focus should be in finding, simple, feasible and fast solutions, translated into adequate and affordable food and pharmaceutical products. Besides, from the market addressed for those in need to treat micronutrient deficiencies, lately, arouse a new market sector of enriched products for consumers worldwide concerned about personal health, wellbeing, and environmental protection. Consequently, for both situations, it is a high demand for fortified products.

This thesis addresses the problem of incorporating sensitive essential nutrients like vitamins into microparticles delivery systems with potential applications for fortified products.

The selection of the vitamins tested includes four water-soluble vitamins as follows: B1, B2, B3 and B12. Their activity keeps health in good parameters, and for this reason, a daily supply from the diet is needed. Good vision and skin, proper functioning of the nervous system and red cell blood formations are just a few of the functions performed by these vitamins.

Before this work, the author participated in some studies with vitamins C and B12 [32] that were finalised with promising results and gave a good image of what can be developed in time. A thorough search of the relevant literature yielded a small number of related publications about these vitamins. So, the output of this work can help fill part of the knowledge gap from the research subject of vitamin microencapsulation through the spray-drying method.

Nowadays, the urge to find feasible solutions for incorporating vitamins in industrial applications is one growing process, and after years of research, microencapsulation has proven to be an ideal way to preserve and deliver sensitive compounds like vitamins.

Among all the microencapsulation techniques, spray-drying is one of the best solutions to avoid stability problems. The most appealing reasons to use spray-drying are efficiency, low cost, flexibility, and the easy way to handle the equipment. Summing up the considerations presented above can be understood the motivation that stands for this research.

1.3. Aim and objectives

This work aimed to produce and characterise microparticles with vitamins B1, B2, B3 and B12 content prepared using the spray-drying method.

This aim was achieved along with this study by pursuing the following four major objectives:

Objective 1:

1.1. Optimisation of microencapsulation process – the selection of operational conditions and encapsulating agents.

1. 2. Microencapsulation of the vitamins – evaluation of the product yield and the encapsulation efficiency.

Objective 2:

Characterisation of the microparticles – examination of external morphology by scanning electron microscopy and determination of the size of the microparticles using laser granulometry method.

Objective 3:

3.1. Optimisation of the analytical method used to evaluate the presence of vitamins in the microparticles and their release from the microcapsules – validation of the spectrophotometric method and selection of operational conditions.

3.2. Release studies done under controlled conditions – evaluate the time necessary for the complete release of the vitamins from the microparticles.

3.3. Stability over time: comparing the behaviour of fresh samples and stored samples – evaluation of the mass loss detected after a determined period of storage.

Objective 4:

The modelling of the release profiles – evaluation of the fitting degree of the experimental data to some specific kinetic models, namely zero order, first order, Higuchi, Korsmeyer – Peppas and Weibull models.

1.4. Structure of the thesis

This thesis is divided into ten chapters as follows: introduction, theoretical aspects and state of the art, materials and methods, optimization of experimental conditions, microencapsulation of vitamin B12, microencapsulation of vitamin B1, microencapsulation of vitamins B2 and B3, comparison study, conclusions, and final remarks. In turn, each of these chapters is subdivided into different subchapters.

The first chapter, introduction, covers the following topics: relevance of studying B vitamins microencapsulation, challenges and background of this topic, the motivation for starting this research, main objectives followed during the work, and it briefly presents the structure of the current thesis.

The second chapter, theoretical aspects and state of the art, describes the most important theoretical aspects of the vitamin B complex, especially vitamins B1, B2, B3 and B12, nutrition and nutrients, microencapsulation, and spray-drying method. This chapter also presents a review of the state of the art with the most relevant studies identified about B vitamins on microencapsulation, mainly focusing on the studies were the spray-drying method was used.

In the third chapter, materials and methods, are revealed the materials and the equipment required for this research, and the experimental details of all the procedures used to prepare and characterise the microparticles.

In the following four chapters are presented the experimental results of this thesis, as scientific articles, in the format of their publication:

- the fourth chapter – optimization of work conditions

"Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12".

- the fifth chapter – microencapsulation of vitamin B12

"Study of different encapsulating agents for the microencapsulation of vitamin B12".

- the sixth chapter – microencapsulation of vitamin B1

"Production of vitamin B1 microparticles by a spray drying process using different biopolymers as wall materials".

- the seventh chapter – microencapsulation of vitamins B2 and B3

"Innovation and improvement in food fortification: Microencapsulation of vitamin B2 and B3 by a spray-drying method and evaluation of the simulated release profiles".

Chapter number eight, entitled comparison study, aims to clarify the differences and similarities between the microparticles with different vitamin content.

The main conclusions of this work are gathered in chapter number nine.

The final remarks of this work can be found in the last chapter. After the evaluation of the entire research, were established the limitations encountered along the time. Were also made some suggestions helpful in continuing the work in the future.

The bibliographic references used in this thesis can be found at the end of the thesis.

Chapter 2

Theoretical aspects and state of art

The second chapter aimed to present important theoretical aspects and to provide an adequate background on the studied subject. For this, various topics related to vitamins, nutrition, microencapsulation, and spray drying were covered. Furthermore, a literature review focused on similar approaches for microencapsulation of B vitamins, especially studies that used the spray-drying method.

2.1. Vitamins

The history of humankind was marked by many diseases, considered without a cure for a long time. However, in the last decades, doctors and scientists worldwide concluded that some of these diseases were caused by poor nutrition. Scurvy, beriberi, rickets, pellagra or night blindness are just a few examples of these illnesses [2,33,34].

As a coincidence, all occurred when large groups of people were constrained by unfavourable circumstances which led to limited food resources, hence the incapacity to ensure a varied diet. And due to the poor medical knowledge of that time, effective treatments were found only very late. However, at the beginning of twenty century, the nutrients known nowadays as vitamins were isolated and their discovery was considered an exceptional scientific achievement for the medical and nutrition research fields because it brought a significant contribution to human health [28,33,34].

Shortly after identifying all vitamins, it was proved that the diseases referred before are the consequence of missing only one compound, namely a specific vitamin, thus not certain food products. The explanation comes from the fact that vitamins can be found naturally in food products.

The term vitamin was coined in 1912 by Kazimierz Funk, a Polish biochemist working in the Lister Institute from London. Just a year earlier he announced he found the cure for beriberi disease and the validation of his finding came from full treatment and recovery in several cases of affected humans and chickens. This antidote factor was an organic base found in natural food sources that also included nitrogenous molecules; therefore, Funk named it vitamine, as a combination of the following words: vital and amine [2,33–35].

So, after the chemical structures were determined, Funk, realised that not all these compounds are amines and for this reason, he changed the name vitamine into just vitamin [2,34].

Several years later, through the involvement of more researchers who continued Funk's work, more nutrients with vitamin status were confirmed, which are implicitly required for general health. Thus, was reached a group of 13 that includes the following vitamins: A, B1, B2, B3, B5, B6, B7, B9, B12, C, D, E, and K [1,2,36].

Each of these compounds follows the definition: "*a vitamin represents an organic compound required in minimal amounts by the human body to perform essential physiological functions and to maintain optimal health"* [1,2].

A daily supply should be ensured through diet since the human body can synthesise just a few vitamins, like the cases of vitamins D, K, B3, and B7, although to some extent [1,2,4,10].

The amount of this daily supply was established since the beginning of 1940 and is known as the recommended dietary allowance (RDA). However, surprisingly it is not a fixed value because it may differ according to several criteria regarding gender, age, diseases and geographical location [2,9,10].

The daily requirements are minimal, of the order of micrograms; therefore, vitamins are recognized as essential micronutrients, along with the group of minerals.

Vitamins can be found easily in many natural sources, so the best way to reach the RDA is to adopt a nutritionally correct diet consisting of various natural food products. However, if the vitamin supply becomes lower than normal, it is mandatory to correct the nutritional vitamin intake, because in this situation people develop an illness known as deficiency syndrome, which can determine severe medical implications if not treated on time [5,12,13].

Vitamin deficiencies occur with predilection in infants, children, women (pregnant or under lactation) and elderly. Besides these target population groups, deficiency can also appear because of the following gaps: insufficient absorption, improper ingestion, unfollowing recommended dosage (increased/decreased requirement), followers of restricted food diets or patients with some chronic diseases.

Vitamins do not share common structural properties and do not perform the same functions, so the only empirical classification recognised to this day is the one based on their solubility. Thereby the most significant difference between vitamins is the type of environment in which can be dissolved, absorbed by intestines, and assimilated in tissues.

Such being the case, the family of 13 vitamins was split into fat-soluble, represented by vitamins A, D, E and K, and water-soluble identified as vitamins C, B1, B2, B3, B5, B6, B7, B9, and B12 [1,2].

The characteristics of the water-soluble vitamins are the solubility in polar solvents (water) and the abundance of both vegetal and animal food sources. The bloodstream makes the absorption to the small intestine, but fast after this stage, the excess of vitamins is eliminated by urine. Therefore, it is mandatory to guarantee daily supply. Another important aspect is related to the low stability of water-soluble vitamins during cooking and storage time and to avoid these situations, and some measures must be taken [1,2,36].

The fat-soluble vitamins dissolve in nonpolar solvents (fats and oils) and can be found in foods with fat content. The absorption occurs in the lymph, and its excess is stored in the liver and the fatty tissues. Contrary to the water-soluble vitamins, the fat-soluble vitamins are not eliminated, thereby, a daily, regular supply is not requested. Another benefit of the fat-soluble vitamins is the fact that after cooking are not registered losses [1,2,36].

In Table 2.1, all the vitamins with some of their most relevant properties are presented.

Table 2. 1. Vitamins and some of their characteristics.

	Generic descriptor	Active forms in the body	RDA (*)	Physiological functions	Examples of good dietary sources
Fat-soluble vitamins	Vitamin A	Retinol Retinal Retinoic acid	900/700 µg	Healthy vision, skin, teeth and immune system, cell and tissue growth, gene transcription and protein formation.	Fish, liver, red meat, butter, cheese, eggs, sweet potato, carrots, spinach, lettuce, mango, grapefruit.
	Vitamin D	Cholecalciferol (D3) Ergocalciferol (D2)	15 µg	Absorption of calcium and phosphorus, mineral metabolism, regulates insulin levels, bone and teeth growth.	Oily fish, veal, beef, egg yolks, cheese, mushrooms.
	Vitamin E	α – Tocopherol γ – Tocopherol	15 mg	Antioxidant, enzymatic activity regulator, prevents oxidative stress, cellular and blood vessel health, keeps skin, hair and eyesight youth.	Vegetable oils, seeds, nuts, goose meat, dairy products, avocado, turnip, broccoli, red sweet paprika, mango, kiwifruit.
	Vitamin K	Phylloquinones (K1) Menaquinones (K2) Menadione (K3)	90/75 µg	Blood clotting, bone formation, calcium metabolism, cardiovascular health, regulation of cellular function.	Meat, liver, dairy products, egg yolks, vegetable oils, spinach, kale, lettuce, broccoli, turnip, parsley, blueberries, grapes.
Water-soluble vitamins	Vitamin B1	Thiamin	1.2/1.1 mg	Energy metabolism; nervous system, muscle, heart and stomach health; promotes normal appetite.	Meat, fish, eggs, yeast, whole grain cereals, nuts, cauliflower, kale, asparagus, oranges.
	Vitamin B2	Riboflavin	1.3/1.1 mg	Energy metabolism; stimulates growth and reproduction; maintains vision and skin.	Meat, whole grain cereals, eggs, milk, dairy products, green leafy vegetables, mushrooms, almonds.
	Vitamin B3	Nicotinic acid Nicotinamide	16/14 mg	Energy metabolism; lowers cholesterol, prevents neurological degeneration; brain function; skin, hair and nervous system health.	Fish, meat, milk, eggs, whole grain cereals, avocadoes, potatoes, nuts.
	Vitamin B5	Pantothenic acid	5 µg	Energy metabolism; red blood cell formation; healthy digestive tract; synthesises cholesterol; creates sex and stress hormones; wound healing.	Meat, eggs, dairy products, whole grain cereals, mushrooms, avocado, kale, broccoli, tomatoes, nuts.
	Vitamin B6	Pyridoxol Pyridoxal Pyridoxamine	1.3 mg	Energy metabolism; prevents vascular accidents, amino acids and proteins metabolism, red blood cell formation; healthy nerve and immunity systems.	Chicken, liver, fish, eggs, chickpeas, maize, beans, potatoes, nuts, bananas.
	Vitamin B7	Biotin	30 µg	Energy metabolism; nervous system; psychological functions, healthy skin and hair.	Liver, fish, egg yolks, milk, dairy products, whole grain cereals, mushrooms, cauliflower, kale, avocado, nuts, banana, raspberry.
	Vitamin B9	Folic acid Polyglutamyl folacins	400 µg	Energy metabolism; growth factor, red blood cells; DNA synthesis. Brain function, mental and emotional health.	Liver, milk, dairy products, egg yolk, yeast, whole grain cereals, spinach, lettuce, asparagus, oranges.
	Vitamin B12	Cobalamin	2.4 µg	Energy metabolism; red blood cell formation; regulates DNA, prevents birth defects; nervous and gastrointestinal system.	meat, liver, shellfish, fish, brewer's yeast, milk, dairy products.
	Vitamin C	Ascorbic acid Dehydroascorbic acid	1.2/1.1 mg	Antioxidant, boosts the immune system, stimulates collagen biosynthesis; absorption of iron, reduces infections and inflammation.	Liver, milk, dairy products, citrus fruits, tomatoes, green leafy vegetables.

(*) Values shown for men/women of adult age between 18 and 50 years old.

2.1.1. The vitamin B complex

In the previous Subchapter 2.1., were briefly presented individuals characteristics of every B vitamins, as follows: generic descriptor name, the active forms in the human body, the specific RDA for adult female and male subjects, part of the physiological functions in which their activity is needed and some aliments rich in these vitamins. As it can be observed the individual influence of every B vitamin is crucial for human health, and this section will be analysed all B vitamins as a unitary whole [1,2,36].

B complex is the generic name given to eight members of the water-soluble group of vitamins, namely for vitamins B1 (thiamine), B2 (riboflavin), B3 (niacin), B5 (pantothenic acid), B6 (pyridoxine), B7 (biotin), B9 (folic acid) and B12 (cobalamin). All these compounds are classified as essential dietary micronutrients for human health, and they perform closely inter-related functions. Besides the designation of vitamin B complex, these vitamins are also commonly known as the B family [1,2,4,35].

The name of each compound from this complex consists of a letter and a number. The letter was chosen because vitamin A was the first vitamin identified in the early 1900s, so for the second vitamin, letter B was designated. This vitamin was called “hydro-soluble vitamin B” due to its solubility in aqueous solutions at the time of discovery. The name had to be changed soon since it was proved that there are several similar compounds and for every new B vitamin was attached a number consecutively, from 1 to 12. However, four compounds out of twelve were eliminated after further investigation because they turned out to be pseudo-vitamins which are not involved in the performance of essential functions [2,33,34].

The vitamin B complex plays an essential role in growth, reproduction, metabolism, and overall health. Moreover, for the fulfilment of these functions, B vitamins contribute as co-enzymes or cofactors.

B vitamins function as co-enzymes for the metabolism of proteins, carbs, and fats, therefore, they are involved in converting food into the energy required by the human body to sustain daily life.

As cofactors, vitamin B complex oversees other important functions like keeping the skin, hair and vision healthy; hormone production; red blood cell formation; normal appetite; boosting

the immunity system; maintain in proper parameters the nervous, digestive and cardiac systems [1,2,36].

The vitamins from B family are soluble in water, and their absorption occurs very fast in the gut, from where are later eliminated via the renal system. So, in these conditions, B vitamins must be supplied mandatory every day.

The daily requirements for B vitamins are minimal and vary from few micrograms to few milligrams, as presented in Table 2.1.

Deficiencies caused by an insufficient supply of the B vitamins are not very common nowadays, mostly because these vitamins are found naturally in many food sources and because many products are fortified by law or voluntary as a precaution measure from health authorities. Although, in some circumstances, people can be diagnosed with deficiency of one or more vitamins from the B complex.

Diseases associated with vitamin B deficiencies develop mainly to people who are in one of the following situations:

- Restrictive food diets – vegetarians and vegans are at high risk because it is difficult to reach the RDA of B vitamins when are excluded part or all food products of animal origin from the diet.
- Pregnant women – the overall nutritional intake increases during this time for women because they must provide the necessary for them and the growing fetus. Likewise, women under lactation should be aware of possible deficiencies that can be easily transmitted to their infants through low nutrient breastmilk.
- Gut malabsorption – which is more common for the elderly because some people absorb essential nutrients like the B vitamins with difficulties with age.
- Alcoholism – this chronic addiction is dangerous for health in general, and one consequence is the incapacity of absorbing vitamins from B complex.
- Acute smoking – along with the previous addiction, alcoholism, smoking, interferes with absorption process and the amount of absorbed B vitamins decreases.
- Consumers of processed food – food products go through a series of operations until they reach consumers and sometimes lead to the destruction of essential compounds from their initial composition.

- Low access to food sources – several reasons like poverty, living in a conflict area or a geographical area with low food sources, leads to the impossibility of ensuring a proper B vitamins intake [6,8,12,13].

In all the cases presented above, it is necessary to confirm the deficiency through blood analysis and to establish the gravity of the problem. The treatment usually includes vitamin supplementation and it is decided according age, sex and medical history [2,10,37].

Besides the medical supplementation, diet changes are also useful and can help maximize the intake of missing vitamins. Therefore, consumption of food products rich in vitamins is highly recommended. And for the case of diets that exclude products of animal origin, can be consumed foods like dark leafy vegetables, fruits, legumes, seeds and nuts, brewer yeast and whole grains, products which are known to have high content of B vitamins. As for cooking tips, it is indicated to prepare the meals at low temperature, with a reduced amount of water for boiling or if possible, to cook with steam [2,4,35].

The most frequent symptoms associated are vitamin B complex deficiency, weakness, fatigue, depression, anxiety, insomnia, diarrhoea, anaemia, dermatitis, eye disorders, crack and sores of the mouth, and high cholesterol digestive and neurological problems [1,2,36].

B complex is essential for many physiological functions, and their influence is felt in many parts of the body, hence, it is necessary to have a balanced diet and a healthy lifestyle.

This research focused on studying four vitamins from the B complex, namely: vitamins B1, B2, B3 and B12. This selection was made considering the importance of the vitamins and their potential for incorporating in pharmaceutical and food products. In the next subchapters will be presented briefly important theoretical aspects about each of these four vitamins, namely information about their history, general properties, functions and health benefits, food sources, RDA and biochemical indicators, absorption and excretion, stability, deficiency and symptoms, treatment and recommendations, and toxicity.

2.1.2. Vitamin B12

Short history

Vitamin B12 was the last dietary micronutrient from the class of vitamins to be discovered. This vitamin is distinguished from the others by three main characteristics: owns the largest and most complex chemical structure, presents a carbon-metal bond and it is distributed mostly in products of animal origin, with very few exceptions. Due to this unique combination of characteristics, the process of discovery of VB12 was long and complicated, but at the same time, it was very prolific in winning Nobel prizes [2,35,38–41].

The history of VB12 started while seeking for the treatment of pernicious anaemia, the most common type of VB12 deficiency registered worldwide. Around the year 1849, the British physician Thomas Addison identified a new kind of untreatable stomach disorder. Later, in 1855, he described this disease as pernicious anaemia (PA) in the monograph book "Diseases of suprarenal capsules" [35,39,42].

From that moment began a series of studies dedicated to treating this type of stomach anaemia, although only in 1920 was published the first study with successful results. The study belonged to the American physician George Whipple who induced PA to dogs by exsanguination. The cure came after he fed them with liver, and this type of treatment led to the conclusion that the anti-pernicious anaemia factor is found in the composition of liver [35,39].

Shortly after, other two American medical researchers, George Minot and Willian Murphy tried the "liver therapy" on human subjects diagnosed with PA. Surprisingly, the patients were declared cured after they followed a treatment with lightly cooked liver. For their contribution, Whipple, Minot, and Murphy won in 1934 a Nobel prize for Medicine and Physiology for the fast and effective remedy for PA. This award was the first one out of four, and it was considered the start point for the discovery of VB12 [2,35,39,40].

The research continued with some attempts to understand what type of compound the anti-pernicious anaemia factor is. In this regard, a second Nobel prize was received by the British biochemist Alexander Todd in 1957. The award was for the chemistry field because he succeeded the isolation, extraction and purification of VB12 from liver composition [2].

Shortly after, in 1964 the British biochemist Dorothy Hodgkin used the X-Ray crystallography and finally found the complete structure of VB12, and the achievement of Hodgkin was rewarded with a third Nobel prize [2,35,39,40].

The last significant event from the history of VB12 was recorded in the year 1965 when the American chemist Robert Woodward managed to synthesize chemically VB12 and for this discovery was received the last Nobel prize associated to this vitamin [2,35].

General properties

Vitamin B12 gathers a family of related compounds called cobalamins containing one central atom of cobalt and a biological active corrinoid structure. The corrinoids are macrocyclic compounds with four pyrrole rings engaged by three methane bridges and one corrin ring, used in VB12 to link the cobalt atom directly [1,2,4,35,40]. The main difference between VB12 types is given by the β position from the upper bound of cobalt. The ligands used to create these different forms of vitamin B12 and their specific names, are listed in Table 2.2, presented below. The first fifth positions from Table 2.2. refer to synthetic forms of VB12, and barely the last two occur naturally as dietary sources [1,2,4,38].

Table 2. 2. Forms of vitamin B12.

Common name	Replaced ligand
1. cyanocobalamin	-CN
2. hydroxocobalamin	-OH
3. aquocobalamin	-H2O
4. nitritocobalamin	-NO2
5. sulfitocobalamin	-SO3
6. methylcobalamin	-CH3
7. 5´-deoxyadenosylcobalamin	-deoxyadenosyl

Cyanocobalamin (CNCbl) and hydroxocobalamin are the only compounds suitable for pharmaceutical and food applications. Between these two, CNCbl is preferred for products like additives, supplements, fortified foods, or medical products, because it has better stability. And

although hydroxocobalamin has lower demand in general, it is explicitly required to treat diseases like tobacco amblyopia and optic neuropathy [1,4,10,38].

The natural forms of VB12 have co-enzymatic status and are metabolically active in the human body. Methylcobalamin works as the coenzyme of methionine synthase which catalyses homocysteine into methionine. Moreover, 5´-deoxyadenosylcobalamin (compound referred in the literature also as co-enzyme B12) is the coenzyme of methylmalonyl-CoA that converts to succinyl-CoA [1,4,35,40,43].

Any of the other forms mentioned above it is associated with the generic name of VB12, however, is used with a predilection for CNCbl, because it is considered to be the most known form of VB12 [4,44,45].

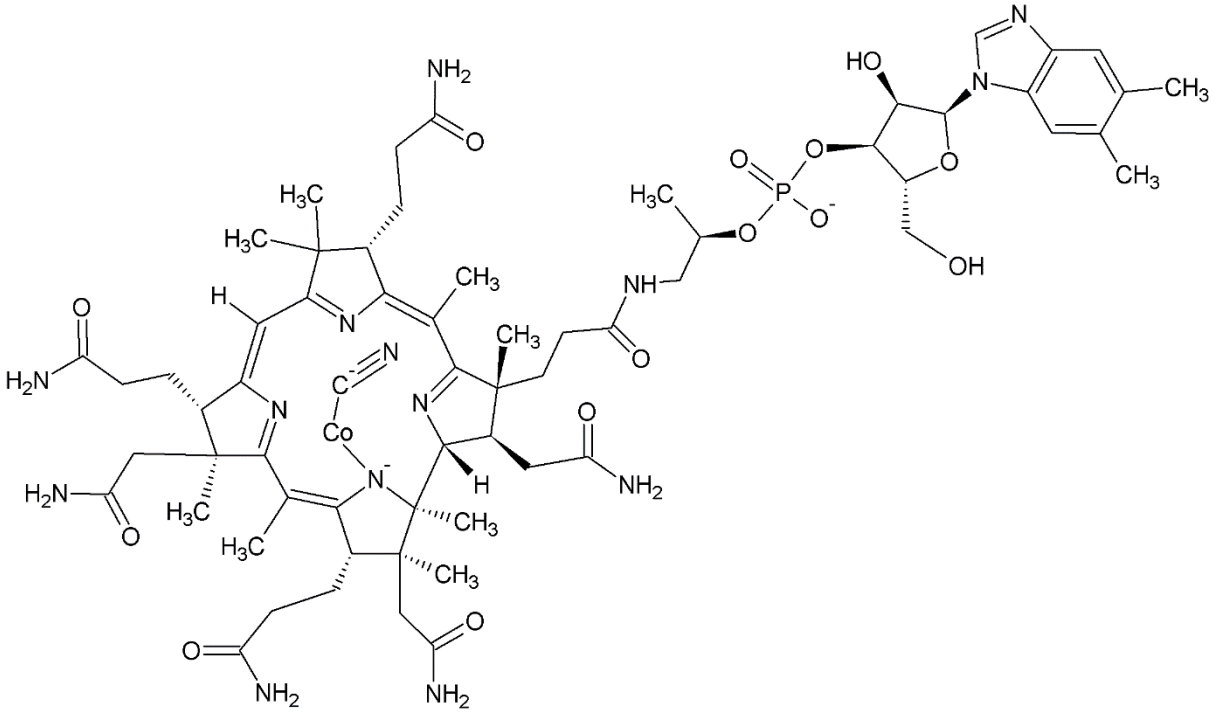

Figure 2. 1. Chemical structure of cyanocobalamin.

The commercial CNCbl is a dark red crystalline hygroscopic powder without taste or odour. Its molecular mass is 1355.4 g/mol and the chemical structure is shown in Figure 2.1 (empirical formula $C_{63}H_{88}CoN_{14}O_{14}P$). As a water-soluble vitamin, CNCbl is characterized by a good solubility in water of 1.25 g/100 ml at 25 °C. Other specific solvents of CNCbl are alcohols, phenols, and polar solvents with hydroxyl groups, but cannot be dissolved in organic solvents like acetone, ether, and benzene. The CNCbl aqueous solutions absorb at 278, 361 and 551 nm, although the specific maximal absorbance is in the interval 350 – 368 nm [1,2,4,35,38,46].

Functions and health benefits

VB12 performs essential metabolic functions, and for this reason, it belongs to the group of life-sustaining micronutrients. The coenzyme forms of VB12, 5´-deoxyadenosylcobalamin and methylcobalamin, play a crucial role in cellular metabolism and help cell division and development. VB12 is required together with folate for the rapid synthesis of deoxyribonucleic acid (DNA) and the red blood cells formation. The influence of this vitamin is also felt in other critical physiological functions related to the neurological system (supporting peripheral and cerebral spinal), human growth (fetal and child development) and bone health (bone marrow tissue responsible) [2,10,38,47,48].

Food sources

VB12 is synthesized solely by anaerobic bacteria and absorbed by animal tissues. Thus, the naturally occurring forms of VB12 come from fermented foods or products of animal origin. The most important food sources of VB12 are meats, fish, seafood, eggs and dairy products [1,2,10,35].

Table 2.3 presents some of these natural sources and their VB12 content per 100 g. Vascularized meat organs like liver, kidney, brain or chicken giblets, herring fish and seafood products like oysters or clams are reported as the most affluent products in VB12 [2].

Although this vitamin is impossible to synthesise by plants, surprisingly, in the last years, a few vegetal products with appreciable VB12 content were discovered. These products belong to the following foods: edible algae, edible mushrooms, fermented soybeans, and tea leaves. Other exceptions include contaminated vegetables from organic fertilizers (like cow manure) or poor hygiene situations (like not washing the products before consumption, using dirty water for cooking or improper storage conditions). Moreover, the most relevant examples are listed in Table 2.4. The new sources are not easily accessible for everyone, but they have attracted the interest to people who have eliminated wholly or partly the animal origin foods from their diets [2,41,45,46,49].

Contrary to the case of other water-soluble vitamins, VB12 can be stored in the liver for up to 2 – 3 years, and the deposit can be used to compensate possible imbalances [1,2].

Table 2. 3. Sources of vitamin B12 of animal origin.

	Food product	Content of vitamin B12 (µg/100g)
Meats	Chicken	0.27 – 0.32
	Chicken giblets	9.48
	Turkey	0.36 – 1.65
	Pork	0.43 – 1.11
	Pork ham	0.65 – 1.06
	Beef	1.38 – 3.17
	Beef liver	83.13
	Beef kidney	24.9
	Beef brain	10.10
Fish	Tuna	2.55
	Salmon	3.26 – 4.48
	Trout	6.3
	Herring	13.14
Seafood	Shrimp	1.21 – 1.87
	Lobster	1.43
	Oysters	16 – 19.13
	Clam	40.27
Eggs	Whole egg	0.89
	Egg white	0.09
	Egg yolk	1.95
Dairy	Milk	0.38 – 0.5
	Yoghurt	0.75
	Cheese	0.29 – 2.28

NOTE: adapted from Combs G. – *The Vitamins. Fundamental Aspects in Nutrition and Health*. 5th edition. Elsevier Academic Press; 2017.

However, not all food sources have biologically active forms of VB12. There are several situations in which the activity can be lost or does not exist:

- Food products with inactive corrinoid compounds in their composition.
- Cooking and processing methods that use heat can lead to the degradation of VB12. In this case, the value of temperature, the exposure time, the interaction with other ingredients, and the retention caused by vacuum processes or microwaves are damaging factors.
- Chemical treatment with light exposure applied to products determines the degradation of VB12[46].

Table 2. 4. Plant-derived food sources of vitamin B12.

Food product	Content of vitamin B12 (µg/100g)
Edible algae	
Dried green laver (*Enteromorpha* sp.)	63.6
Dried purple laver (*Porphyra* sp., nori)	32.3
Microalgae *Chlorella* sp.	0 – 200
Edible mushrooms	
Porcini mushrooms (*Boletus sp.*) Parasol mushrooms (*Macrolepiota procera*) Oyster mushrooms (*Pleurotus ostreatus*) Black morels (*Morchella conica*)	0.01 – 0.09
Black trumpet (*Craterellus cornucopioides*) Golden chanterelle (*Cantharellus cibarius*)	1.09 – 2.65
Shiitake mushroom (*Lentinula edodes*)	5.61
Fermented foods	
Tempe – Korean fermented soybean food	0.7 – 8.0
Natto – Korean fermented soybean food	0.1 – 1.9
Kimchi – Korean fermented cabbage	0.18 – 2.4
Tea leaves	
Fermented black tea leaves	0.1 – 0.5
Contaminated vegetables	
Spinach leaves, asparagus, broccoli	0.14

RDA and biochemical indicators

The RDA established for healthy children older than 14 years and adults is of 2.4 μg per day. For pregnant women or under lactation, the requirements grow a bit, reaching the values of 2.6, and respectively 2.8 μg. Surprisingly, infants, children and young teenagers the need a lower daily VB12 intake, as presented below in Table 2.5. [2,10,35,44]

Table 2. 5. RDA values for vitamin B12 presented by age and sex groups.

Age group	RDA (μg/day)
0 – 6 months (*)	0.4
7 – 12 months (*)	0.5
1 – 3 years	0.9
4 – 8 years	1.2
9 – 13 years	1.8
14+ years	2.4
Pregnant women	2.6
Lactating women	2.8

(*) For infants, there are no official RDA data, and the presented values are adequate intakes recommended by FAO/WHO.

Analysing Table 2.1., where are presented the reference values for the RDA of each vitamin can be observed that for vitamin B12 must be ensured the lowest value for daily supply. Although the intake value is low, it is difficult to rich in some situations, and health problems can be developed quickly.

Absorption and excretion

From food, VB12 is transported with the help of proteins. After VB12 is released inside the stomach in the presence of hydrochloric acid and pepsin, from there, VB12 will link to a glycoprotein called intrinsic factor, and then the vitamin is absorbed [1,2,10].

The excretion of VB12 is made daily through the renal and biliary tract. Although in the case of other water-soluble vitamins the excess is eliminated from the body, VB12 will eliminate only 0.1 – 0.2% of the body deposit, found mostly in the liver [2].

Stability

Aqueous solutions and crystalline forms of VB12 are considered stable products when they are protected from light exposure. Though, the most stable form of VB12, CNCbl transforms into hidroxycobalamin if left exposed to light. Enhanced stability of CNCbl was registered at values of 4.0 – 4.5 pH and 4.0 – 7, during autoclaving at 120 ºC. In the presence of compounds like ascorbic acid, thiamine, niacin, strong acids, metals, strong UV or visible light, VB12 is susceptible to degradation.

Generally, VB12 should be stable after food processing if it is not overpassing thermic treatments with higher temperature of 120 ºC. Although, are reported large losses of VB12 due to leaching in the boiling water [1,2,4].

Deficiency and symptoms

VB12 deficiency is determined by two main reasons: a poor diet and malabsorption disorders. Each type of deficiency is specific for different population segments, also known as risk groups [2,35].

Some of the frequent symptoms of VB12 deficiency are loss of appetite, weight, taste and smell, inflammation of the tongue (glossitis), weakness, impotence, irritability, mild depression, memory impairment and hallucinations. Thus, only in severe cases, VB12 deficiency will determine megaloblastic anaemia [4,35,50,51].

The first type of VB12 deficiency is called dietary deficiency and is affecting those who follow vegetarian diets (lacto–vegetarian, ovo–vegetarian lacto–ovo–vegetarian, vegan, pescatarian, vegan and other combinations) [35,52].

According to the number of aliments rich in VB12 that are excluded from their daily diet, vegetarians are more, especially the vegans, or less, pescatarians and lacto–ovo–vegetarians, predisposed to develop VB12 deficiency [10,35].

Moreover, from the class of vegetarian people, women who are pregnant or during lactation represent one of the critical risk groups. A low VB12 level can lead to birth defects and abnormal growth due to cognitive and motor consequences. The children raised exclusively

with vegetarian diets or even with low intake of meat products are considered to have a high risk of VB12 deficiency [35,44,53].

In VB12 deficiency caused by malabsorption several risk groups are identified, namely people who suffer from PA, with food-bound B12 malabsorption, with Crohn or celiac disease and those who had gastric bypass surgery [44].

PA is the most common type of VB12 deficiency registered worldwide. It is classified as an autoimmune disease caused by the atrophy of the gastric mucosa and the reduction of the parietal cells responsible for the intrinsic factor production. The poor function or lack of the intrinsic factor leads to the incapacity of VB12 to bind to this factor and therefore the vitamin cannot be absorbed by the body from food. PA can produce damages to the hematological and neurological systems in most severe cases. [40,42,50,51,54].

People above 60 years tend to be more exposed to PA due to the increased possibility of developing chronic inflammation of the stomach. Some cases of Helicobacter Pylori, thyroid diseases and type I diabetes are also associated with PA [35,42,43,51,53,55].

PA diagnosis is based on hematological abnormalities that prove the VB12 and the intrinsic factor deficiency. The first clinical signs are very similar to normal anemia and include a general state of weakness, tiredness and headaches [42].

Lack of VB12 is also common for people with food-bound B12 malabsorption because this is a gastric dysfunction like atrophic gastritis known for a low stomach acid secretion. The incidence of this disease is also specific to older adults [4,43,47,52,55].

Patients with Crohn or celiac disease and the ones with gastric bypass intervention can suffer from this deficiency because of decreased intestinal absorption of VB12 [44].

Another important factor considered in the diagnosing of deficiency is the interaction of VB12 with other vitamins. Some epidemiological studies suggest that a high status of folate can hide a low status of VB12. Moreover, if the deficiency of VB12 is not found on time, it might determine some severe health problems. The groups affected the most by this condition are children, pregnant women and elderly [56].

Treatment and recommendations

In the case of VB12 deficiency without severe complications, are recommended supplements of vitamin B12 and some periodic check-ups. If possible, people should also introduce more food sources with high levels of VB12 [44].

The supplementation recommendation generally applies to all vegetarians that can avoid health problems by filling nutritional deficiencies with medication.

During lactation, pregnant women should increase the standard RDA to some higher values prescribed by doctors, and those who follow a vegetarian diet should take more measures so the fetus or the new-born infant not to be affected [44].

Elderly people should pay more attention on achieving the RDA of VB12 from supplements and regulated fortified foods, then from dietary sources [10].

Vegans, which are the strictest vegetarian should try to include more food products in their diet from time to time because a rich source of VB12 can compensate overtime even though it is not a daily source. In this context, regular or even occasional fish and seafood consumption can help ensure proper VB12 intake. Therefore, these classes of food should be considered by those who exclude meat from their diets. Also, fish and seafood are healthy options because they are rich in other essential micronutrients and are low in saturated fat [47].

Only severe cases must be treated with high doses of oral medication and with cyanocobalamin (for the United States) and hydroxicobalamin (for Europe) intramuscular injections [35,53,55].

Toxicity

Toxicity levels are not rated for VB12 because no data proves harmful effects for overdoses. Therefore, there are no health disorders associated with VB12 hypervitaminosis. As well there is no official safe limit, but doses higher than 1000 times the RDA were safe for both human and animal subjects [2].

2.1.3. Vitamin B1

Short history

Vitamin B1 represents the first compound from B complex classified as a vitamin. Beriberi is the nutritional disease associated with the deficiency of VB1, and its history starts in China in the year 2697 B.C. [2,4,35,57].

Even though beriberi was known for a long time, the first description of the disease dates from the 17th century, around the year 1630, and was made by a Dutch physician named Jacobus Bontius. Back then, it was noticed a tendency for beriberi disease in men from Asian countries enrolled in the army or navy. All these men were living in the same conditions, were consuming mostly white polished rice, and they presented severe neurologic and cardiovascular symptoms [2,58,59].

Over the years 1883 – 1884, a Japanese naval doctor, Kanehiro Takaki, compared the effects of polished and unpolished rice diets served to the men from two different ships. More than half of the men who consumed polished rice got sick and developed beriberi, but very few men were affected in the other group's case. Comparing these two situations, scientists concluded that beriberi appeared as an effect of an improper diet. Moreover, from this moment started the challenge of finding the cure for beriberi. Men affected by beriberi, who then changed their diet from polished rice to various meat types, fruits, vegetables, and cereals showed a fast recovery. Thus, it was understood that the anti-beriberi factor is found in these products and in the outer layer of the rice lost by polishing operation [2,58–61].

Later a Dutch pathologist, Christiaan Eijkman, made a similar study with chicken and he proved the nutritive value of unpolished rice since only the chicken who were fed polished rice got sick. This study turned out to be very valuable and in 1929 was awarded a Nobel Prize for finding the solution to prevent beriberi [2,59,60].

In 1912, the Polish biochemist Kazimierz Funk successfully extracted the anti-beriberi factor from rice and used his discovery to treat both human and chicken subjects. The cure factor was an organic base with nitrogenous molecules, so Funk decided to name it “vitamine”, a conjunction of the words vital and amine. Later the name was simplified to vitamin and because were found more compounds with common characteristics the name was changed again into vitamin B1 [2,59,61].

The work dedicated on understanding this vitamin continued with the isolation of VB1 from rice in 1926 by Jansen and Donath, though the formula turned out not to be complete. Ten years later, in 1936, was possible the synthesis of VB1 and the final chemical formula was revealed by Williams and Cline who attach to the older version a missing sulphur molecule [58,61].

General properties

Aneurine and thiamine (with or without the last letter "e") are common names of VB1 used interchangeably. The natural types of VB1 occur as free-base thiamine and as three different phosphorylated forms [2,35].

The free base thiamine has the following empirical formula $C_{12}H_{17}N_4OS^+$: 3–[(4–amino–2–methyl–5–pyrimidinyl) methyl]–5–(2–hydroxyethyl)–4–methyl thiazolium and the molecular mass of 265.35 g/mol. The chemical structure of thiamine includes three parts: a pyrimidine, a thiazole linked through a methylene bridge and a hydroxyethyl chain in the fifth position of the thiazole ring that gets phosphorylated as shown in Figure 2.2 [1,2,35].

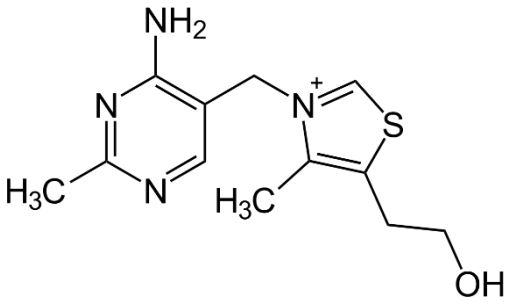

Figure 2. 2. Chemical structure of vitamin B1.

After the phosphorylation process thiamine changes into three phosphate esters with biological activity[35], and these esters are thiamin monophosphate – TMP, thiamin diphosphate or pyrophosphate – TPP and thiamin triphosphate – TTP. The second one TPP is the most important from these forms because it acts as a cofactor for the following enzymes: pyruvate dehydrogenase, α–ketoglutarate dehydrogenase (α–KGDH) complex, transketolase and branched-chain α–keto–acid dehydrogenase complex [2,35,58,60].

The natural form of VB1 is a white powder with a sulfurous odor and bitter taste. The solubility of vitamin B1 is rated as very high for water and 0.1M HCl solutions since it will dissolve

immediately. However, in alcoholic solutions, the solubility is very low and will require around 2 hours for VB1 to dissolve [2,4,35].

The free base thiamin is very unstable and oxidizes quickly, therefore is difficult to work with VB1 in its pure form. For commercial purposes is preferred to use thiamine hydrochloride due to its high stability. The synthetic hydrochloride salt of thiamine is a colourless crystal hygroscopic compound, highly soluble in water (1 g/mL). Figure 2.2 presents the chemical structure of thiamine hydrochloride ($C_{12}H_{18}Cl_2N_4OS$, 337.3 g/mol) [4,35,62].

Because of its solubility thiamine hydrochloride is widely used for pharmaceutical products with parental administration and fortified food products. Besides thiamine hydrochloride, there is a second commercial form of VB1, namely thiamine mononitrate. This compound is much more stable than the first one because it is less hygroscopic; but has lower water solubility, of just 27 mg/mL. Thiamine mononitrate is preferred for food and feed supplementation and in the pharmaceutical industry for dry products [2,4,35].

The synthetic forms, thiamine hydrochloride and thiamine mononitrate, have the stinging smell, and the solubility in alcohols and nonpolar solvents. Both are moderately soluble in methanol, ethanol, glycerol, and insoluble in acetone, ether, acetone and benzene [35].

The VB1 solutions' pH has a significant impact on the maximum value that can be absorbed by the UV-light. A solution with a pH value around three VB1 will absorb at 246 nm, and in a solution with pH five can absorb at two different values, namely 235 and 267 nm [4,35,36].

Functions and health benefits

The functions in which VB1 is involved, reflect its importance for human health. As a co-enzyme precursor, TPP is responsible for different cellular enzymatic functions involved in the metabolism of carbohydrates, lipids, branched chained amino acids and glucose.
Besides, VB1 plays an essential role in:

- the brain and nervous system by preventing neurological disorders.
- the cardiovascular systems enhancing its well-functioning and avoiding heart failure.
- the immune system strengthening specific parameters, withstanding stress, improving mood and memory [2,57,58,60].

Food sources

Fungi and bacteria produce VB1, and it is present in a variety of plant and animal products. Although most of these natural sources contain low amount of VB1, adopting a balanced food diet should be enough to ensure the mandatory daily intake [1,2,35].

Most of the plant origin food products contain VB1 in its free form and as TMP. TPP represents up to 85% of the total thiamine from cells of animal origin and the rest of comes other phosphorylated forms [2,35,63,64].

The most important natural sources of VB1 are yeast, grains, and meat. Though VB1 can also be found in other food categories, like fruits, vegetables, and dairy products, it is even smaller. Table 2.6 presents the richest food sources of VB1 and the content of the vitamin expressed in mg per 100 g of the respective food product [1,2,35].

Worldwide grains are among the most common ingredient to produce primary food products like bread and cereal derivatives. So, this vitamin's requirements can be easily achieved through a high carbohydrate intake, a fact confirmed especially by Asian diets that are rich in rice. Another example is the US where around a third of VB1 supply is provided from grain product consumption [1,2].

The food products with VB1 content suffer thermal breakdown during boiling or blanching. For example, boiling rice and green vegetables were reported high losses of up to 90%. Therefore, it is recommended to change classical cooking methods with steaming that requires a low volume of water or none. Another important aspect regarding food preparation is the pH. The best option is to maintain the food's pH at low values because, in neutral or alkaline conditions, VB1 will degrade fast [1,2,35,64,65].

Table 2. 6. Food sources of vitamin B1.

	Food product	Content of vitamin B1 (µg/100g)
Meats	Beef	0.02 – 0.10
	Beef liver	0.19
	Duck	0.26
	Pork	0.41 – 0.92
	Pork liver	0.28
	Cured ham	0.82
Fish	Salmon	0.02 – 0.16
	Trout	0.15
Dairy	Cheese	0.01 – 0.15
	Milk	0.04 – 0.05
Eggs	Whole egg	0.04
	Egg white	0.01
	Egg yolk	0.30
Grains	Cornmeal	0.39
	Whole grain wheat	0.52
	Rye flour	0.29
	Brown rice	0.18
	White rice, cooked	0.02 – 0.20
Vegetables	Cauliflower	0.05
	Cabbage	0.06
	Green beans	0.07
	Broccoli	0.07
	Carrots	0.07
	Potatoes	0.11
	Asparagus	0.16
	Green pees	0.27
Fruits	Apricots	0.03
	Bananas	0.03
	Oranges	0.07
	Pineapples	0.08
	Grapes	0.09
Other	Baker yeast	1.89
	Peanuts, uncooked	1.14
	Peanuts, roasted	0.18

NOTE: adapted from Combs G. – *The Vitamins. Fundamental Aspects in Nutrition and Health*. 5th edition. Elsevier Academic Press; 2017.

RDA and biochemical indicators

In Table 2.7 the values for the recommended VB1 intake expressed as RDA are presented. Female adults are accepted an RDA of 1.1 mg/day and for male adults, a higher amount of 1.2 mg/day [2,10].

Table 2. 7. RDA values for vitamin B1 presented by age and sex groups.

Age group	RDA (mg/day)
0 – 6 months (*)	0.2
7 – 12 months (*)	0.3
1 – 3 years	0.5
4 – 8 years	0.6
9 – 13 years	0.9
14 – 18 years, males	1.2
14 – 18 years, females	1.0
19+ years, males	1.2
19+ years, females	1.1
Pregnant women	1.4
Lactating women	1.4

(*) For infants there no official data of RDA and the presented values are adequate intakes recommended by FAO/WHO.

Absorption and excretion

VB1 is released quickly from natural sources, and its digestion occurs without difficulties. The absorption takes part in the jejunum and ileum (small intestine), from where it is further transported to the liver via portal vein by active and passive uptake. This last part of the absorption process is done with a thiamine-binding protein (a plasma carrier protein) [1,2,35,66].

Primary deposits are found in the liver, kidneys, heart, brain, and muscles; however, these accumulations are temporary and quantitative insignificant. The human body storage of VB1 is kept to a minimum to ensure the proper performance of metabolic functions, and any surplus is fast excreted by the urinary system [2,35,66].

Stability

The stability of VB1 is easily affected by the following external factors pH, humidity, temperature, oxygen, and metal ions (as solutions with Fe^{3+} and Cu^{2+}). The thiazole ring opens in the presence of alkali, at room temperature and a pH value higher than 7. In a moisture atmosphere, VB1 will degrade faster than in a dry atmosphere in which the vitamin is known to be stable for several hours at 100 ºC. As well, the autoclaving process destroys VB1[2,4,36,62].

In the case of oxidation, VB1 will form compounds like thiamin disulfide or tiochrome. The last one, tiochrome, is a widely known yellow substance, without biological activity but used to quantify VB1 due to its intense blue fluorescence [1,2].

It is recommended to keep VB1 in sealed containers at low temperature, below -20 ºC, to maintain its stability [2,4].

Deficiency and symptoms

VB1 deficiency is commonly known as beriberi disease and appears primary when the intake of vitamins does not satisfy the organism's real needs. Although it can also be the consequence of secondary causes which refer to the following risk groups: chronic alcoholics, elderly, pregnant women and their future infants or patients with a chronic disease like (HIV, malaria, Wilson, Parkinson or Alzheimer) [2,58,66].

The occurrence of Beriberi disease is not very common for developed countries, and most recently reported cases are from low industrialized countries with limited living conditions. Though a relevant example are the Asian countries in which adults have a poor diet based on milled rice [2,58].

Alcoholism is associated with poor nutrition and malabsorption; therefore, VB1 deficiency occurs rapidly. Besides chronic alcoholics, the segment of elderly people is also affected by malabsorption of VB1 or other essential compounds [2,58].

Infant Beriberi appears in the early months of the infants and is a consequence of deficient mothers in VB1. As the infants' nutrition depends on the milk of their mothers, the RDA of this vitamin should be ensured to avoid getting sick [2,35].

The general symptoms of this disease can gradually worsen and include low appetite (anorexia), decreased overall growth, weakness and mental fatigue, cheilosis, edema, gastrointestinal disorders (ulcer), anaemia (low level of erythrocytes), cardiac problems and some neurologic affections [2].

Two big classes of deficiency have been established after was observed that this disease could manifest differently. The first one is distinguished by cardiac system symptoms and the other one by signs in the nervous system. Moreover, among the most known diseases related to this classification are Wet Beriberi for the first class and Dry Beriberi with Wernicke–Korsakoff syndrome for the second one [2,35,58,60].

Treatment and recommendations

VB1 deficiency can be treated with a medical treatment, and the medication differs quantitatively according to the gravity of the deficiency, so for mild cases will be given to the patients' doses of 10 -20 mg/day, and for severe cases, the doses can reach up to 300 mg/day. The duration of each treatment may differ according to the response of the body. At the end of medication treatment, it is always recommended that the patients continue with a food diet rich in VB1, if possible from natural sources, if not from fortified food products [2,35,67].

Toxicity

For human subjects, there is no official data for the tolerable upper limit of VB1. Besides, the limited absorption of VB1 and the rapid urinary excretion of possible surpluses makes this vitamin practically nontoxic [1,2,10,63].

2.1.4. Vitamin B2

Short history

The first steps for the discovery of vitamin B2 were done in 1879 by the English chemist Alexander Wynter Blyth when he isolated the vitamin known nowadays. The compound was found in milk whey and was classified as a water-soluble yellow fluorescent pigment. And due to its properties, VB2 was initially named lactochrome (Lacto = related to milk, and chrome = colour) [2,68,69].

However, until the early 1930s, the studies developed by two biochemist Americans, McCollum and Kennedy, hypothesized that VB2 could be used to cure pellagra. People affected by this severe disease manifested by dermatitis, dementia, and diarrhoea. Although they showed improvements after the administration of lactochrome, they did not heal completely. While the discovery of vitamins continued, later was established that the compound responsible for pellagra was vitamin B3 (nicotinic acid) [2,35,68].

In the year 1933, lactochrome was isolated from other food sources (egg white, yeast, vegetables) and after it was administrated to rats, was concluded that this compound promotes growth. This research work was accomplished by done the Austrian-German biochemist Richard Kuhn and Swiss chemist Paul Karrer, and their findings were considered important for health and nutritional disorders [4,68,69].

Lactochrome was recognised as part of the B complex in 1934. Then the chemical structure was revealed, and the name was changed. The new name given to the vitamin was riboflavin and refers to the Latin words: ribityl – for the radical from the structure of VB2 (ribo-) and flavus – for the characteristic yellow colour. In 1935 Kuhn achieved the synthesis of VB2 [68,69].

Later in the year 1938, Kuhn and Karrer were awarded a Nobel prize for their impressive work dedicated to vitamins. During that year, VB2 was recognized as an essential micronutrient for human health [70].

General properties

VB2 gathers a family of flavin compounds and is well-known under different names like riboflavin, lactoflavin or vitamin G [1,2].

The VB2 molecule (Figure 2.3) is composed by an isoalloxazine ring (flavin ring), methylated in the positions number 7 and 8, and has a D-ribityl radical in position number 10. The chemical

formula of VB2 is $C_{17}H_{20}N_4O_6$: 7,8-dimethyl-10-(1´-D-ribity) isoalloxazine and the molecular mass is 376.36 g/mol [1,2,4].

VB2 is a yellow crystalline compound with fluorescence properties, odorless and with an unpleasant bitter smell in the form of free base. VB2 shows the maximum values of absorption at 223, 266, 373 and 445 nm.[1,2]

The solubility of VB12 in water is moderate and increases with the temperature, as follows: 0.10 – 0.13 mg/ml at 25 – 27.5 °C, 0.19 mg/ml at 40 °C and 2.30 mg/ml at 100 °C. Contrary to the free form, the co-enzymes FMN and FAD present a high water-solubility, due to their ionic phosphate groups. VB2 is slightly soluble in ethanol, 0.045 mg/ml at 27 °C, though it is insoluble in other compounds like ether, chloroform and acetone [1,2,35,71].

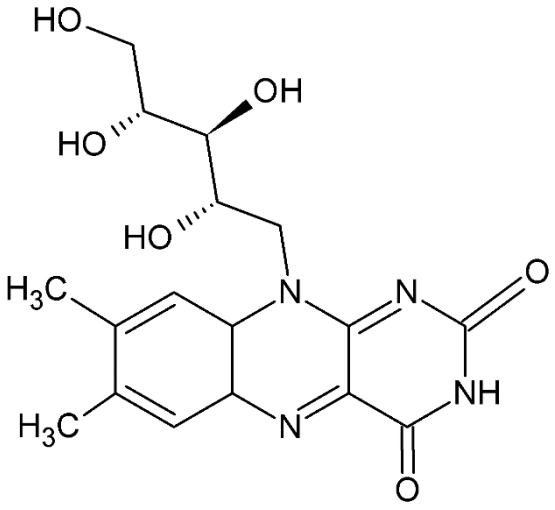

Figure 2. 3. Chemical structure of vitamin B2 form.

The most important biologically active forms of VB2 are flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The FMN molecule is formed by phosphorylation of the 5´ position of the ribityl side chain of VB2. Moreover, by adding adenosine-5´-monophosphate to FMN molecule FAD is produced [1,2,4].

Both compounds serve as co-enzymes for some flavoproteins and are involved in several oxidation-reduction reactions (redox reactions) of metabolism. Some examples of these reactions are electron transport chain for NADH and succinate dehydrogenase, oxidation of saturated fatty acyl-CoA dehydrogenase, glutathione reductase and fumarate dehydrogenase. Furthermore, the influence of VB2 is significative also for the metabolism, synthesis and activation of other water-soluble vitamins as pyridoxine, niacin and folate [1,4,72].

The synthetic forms of VB2 are produced through microbial synthesis using strains like bacterium *Bacillus subtilis*, the yeast *Candida famata* and the mold *Ashbyagossypi*. From the global production of VB2, 30% go to pharmaceutical and food industries and 70% to feed additives [69,72].

Functions and health benefits

The cofactors FMN and FAD are essential for carbohydrates, lipids and proteins metabolism; therefore, they convert the food into energy. FMN and FAD also help activate other vitamins, namely VB6 and VB9 [2,10,35,72].

The role of VB2 is very versatile for human health and counting all its benefits, and it is easy to understand the importance of this vitamin. Among the most known VB2 functions are:

- growth of healthy skin, nails and hair.
- responsible for good vision and healthy eyes.
- antioxidant and anti-inflammatory properties.
- strengthens immune and digestive systems.
- reduces blood pressure [1,2,71,73,74].

Food sources

VB2 is synthesized by plants and by microorganisms. The natural food sources of VB2 contain the phosphorylated forms, FAD in higher proportions and FMN in lower amounts predominantly. VB2 as a free compound can also be found in naturals products, although it is not very common and in minimal quantities [69,72].

The daily VB2 intake can be reached easily from a normal diet, due to its bioavailability in various natural products.

The most important natural sources for the Western population of VB2 are eggs, dairy products, and meats. As shown in Table 2.8, these three types of products are also the ones with a higher concentration of VB2. The opposite case is developing countries which rely mostly on cereals, vegetables, and fruits to achieve the minimum VB2 intake. And although the concentration of VB2 is significantly lower in products of vegetal origin, many countries can rely upon a few of these foods. [2,36,72,73].

Table 2. 8. Food sources of vitamin B2.

	Food product	Content of vitamin B2 (µg/100g)
Meats	Beef	0.24
	Beef liver	3.50
	Cured ham	0.19
	Pork	0.27
	Chicken	0.19
	Lamb	0.22
	Lamb's liver	5.11
Eggs and dairy	Whole egg	0.30
	Yoghurt	0.16
	Milk	0.17
	Cottage cheese	0.28
	American cheese	0.43
	Cedar cheese	0.46
Grains	Rice	0.01
	Oatmeal	0.02
	Rye	0.08
	Wheat hole	0.11
Vegetables	Potatoes	0.04
	Cabbage	0.06
	Carrots	0.06
	Corn	0.06
	Cauliflower	0.08
	Lima beans	0.10
	Spinach	0.14
	Aspargus	0.18
	Broccoli	0.20
Fruits	Apples	0.01
	Oranges	0.03
	Bananas	0.04
	Peaches	0.08
	Strawberries	0.09
Other	Baker yeast	5.41
	Torula yeast	5.06
	Almonds	0.93

NOTE: adapted from Combs G. – *The Vitamins. Fundamental Aspects in Nutrition and Health*. 5th edition. Elsevier Academic Press; 2017.

Cooking methods that use heat, as canning or sterilization do not affect the stability of VB2, because of this a compound very stable to temperature. Though if exposed to direct light, the vitamin becomes very sensitive and shortly will be destroyed. One of the most common causes of VB2 photodegradation is the milk packed in transparent bottles. To avoid this type of situation, worldwide it is recommended to keep milk in closed opaque containers. Besides milk, also fruits and vegetables exposed to sunlight for drying processes are prone to lose VB2. Milling methods affect VB2 concentration like in the case of VB1; therefore, it is preferred not to process cereals too much, or make fortification mandatory [2,72,73,75].

RDA and biochemical indicators

VB2 cannot be stored in the human body, and it is mandatory to ensure a minimum daily dose from the diet. The adult RDA is of 1.1 mg/day for women and 1.3 mg/day for men. All age requirements of VB2 are presented below in Table 2.9 [10,35,75].

Table 2. 9. RDA values for vitamin B2 presented by age and sex groups.

Age group	**RDA (**mg/day**)**
0 – 6 months (*)	0.3
7 – 12 months (*)	0.4
1 – 3 years	0.5
4 – 6 years	0.6
7 – 9 years	0.9
10 – 18 years, males	1.0
10 – 18 years, females	1.3
19+ years, males	1.1
19+ years, females	1.3
Pregnant women	1.4
Lactating women	1.6

(*) For infants there no official data of RDA and the presented values are adequate intakes recommended by FAO/WHO.

Absorption and excretion

Natural food sources contain very low amounts of VB2 in its free form and to obtain it the co-enzymes FMN and FDA must be hydrolysed. After the absorption of VB2 it is made with the help of carrier-mediated processes in the upper part of the small intestine and colon [1,2,35,72].

Like other water-soluble vitamins, VB2 cannot be stored in the human body in appreciable amounts because it is excreted in the urine very fast [2,35,72].

Stability

VB2 is relatively stable to heat or variations of pH. Nevertheless, the stability is easily affected by the exposure to light. Photodegradation leads to biologically inactive compounds as lumiflavin, in alkaline conditions, and lumichrome, in acid conditions. VB2 handling must be done in the dark or under subdued red light [2,4,35,72,73].

Deficiency and symptoms

The nutritional disorder provoked by the lack of VB2 is named ariboflavinosis. This deficiency is more common for developing countries from Asia and Africa, where people have poor diets. Rarely, cases of VB2 deficiency are registered in industrialized countries, especially inside communities with low income [2,10].

At high risk are the following groups: people who experience poor diets or stress situations, pregnant women or during lactation, infants and elderly people who might suffer from malabsorption. It can also affect people with selective food diets, alcoholics, athletes and patients with cancer [72,74,75].

General symptoms of ariboflavinosis include dermatological and ophthalmological disorders, appetite and growth decrease, muscular weakness, anaemia, fatigue, anxiety with signs of depression and gastrointestinal inflammation. The dermatological and ophthalmological signs are easier to recognize because attack the parts of the body rarely covered. Some common examples are cheilosis, stomatitis, glossitis (cracks of the lip, mouth or tongue) and blurred vision or photophobia [2,35,75].

Another feature of ariboflavinosis is the association with deficiencies of vitamins B3, B6 or B9. In such case of multivitamin deficiency, the symptoms are inevitably interrelated and addressing this health problem is much more difficult [4,72,75].

Treatment and recommendations

Most of the times, ariboflavinosis occurs in combination with deficiencies of other B-complex vitamins. Therefore, the first recommendation before starting any treatment is to check if the deficit is assigned only to VB2 or other vitamins. The typical treatment for VB2 deficiency includes oral medication with a maximum concentration of 25 mg. Higher doses are not more effective because the human body cannot absorb them [72,74].

Besides specific medication, the treatment will also relay on a proper diet, rich in natural foods. If available on the market, fortified products are also recommended, because they can ensure controlled amounts of VB2 [4,72,76].

Toxicity

The limited intestinal absorption of VB2 makes this vitamin nontoxic. The maximum amount of VB2 that can be absorbed by the human body is about 25 mg, and the surplus of normal intake is later fast excreted [2,10,35,74].

2.1.5. Vitamin B3

Short history

Vitamin B3, the third essential compound from the B complex, was identified only in the year 1937 after was discovered the treatment for pellagra. Pellagra disease is caused by the deficiency of vitamin B3 and was one of the most severe nutritional diseases that affected humanity. Over 100.00 people died, mainly from southern states of America, so the high mortality rate was declared an epidemic situation until the beginning of the 20th century [2,35,77,78].

Around the year 1500, Christopher Columbus brought corn seeds to Europe from his journey to the "New World" (America). He promoted the benefits of this staple food, so shortly after people started to cultivate it intensively [79,80].

Pellagra was described for the first time in 1735 when a Spanish physician wrote a document about a disease affecting poor farmers. The specific signs were dermatitis, diarrhea and dementia, and in severe cases would lead to death. In 1771, an Italian physician analysing the manifestation and evolution of the disease, Francesco Frapolli coined the name pellagra that comes from "Pelle Agra" which means rough skin [2,35,79].

In 1914, the American physician and epidemiologist Joseph Goldberger started to investigate pellagra, and he is the first to suggest that this disease is a consequence of poor diet, specifically the corn-based diet. His later studies from 1920 led to the conclusion that pellagra is caused by the absence of a water-soluble compound found in corn. This discovery was known as "pellagra preventing factor" and was used with success for a trial treatment of "black tongue disease, " a similar health impairment with pellagra for dog subjects [77–80].

The treatment model used for dogs was used in human subjects and turned out to cure those who were suffering from pellagra. In 1937 the American biochemist Conrad Elvehjem identified the "pellagra preventing factor" that received a new name: nicotinic acid – vitamin B3 [4,35,77,78].

General properties

Vitamin B3 is commonly associated with names like vitamin PP, from the old name "pellagra preventing factor", and niacin, which is the short form of nicotinic acid. In nature, vitamin B3 exists as two different vitamers – forms of the vitamin which exhibit the same biological activity, namely nicotinic acid and nicotinamide, presented below in Figure 2.4 [35,79–81].

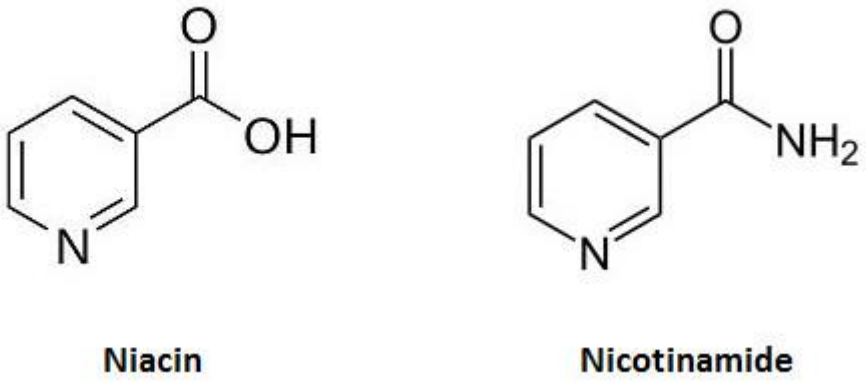

Figure 2. 4. Chemical structure of vitamin B3 forms: niacin and nicotinamide.

Nicotinic acid refers to pyridine-3-carboxylic acid, $C_6H_5NO_2$, and has a molecular weight of 123.11g/mol. Nicotinamide is the descriptor of pyridine-3-carboxylic acid amide, $C_6H_6N_2O$, with the molecular weight of 122.12g/mol. Both compounds are white crystalline odourless substances and present high stability in their solid form. Nicotinamide powder has a specific unpleasant bitter taste that should be masked, especially in food products [1,2,35,79,81].

Nicotinic acid is classified as sparingly soluble in water (1.67 g/100 ml) and ethanol (0.73 g/100 ml) at room temperature, although insoluble in acetone or diethyl ether. In contrast with nicotinic acid, nicotinamide is highly soluble in water (100 g/100 ml) and moderately soluble in ethanol (67 g/100 ml) at room temperature; and slightly soluble in acetone, diethyl ether and chloroform [1,4,35,81].

The maximum ultraviolet absorption value is also similar for nicotinic acid and nicotinamide, approximately 262 nm [2,4,35,81].

NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) are the co-enzymes of VB3 found in most unprocessed foods and only in few fortified products. These co-enzymes are required for metabolism and work as proton electron carries in many cellular oxidation-reduction processes. Trough phosphorylation NAD can be transformed into NADP in the presence of NAD^+ kinase enzyme [1,4,79] .

Functions and health benefits

VB3 play an essential role in the metabolism of carbohydrates, lipids and amino acids. The co-enzymes of VB3 have many similarities of structure and mechanism, but they execute different

types of redox reactions. The redox couple NAD(H) – NAD^+/NADH is responsible for oxidative and catabolic reactions: glycolysis, anaerobic glucose oxidation, β-oxidation of fatty acids, amino acid catabolism and Krebs cycle. Furthermore, the second redox couple NADP(H) – $NADP^+$/NADPH is used for reductive and anabolic reactions: fatty acids, cholesterol, bile acid and steroid synthesis. In a few cases, NAD and $NADH/H^+$ are used together for anabolic processes: glyconeo-genesis, fat, steroid and cholesterol synthesis [2,77,79,80].

Other essential functions of vitamin B3 are keeping the skin healthy, boosting brain activity, improving cholesterol levels, reducing the risk of heart diseases, and preventing birth defects [2,35,78].

Food sources

VB3 differs from other water-soluble vitamins because the human body can synthesize it with the help of L-tryptophan, an essential amino acid. L-tryptophan can be ensured through protein intake, so plant and animal foods with protein content are valuable sources of VB3. For every 60 mg of L-tryptophan will result 1 mg of VB3 [1,79,80].

VB3 can be found in natural food products as nicotinic acid and nicotinamide; therefore, it can be quickly supplied from the diet. High bioavailability of VB3 can be observed in the variety of common food products with VB3 content presented in Table 2.10 [2,35,81].

The richest natural sources are meat (red lean meat and organs), fish (herring and tuna), cereals, some vegetables (mushrooms, asparagus, pepper and lentils), yeast and peanuts.

Most dairy products, eggs, and vegetal origin foods do not contain high amounts of VB3, although they are considered essential sources, especially for followers of different types of vegetarian diets, if consumed with regularity [1,2,79,80].

Like other water-soluble vitamins, VB3 is lost during the milling process applied to cereals. And because this vitamin is essential for human health belongs to the mandatory fortification program adopted worldwide. Flour and grain derivates are fortified with VB3 and other nutritional compounds to help avoid deficiency disorders [1,79,81].

Table 2. 10. Food sources of vitamin B3.

	Food product	Content of vitamin B2 (µg/100g)
Meats	Beef	4.6
	Beef kidney	6.4
	Beef heart	7.5
	Chicken	4.7 – 14.7
	Turkey	8.0
	Pork	0.8 – 5.6
	Lamb	4.5
Fish	Cod	2.2
	Herring	3.6
	Tuna	13.3
Eggs and dairy	Eggs	0.1
	Yogurt	0.1
	Milk	0.2
	Cheeses	1.2
Grains	Polished/unpolished rice	1.6/4.7
	Barley	3.1
	Buckwheat	4.4
	Wheat grain/bran	3.4 – 6.5/8.6 – 33.4
Vegetables	Mushrooms	4.2
	Peas	0.9 – 25.0
	Peppers	1.7 – 4.4
	Lentils	2.0
	Corn	1.7
	Asparagus	1.5
	Potatoes	1.5
	Broccoli	0.9
	Tomatoes	0.7
Fruits	Peaches	1.0
	Bananas	0.7
	Apples	0.6
	Oranges	0.4
Other	Yeast	50.10
	Most nuts	0.60 – 1.80
	Peanuts	17.20

NOTE: adapted from Combs G. – *The Vitamins. Fundamental Aspects in Nutrition and Health*. 5th edition. Elsevier Academic Press; 2017.

RDA and biochemical indicators

The RDA for healthy adults of vitamin B3 is 14 mg/day for women and 16 mg/day for men. These values are the highest required for water-soluble vitamins and must be reached daily from the diet because the human body cannot store it. For every age group are presented in Table 2.11 the specific RDA values [1,2,10,36,77,79].

Table 2. 11. RDA values for vitamin B3 presented by age and sex groups.

Age group	RDA (mg/day)
0 – 6 months (*)	2
7 – 12 months (*)	4
1 – 3 years	6
4 – 6 years	8
7 – 9 years	12
10 – 18 years, males	16
10 – 18 years, females	16
19+ years, males	16
19+ years, females	14
Pregnant women	18
Lactating women	17

(*) For infants there no official data of RDA and the presented values are adequate intakes recommended by FAO/WHO.

Absorption and excretion

VB3, in the form of co-enzymes, is absorbed fast in the stomach and the small intestine. The process is eased by sodium-dependent diffusion, and for higher concentrations of VB3 is done via passive diffusion [2,79,80].

VB3 is not stored in the human body so the urinary system will excrete any excess. All extra intake of VB3 is methylated in the liver and forms the compound 1-methyl nicotinamide after being released in the urine in this form or as an oxidized metabolite, 1-methyl-6-pyridone-3-carboxamide [2].

Stability

VB3 is the most stable water-soluble vitamin because its stability is not affected by external parameters like temperature, light, oxidation, acid or alkali. Food products with VB3 in their

composition are not affected general cooking methods, except leaching, in which losses up to 40% are registered [1,2,4].

Deficiency and symptoms

The deficiency of VB3 will cause pellagra, a severe nutritional disease considered rare in developing countries. Nowadays, pellagra cases are recorded in poor communities in which people have inadequate diets and pass through stressful situations. Such cases occur especially in countries from Asia and Africa [2,10,79].

The most vulnerable to develop this type of deficiency are chronic alcoholics, patients with schizophrenia, Hartnup disease, HIV infection, gastrectomy or cancer. The following groups may also be affected, but have a lower incidence: elderly people who suffer from malabsorption disorders, pregnant or during lactation women and their infants, and people with selective food diets [2,77,79,80].

Depending on the type of symptoms, VB3 deficiency can be mild or severe. The signs of mild deficiency include dermatologic symptoms, slow metabolism, fatigue with loss of appetite, high cholesterol, digestive problems and brain impairment. Severe deficiency leads to wasting disease pellagra. Besides the signs of mild deficiency, pellagra is characterized by the "4D": dermatitis, diarrhea and dementia that in the final stage, can lead to death. The symptoms of dermatitis are easily observed because they are symmetrical and appear on the skin parts exposed to sunlight (face, neck, hands and legs). Diarrhea is the result of gastrointestinal inflammation and glossitis. And dementia appears due to the mental changes, as delirium, depression and irritability, that attack the nervous system strongly [2,10,79,80].

People who suffer from VB3 deficiency are also prone to other deficiencies such as vitamins B2 or B6. Therefore it is recommended to check if it is the case of multi-vitamin deficiency or not [2,10,80].

Treatment and recommendations

The diagnosis of vitamin B3 deficiency can only be made after a clinical examination. The medication doses used as treatment can include up to 300 mg/day of VB3. Besides, the medication should be consumed with regularity natural products rich in VB3 and fortified products.

VB3 can also be used as an adjuvant to treat different diseases, like dyslipidemia, acute migraine, chronic tension, anxiety, depression, schizophrenia, diabetes and cancer [2,77,79,80].

Toxicity

High doses of oral medication of VB3 are very efficient in treating several health problems presented in the last section of treatment and recommendations. However, besides the therapeutical outcome can provoke hepatotoxicity, skin flushing, itching urticaria and gastrointestinal discomfort. Therefore, it was adopted as an upper limit of 10 mg/day for children and 35 mg/day for adults to avoid these unpleasant side effects [2,10,63,80].

2.2. Nutrition and nutrients

Nutrition is the process of providing, metabolising, and using nutrients from food to support human physiologic functions. Constant nourishment is mandatory to sustain life and to keep health in proper parameters. The word "nutrition" was first mentioned around the year 1550, and its etymology goes back to the Latin verb "*nutrire*", translated as "to feed or to nourish" [82].

Nutrients are defined as compounds found in the composition of food and required by the human body to grow, reproduce, and survive. The most recommended and easy method to obtain nutrients is the direct supply from food because the human body cannot synthesize them at all or in enough amounts. However, the inability to provide sufficient nutrients or excessive consumption leads to various severe health problems such as deficiencies, nutritional or certain degenerative diseases [82,83].

The nutrients are divided into micro and macronutrients, and these types of nutrients are distinguished by the quantitative intake required by the human body [84,85].

The class of macronutrients is divided into proteins, carbohydrates, and fats. All of them are consumed in significant amounts, usually quantified in grams, and their most important function is to help the human body generate enough bulk energy. Besides the energetic value, proteins build, maintain and repair tissues; carbohydrates have energy storage capacity and support the muscular, digestive, and central nervous systems; and fats have a thermic role and are used as carrier mediums for fat-soluble compounds [83,84].

Although it has no energetic value, water is classified as a macronutrient because is needed in considerable amounts. The water is responsible for regulating the body temperature, eliminating toxins, and transporting several nutrients around the body until specific organs [3,84].

The micronutrients include vitamins and minerals. Both types of micronutrients are needed in minimal amounts, which can be quantified with milligrams or micrograms. Vitamins and minerals support and regulate many biochemical and physiological functions, and both are responsible for a healthy immune system. Vitamins act as co-enzymes or cofactors and contribute to good metabolism. Minerals are electrolytes or constitutes of several body fluids and also maintain bone and teeth health.

Of all the nutrients mentioned above, some are considered essential, which means that a certain mandatory amount must be ensured daily for proper health. The list of essential nutrients is presented below in Table 2.12. It includes the deemed indispensable to life: nine amino acids, two fatty acids, thirteen vitamins, fifteen minerals and the water, which is universally known as the most important essential nutrient [3,83,84].

Table 2. 12. The list of essential nutrients.

Essential macronutrients			**Essential micronutrients**	
Proteins (amino acids)	**Fats (fatty acids)**	**Water**	**Vitamins**	**Minerals**
1. Phenylalanine 2. Valine 3. Threonine 4. Tryptophan 5. Methionine 6. Leucine 7. Isoleucine 8. Lysine 9. histidine	1. linoleic acid (omega - 6 fatty acid) 2. alpha-linolenic acid (omega - 3 fatty acid)		1. vitamin A 2. vitamin D 3. vitamin E 4. vitamin K 5. vitamin B1 6. vitamin B2 7. vitamin B3 8. vitamin B5 9. vitamin B6 10. vitamin B7 11. vitamin B9 12. vitamin B12 13. vitamin C	1. potassium 2. chloride 3. sodium 4. calcium 5. phosphorus 6. magnesium 7. iron 8. zinc 9. manganese 10. copper 11. iodine 12. chromium 13. molybdenum 14. selenium 15. cobalt

The key to healthy nutrition is a balanced diet in terms of nutrient intake proportions. The meals of a balanced diet should be composed of natural or processed as little as possible products. Besides the diet, it is mandatory to have unrestricted access to food products and well-proportioned meals at appropriate times during the day. The ideal situation is for this type of diet to be part of a healthy lifestyle also characterized by constant physical activity, active social life and enough sleep time [3,85].

The most common method to supplement the human body with essential micronutrients used to be through normal staple products from the food diet and pharmaceutical products. However, from 1920 onwards, mandatory fortification programs for basic foodstuffs were introduced at the global level. Thus, from that year the micronutrient supplementation has become possible through both pharmaceutical and food products [19,86].

The pharmaceuticals are divided into drugs and supplements, and in turn, the foods can be fortified or functional products, and nutraceuticals.

The pharmaceutical treatments are usually very efficient, although the cost may vary depending on the type of product, period of treatment and availability. As well, pharmaceutical products need medical approval, therefore a previous appointment and analysis might also be requested.

All types of food products play an important role in preventive medicine and are considered alternative treatments. Using food supplementation and fortification procedures to manufacture new products with enhanced nutritional value is a simple, efficient, and affordable approach, popular among many countries and also for different population segments. When consumed with regularity, these products contribute to the control and prevention of deficiencies [6,10,86].

2.3. Microencapsulation

Over time, different compounds were “labelled” as unfeasible, as they were losing stability or changing their properties in certain situations. Therefore, it was necessary to incorporate these sensitive materials into products by creating new protective systems. One of the methods developed to overcome these compounds' limitations is the encapsulation technology that seeks to obtain functional products with enhanced properties [27,30,87–89].

Encapsulation is defined as a process in which active compounds are coated within secondary substances, and thus protective capsules are formed. The active compound is entrapped inside the capsule, and it becomes isolated from the external environment. The conversion of the sensitive active compounds into stable solid capsules or particles offers the advantage of protection, stability and controlled release [27,29,90–92].

The generic name given to the encapsulated active is core material and for the encapsulating substance is wall material. The outcome products of the encapsulation process are known as capsules or particles, and one of the most common criteria for differentiation is their final size. Hence, are distinguished the macroparticles, the microparticles and the nanoparticles [27,29,87,90,93].

The macroparticles have a diameter higher than 5000 μm, the microparticles range in diameter ranging 1 and 5000 μm, and the nanoparticles are the smallest as their diameter is below one μm. Although, in some studies, the lower limit of the macroparticles and the upper limit of the microparticles is only 1000 μm and not 5000 μm.

The micro and nanoparticles are the most studied, and the encapsulation processes associated are generically called microencapsulation and nanoencapsulation [29,90,91,94].

The purpose of this thesis was to obtain products through microencapsulation technology; therefore, further in this chapter, all references to the encapsulation and microencapsulation processes will be made interchangeably.

The first commercial application of the microencapsulation technology belongs to Barrett Green, which worked from the late 1930s until the 1950s to develop the first carbonless paper. This type of paper used the complex coacervation as microencapsulation method with gelatin and arabic gum as encapsulating agents. The invention of Green was based on pressure

activated release of encapsulated dye-precursor to papers, making it possible to copy multiple papers. In 1955, Green received a patent for his invention, and after the microencapsulation process started to be applied for other applications [89,93,95,96].

The interest in this technology has grown considerably over the years since the encapsulation proved to be a valuable production toll on both laboratory and industrial scale. Among the most common products containing encapsulated materials are foods, beverages, pharmaceuticals, health care, household, personal care, veterinary, textile, agrochemical products or construction [26,27,89,93].

2.3.1. General aspects about microencapsulation

The microencapsulation is presented as a packaging and delivery method suitable for different types of compounds, especially sensitive ones or with undesirable properties. Hence, the microencapsulation aims to confer protection and stability by coating the encapsulated material with an encapsulating agent [27,29,91,97].

The micron-sized products obtained after microencapsulation consist of two components: the core and the wall material. The material entrapped inside the microparticle is the core, and it can be in a solid, liquid, or gaseous state. The core can also be found in the literature under different names like internal/inner phase, active/bioactive or fill material. The wall material is a polymeric nature compound, referred to as the encapsulating agent, the external/outer phase, carrier or shell material [94,97–99].

The size, shape, internal structure, and morphology of the microparticles depend on the encapsulation process. Thus, selecting materials, equipment, and experimental parameters easily influences the microparticles' characteristics [27,29,89].

The size can range from a few micrometres up to a few millimetres, as mentioned in the previous section (2.3.). Regarding the final shape, the microparticles can be regular or irregular, as presented in Figure 2.5. The regular shaped microparticles can have spherical, oval, or cylindrical aspect [29,94,100].

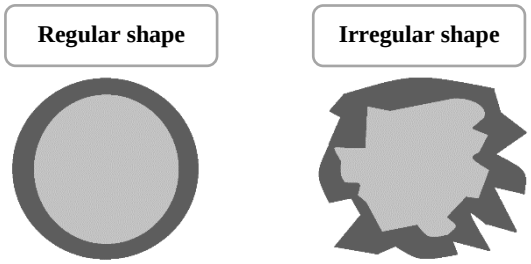

Figure 2. 5. Representation of microparticles with regular spherical shape and irregular shape; the dark shade of grey represents the wall and the light shade of grey the core (adapted from Estevinho et al., 2013) [27].

In Figure 2.6. can be observed the different variations of the internal structure of microparticles as follows: simple (type reservoir), multiwall, multicore, aggregate and matrix (microsphere).

For simplification, the representation of each type of microparticles is made only with spherical, although it is possible to obtain other kinds of shape, namely oval, cylindrical, or irregular.

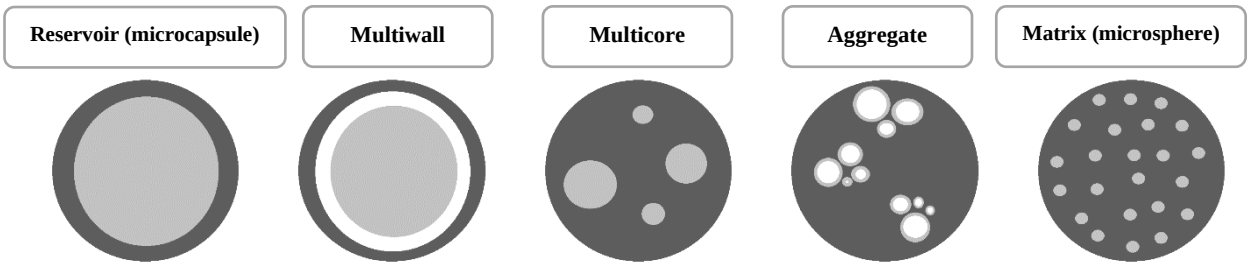

Figure 2. 6. Representation of microparticles internal structures (adapted from Estevinho et al., 2013) [27].

Regarding the morphology are distinguished the reservoir type (microcapsules) and the matrix type (microspheres). Both microsized systems are represented in Figure 2.7 [89].

The first microparticle from Figure 2.6 is known as the simple microcapsule or reservoir, and it is the primary type, characterized by a core surrounded by one coating layer with a uniform thickness. The configuration single-core with single-wall is not the only one accepted for the reservoir microparticle; thus, there are possible configurations with multi-core and multi-wall materials.

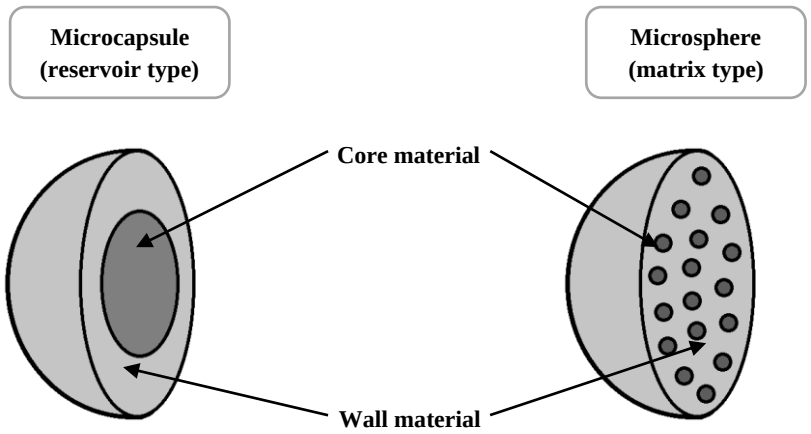

Figure 2. 7. Representation of microparticles morphologies (adapted from Paulo and Santos, 2017) [89].

Thus, besides this basic structure, there are other complex structures of microparticles like aggregate or polynuclear matrix structures. For the matrix microsphere, the core material is dispersed unitary inside the protective wall material shell [27,29,98,101].

Considering the possibilities of size, shape, structure, and morphology, the microencapsulation process can lead to the formation of a wide variety of microparticles [27,89,94].

The use of the microencapsulation technique brings the following significant benefits:

1. protection of the core from the outside environment during processing and storage by conferring long term stability:

=> capacity of the wall material to avoid the core degradation by reducing its reactivity when exposed to environmental factors like heat, moisture, air, or light.

=> the transfer rate and the evaporation of the core are reduced or retarded, especially for volatile core materials.

2. easy handling of the core materials as the final product has improved physical properties:

=> the conversion of the core material into a stable powder offers flexibility for product manipulation and storage.

3. the microparticles can be tailor-made to achieve desired release and properties:

=> the release can take place slowly or fast and can be determined by a particular stimulus's action.

=> the size, structure, and solubility of the microparticles are adjustable.

4. unpleasant organoleptic properties, like the taste, smell or even colour, are masked.

5. the core material can be diluted, if during the process are required only small amounts, yet after the microencapsulation, the core will be dispersed uniform inside the microparticles [27,29,94].

These advantages turned out to be suitable for many industrial applications like foods, pharmaceutical, cosmetics, agrochemicals, or other types of products. Although obtaining microparticles with desired properties, it is mandatory to analyse a serial of factors and determine the best production configuration. Therefore, before starting the microencapsulation process must be considered, the following factors:

1. Function of the microparticles: what benefits should be provided by the chosen materials (core and wall materials) so the product to have a viable application.
2. Core concentration: the ideal value for the active ingredient concentration should be selected after preliminary tests and fulfil its function after the controlled release.

3. Properties of the encapsulating agent: the protective layer must provide a reliable cover until the outburst of the release starts.
4. Controlled core release: what is the best release mechanism for the obtained microparticles.
5. The product cost: what materials and methods to select for a fair final price of the microparticles [29,94].

After considering these aspects, the proper method for a specific product application can be selected.

2.3.2. Applications of microencapsulation

The encapsulated products are enhanced formulations of the original active compounds because, after microencapsulation, they gain new features like protection from external factors, undesirable masked properties, stability over time, easy handling, and sustained release. Thereby, the microencapsulation technology is used to manufacture a wide variety of "added-value" products suitable for academic research and industrial applications.

A global report made in 2018 by Adroit Market Research reported the share size of the microencapsulation technology by industrial applications (Figure 2.8). The highest share size corresponds to the pharmaceutical industry, and it is estimated to own more than 71% of the whole microencapsulation market. The next industrial segments are the household and personal care products with almost 12%, foods and beverages over 8% of the market and agrochemicals with around 5%. A much smaller segment of nearly 3% belongs to other applications from textile, construction, printing, or electronics industries (source www.adroitmarketresearch.com).

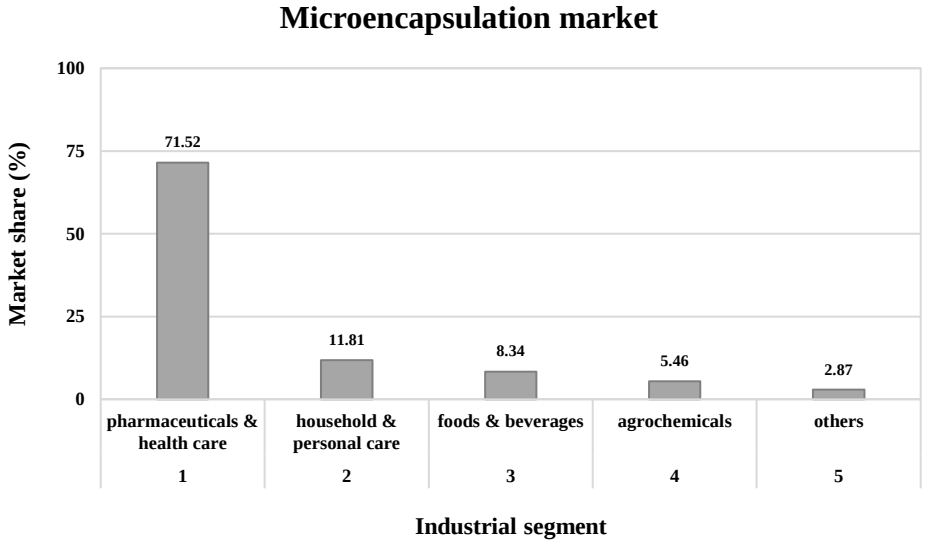

Figure 2. 8. The market share size for microencapsulation technology from the year 2018 (adapted from source www.adroitmarketresearch.com)

The products classified as pharmaceutical, or healthcare own the largest market share because of the wide variety of products that uses microencapsulation for drugs and other medical actives. Among the benefits of microencapsulating pharmaceuticals are the increased

bioavailability of drugs, targeted delivery, controlled release, and masking unpleasant smell, taste, or appearance of the active compounds.

The medical field's indispensable contribution was observed from 1970 when with the help of microencapsulation process was possible to develop innovative products for drugs and vaccine delivery. From this point was registered high demand for microencapsulated products. Allowing controlled release of medication to the patients was considered an essential step for medicine in general, and later this principle turned out to be very useful for food products with added value [91,93].

The household and care products industry uses microencapsulation, especially for the protection and controlled release of fragrances, additives, dyes, or essential oils. The demand for these products is also growing, and it is addressed mostly to costumers interested in high quality and sustainable cosmetics and detergents [100].

The market for foods and beverages is addressed to encapsulated flavours, additives, essential nutrients, colourants enzymes, probiotics, antioxidants, or compounds that can improve the overall nutritional value. Moreover, the recent trend of consumers focused on consuming better food and living a healthy lifestyle helped because it determined the production of functional foods, fortified foods, and nutraceuticals with encapsulated ingredients. In 2018, the food industry's overall revenue was only in third place with 8%, but it is predicted to register till 2025 the fastest growth. Some of the critical factors for this growth are the advantage of a relatively low production price, the capacity to protect food products from nutritional losses, preservation of flavours, and the possibility of avoiding nutritional diseases by consuming fortified foods [26,29,87,91].

The interest in microencapsulation is still growing, fact supported by the versatility of technology and promising results. For 2018 the microencapsulation market was evaluated to 7,158.3 USD millions, and it is predicted to reach 15.291.9 USD millions by 2025. This economic growth is due to the increased demand for products like medication with controlled drug release, supplements with natural ingredients, fortified food products or cosmetics.

2.3.3. Microencapsulation techniques

Before selecting the microencapsulation technique, the physico-chemical properties of the core and the wall materials, the expected release mechanism, and the purpose of the encapsulation process with the final application of the microparticles must be considered. Furthermore, after choosing a method, it is important to perform optimization tests of the parameters that define the encapsulation process to obtain an optimal product in terms of process efficiency and efficiency, size and shape of microparticles [27,87,90,102].

All the microencapsulation methods aim to achieve stability for the core and confer a new functionality for the microparticles. Despite the significant number of available methods, none of them is universally applicable for all kinds of materials or applications [26,94,103,104].

The available microencapsulation techniques are conventionally divided into three categories: the chemical, physico-chemical and physico-mechanical processes, according to the nature of the main process determining the encapsulation. Table 2.13 presents the classification of the microencapsulation techniques and some of the most used microencapsulation methods [90,93,105].

Table 2. 13. Classification of microencapsulation techniques and their different types of methods.

Chemical processes	**Physico-chemical processes**	**Physico-mechanical processes**
Interfacial polymerization *In-situ* polymerization Polycondensation	Coacervation (phase separation) Emulsification Supercritical fluid Co-crystallization Inclusion complexation	Spray drying Spray cooling/chilling Electrospinning Lyophilization (freeze-drying) Fluidized bed coating Extrusion Liposome entrapment

The microencapsulation methods relying on chemical processes are interfacial polymerization, *in-situ* polymerization, and polycondensation. The group with physico-chemical processes includes the following methods: coacervation (phase separation), emulsification, supercritical fluid, co-crystalization and inclusion complexation. Furthermore, the last group with physico-mechanical processes contains probably the most significant number of methods like spray

drying, spray cooling/chilling, lyophilization (freeze-drying), fluidized bed coating, extrusion, and liposome entrapment.

Chemical processes

The chemical processes used for microencapsulation techniques are based on chemical reactions and imply monomers' polymerization with small molecules to form the protective polymeric wall of the microparticles.

The interfacial and *in-situ* polymerization is among the most known chemical methods for the encapsulation of active ingredients. Both methods have the advantages of being fast processes, easy to scale-up and generally with high encapsulation efficiency. Although the polymerization methods have the inconvenience of using large quantities of organic solvents, not all monomers are biodegradable and biocompatible, and the overall polymerization process is difficult to control [100].

- **Interfacial polymerization**

The encapsulation through interfacial polymerization refers to the formation of the polymeric wall material at the interface of two phases of liquids, one oil-soluble and another water-soluble. The polymerization of the reactive monomers will determine the wall material's production at or on the surface of a droplet or a particle. The materials used for this process are multifunctional monomers like isocyanates and acid chlorides, which can be used individually or in combination. The selected multifunctional monomer is dissolved in the solution with the core material, and after it is dispersed in an aqueous phase, with the desired droplet size. This phase of the encapsulation process involves a dispersing agent and a multi-functional amine. The polymerization reaction occurs rapidly at the interface and generates the formation of the microparticles wall. If possible, the monomers should be soluble only in one phase, so the polymerization only occurs at the interface [90,93,100,106].

- ***In-situ* polymerization**

The procedure used for *in-situ* polymerization is similar to the one of interfacial polymerization. Although, for this method is not necessary to use reactants for the core material solution. The polymerization reaction requires only one monomer that will slowly precipitate, under the action of heat and catalysts, on the core material's surface. A typical wall material for *in-situ* polymerization is the melamine-formaldehyde resin [90,100,107].

Physico-chemical processes

For the physico-chemical processes, the materials used as encapsulating agents are pre-dissolved and further precipitate due to the variation of temperature, pH, or electrolyte concentration. After, in the last stage of the encapsulation process, the encapsulating material will gradually deposit on the core's surface to form the protective capsule.
This section will analyse the following physico-chemical methods: coacervation, emulsification, supercritical fluid, co-crystalization and inclusion complexation.

- **Coacervation (phase separation)**

The first commercial application of microencapsulation dates from 1950 when was produced a type of carbonless paper by coacervation, as previously presented in Subchapter 2.3 [89,93,95,96].

The coacervation relies on the phase separation of one or more hydrocolloids (a liquid phase that consists of the encapsulating agent) from a polymeric solution followed by the deposition of the newly formed coacervate phase core material suspended or emulsified in the same reaction medium [26,30,90,100,106].

According to the number of polymers used, the coacervation method can occur as simple or complex. During the simple coacervation process, it is used only one polymer, like gelatine, while the complex coacervation requires two or more polymers, such as gelatine and arabic gum. Between these two versions of coacervation, the complex one is often used due to its functionality, especially in food or pharmaceutical applications [108–111].

The encapsulation steps of the coacervation method are: (1) the development of three immiscible phases – mixing of the core, encapsulating agent and the continuous liquid phase, (2) the deposition of the encapsulating agent as a coating layer around the core – by maintaining parameters like temperature, pH, ionic strength, the concentration of the encapsulating agents, and molecular weight of the solution, and (3) the solidification of the coating – by heating, de-solvation or cross-linking techniques [26,90,104,112]. At the end of the coacervation process, the newly formed microparticles are collected by centrifugation or filtration, washed with a proper solvent and dried with the help of spray-drying or fluidised bed drying methods [108,109].

Coacervation is a highly effective method that can reach up to 90% of encapsulation efficiency. The obtained microparticles are usually spherical, with good stability and controlled release

properties. As well the features of the coacervation process are suitable to produce microparticles with heat-sensitive core materials [26,108,109,111,113].

Among the most significant limitations of the process are the overall high cost, the complexity of the mechanism, the complicated scale-up system, and the particles' agglomeration. Moreover, the use of organic solvents is incompatible with many applications, and in some cases, the formed microparticles are sensitive to environmental conditions [26,108,109].

- **Emulsification**

The emulsification method can be achieved through different emulsion-based technologies, suitable for the encapsulation of both hydrophilic and hydrophobic active core materials. The food industry uses this method with a predilection for products like butter, margarine, ice-cream, or mayonnaises.

Emulsions are defined as colloidal delivery systems, which include a mixture of at least two immiscible liquids (oil/s and water). In the emulsion system, the particles of one phase (the dispersed phase) are dispersed as small spherical droplets within the other remaining phase (the dispersant phase) [100,104,112].

According to the organization of both phases, oil and water, the emulsions can be divided into single, double or multi versions. The single emulsions can be water-in-oil (W/O), meaning that the water droplets are dispersed in the oil phase, and oil-in-water (O/W), in which oil droplets are dispersed in the water phase. The double and multi-emulsions are complex systems, and some known examples are water-in-oil-in-water (W/O/W), oil-in-water-in-oil (O/W/O), water-in-oil-in-oil (W/O/O), and water-in-oil-in-oil-in-water (W/O/O/W) [112].

The main three steps followed for the single emulsion process are (1) the dispersion of the core material into an organic solvent with the encapsulating agent, (2) the emulsification in water or oil, with the help of an emulsion stabilizer, and (3) the evaporation of the organic solvent under stirring, which leads to the formation of the microparticles. In the case of multi-emulsions versions, the emulsification step will be repeated as many times as necessary [29,90,93,105].

The emulsion methods can also be divided into low energy and high energy approaches. The low energy approach is suitable, especially for thermo-sensitive ingredients that require microencapsulation. In this case, the most common is spontaneous emulsification, which

produces emulsions by mixing oil, water, surfactant, and a co-surfactant in certain situations. Furthermore, to obtain a specific emulsion size, droplets can be applied mechanical mixing (blending, microfluidization or sonification) or variations of temperature. Contrary to the low energy, the approach with high energy is achieved by high-pressure homogenization, which allows a narrow size distribution of the particles [26].

Overtime new types of emulsifications were developed, starting from these technologies, namely the emulsion-solvent evaporation, emulsion diffusion, emulsion-ionic gelation, and emulsion interfacial protein cross-linking.

The emulsification method offers the advantages of easy scale-up and the production of small spherical particles characterized by a narrow size distribution. The cost of this method is considered intermediate; however, it can fast increase due to the use of expensive materials like surfactants. Moreover, when selecting emulsions as suitable systems for microencapsulation, it must be considered that this is a process difficult to control, which achieves low values of encapsulation efficiency and will determine a high quantity of residual solvent.

- **Supercritical fluid**

Supercritical fluids exist above a critical point of temperature and pressure, after which the liquid and gas phases of the material are no longer distinguishable. Specific for supercritical fluids are intermediate properties of liquids and gases, such as gas-like viscosity and compressibility, liquid-like density, higher mass transfer and diffusivity. A small variation of temperature or pressure causes a large change in the supercritical fluid density near the critical point [30,90,102].

Different materials, such as carbon dioxide (CO_2), nitrous oxide (N_2O), propane and water, can be brought into the supercritical state. However, CO_2 is the material most frequently used for microencapsulation processes. As encapsulating material, CO_2 brings the following benefits: it requires reasonable values of pressure and temperature, it is environmentally friendly, very abundant and cost-effective [26,30,90,102].

Supercritical fluid method starts by preparing the core material's dispersion in the supercritical fluid, after the fluid is released and will precipitate the wall covering the core.

The most known technologies are rapid expansion of supercritical solution (RESS), gas anti-solvent (GAS), supercritical solvent impregnation (SSI), supercritical fluid extraction of emulsions (SFEE), particles from gas-saturated solution (PGSS), supercritical assisted atomization (SAA), and aerosol solvent extraction (ASES) [26,90,102].

Sensitive materials (e.g. volatile flavours, enzymes) can benefit from the supercritical fluid method because they do not require organic solvents, high temperatures, or any type of mechanical stress during the encapsulation process. Another significant advantage of the technique is the high encapsulation efficiency that can reach a value of 100%. Though the whole process is difficult to control, and the obtained microparticles tend to form agglomerations [90,102].

- **Co-crystalization**

Co-crystalization is a simple microencapsulation method that uses sucrose syrup as the protective coating material. For the first phase of the process, the sucrose syrup must be concentrated until it reaches the supersaturated state and after it must be maintained at a high enough temperature to avoid the crystalization. The incorporation of the active core material is made in the second phase, under mechanical agitation. Thus, these conditions will cause the nucleation process and will trigger the spontaneous crystalization of the supersaturated sucrose syrup – core mixture. In the last phase, the sucrose reaches the proper temperature for crystallization, determining the emission of a substantial amount of heat. While cooling, the microparticles are dried to the desired moisture and screened to a uniform size. By applying this method, the sucrose crystals can be modified to form aggregates of relatively small dimensions: water-soluble crystals containing the core either inside the crystals or by entrapment [29,87,114].

Co-crystalization has the advantage of converting liquid core materials into dry stable powders without implying other drying processes. The method is flexible and economical; however, food and pharmaceutical industries choose to avoid it because of the health concerns regarding sucrose's properties. Another drawback of co-crystalization is represented by the core selection, as the heat-sensitive materials can quickly degrade during the process.

- **Inclusion complexation**

Inclusion complexation is a microencapsulation method known as molecular inclusion because it occurs at the molecular level and implies the insertion of the bioactive compounds into specific encapsulation agents like the cyclodextrins [29,30,102].

The cyclodextrins are naturally occurring oligosaccharides with six, seven or eight glucose residues in their composition, interlinked by α (1→4) glycoside bonds in a cylinder-shaped structure. The internal part of cyclodextrin is hydrophobic, and the outer part is hydrophilic. The cavity of the cylinder structure has a depth of ~0.8 nm and variable diameter. These cyclodextrins are labelled as α, β, and γ [26,102].

The most used as an encapsulating agent is β-cyclodextrin, a cyclic derivate of starch with seven glucopyranose units, prepared by partially hydrolysed starch (maltodextrin) by an enzymatic process. In this case, the cavity has a diameter of 00.65 nm. The reduced use of β-cyclodextrin, especially in the food industry, is explained by the limited regulatory rules, mostly from Europe [29,102].

The microencapsulation methods that use inclusion complexation are generally simple co-precipitations and require stirring, sonication or heating of the mixture with cyclodextrins and the active core, so the complexation occurs [111].

This method's main advantages using cyclodextrins are the cost (low to medium), enhanced stability of poorly water-soluble core materials, and the good controlled release properties [30,111].

Physico-mechanical processes

The last class of techniques is based on physical and mechanical principles determining the encapsulation process and microparticles production. The formation of the capsules depends on the solid-liquid phase transition provoked by heating or solubility reduction due to solvent evaporation.

- **Spray drying**

Spray-drying is one of the most known and widely used microencapsulation methods for food industry. During the spray-drying process, the dispersion wall material-core is sprayed under high inlet temperatures and at high feed velocity, and this action leads to the dehydration of the

atomized particles and the formation of dried powders microparticles. Characteristics like fast, easy scale-up, flexible, low cost and reproducible, recommend spray-drying technology for different applications [26,27,30,115].

More information about spray-drying can be found further in this thesis in Subchapter 2.4, which includes a detailed presentation of this method selected to produce B vitamins microparticles.

- **Spray cooling/chilling**

Spray cooling and spray chilling are two similar encapsulating methods, differentiated only by the melting point of the wall material. For these methods, the mixtures with the core and the wall materials are atomized into cooled or chilled air, determining the solidification of the wall around the core. Unlike spray-drying, the principle of spray cooling and spray chilling does not involve water evaporation [26,29,109,111].

The characteristic wall materials for spray cooling have a melting point of 45 – 122 °C, and some typical coating materials are fat, stearin, mono- and diacylglycerols.

For the spray chilling method, the melting point has a lower value of 32 – 42 °C, and the most common wall materials are fractionated or hydrogenated vegetable oil. Microencapsulation by spray-chilling results in free-flowing powders with dense, not porous and almost perfect spheres because the process does not involve mass transfer (evaporation from the atomized droplets) [26,29,30,109].

Due to their lipid coatings, the microspheres produced by spray cooling/chilling are insoluble in water; therefore, most used core materials are sensitive water-soluble compounds like enzymes, flavours, acidulants, minerals and water-soluble vitamins. Typical food industry applications include dry products capable of conserving thermosensitive active core materials, such as foods with high levels of fat, bakery products, or dry soups mixes [26,109,115].

The overall encapsulation processes by spray cooling/chilling are considered rapid, safe, reproducible, low cost and with high encapsulation efficiency values. Although it is difficult to control the particle size with these two methods, the yields have moderate values, release occurs too fast and is required special conditions for handling and storage [26,30,109].

- **Electrospinning**

Electrospinning represents a versatile encapsulation method that transforms solutions into continuous fibers with variable diameters from few nanometers to few micrometers. On industrial level, this method has been implemented since 1990 for the delivery of bioactive compounds [116,117]. Compared to other encapsulation methods, electrospinning presents the following advantages: is not using extreme conditions, in terms of temperature and solvents, the obtained products have a small size, and the process is usually rated with high encapsulation efficiency [118]. However, there is a limitation of the process regarding the encapsulating agents because it is more challenging to work with biopolymers due to their molecular weight and complex chemical structures, and the use of synthetic polymers is preferred [117].

The technology of electrospinning requires electrostatic forces to produce the polymer fibers and consists of three elements: a capillary to pump the liquid, a high voltage source with a positive or negative polarity that generates a charge in the polymer solution, and a grounded collector, which is brought into contact with the counter electrode [119]. And the parameters that influence the electrospinning process are the viscosity, the voltage, the temperature, the pressure and the flow rate [117].

For this process, a polymer solution is supplied from a spinneret that produces a droplet at the spinneret exit. Next, an electric field is applied, and the electric charges gather on the surface of the droplet. Further, the droplet is deformed and gets the shape of a cone, known as the Taylor cone. A fluid jet is formed under the electric field to the spinneret tip, and it moves to the grounded collector. After, the solvent evaporates fast due to whipping and the high surface charge jet under the electric field. The outcomes of the process are thin solid fibers in the form of nonwoven mats [105].

Two similar encapsulation methods to electrospinning are emulsion electrospinning [120] and electrospray [105,119]. The first method, emulsion electrospinning, was developed to enable the incorporation of hydrophilic compounds into hydrophobic polymeric fibers [120]. The second method, electrospray, produces particles, not fibers, and is defined as liquid atomization applied through electrical forces [105,119].

- **Lyophilization (freeze-drying)**

Lyophilization or freeze-drying, is the most appropriate method for both microencapsulation and dehydration of heat-sensitive materials, such as proteins, pigments, essential oils, or microorganisms [29,92,104].

Freeze-drying helps extract the water by sublimating ice from the frozen product at reduced temperature and pressure. Thereby, chemical reactions are avoided, and heat-sensitive food products and other biological materials can be used for long-term preservation. An efficient encapsulation by lyophilization manages to preserve most of the initial properties of the raw core material, like the shape, texture, size, appearance, colour, taste, flavour, or biological activity [92,94].

The most common materials used as encapsulating agents for freeze-drying are maltodextrin, arabic gum, emulsifying starches, and whey protein.

The most significant limitations of this encapsulation method are the cost, which is evaluated as intermediate to high, the process is energy and time-consuming, and the obtained products are powders with high hygroscopicity [94,102,104].

- **Fluidized bed coating**

The fluidized bed coating method produces granules or coated particles by spraying a binder in a solution, melt, or suspension onto a fluidized powder bed [26,92,102].

The core materials particles are suspended in an airstream at a defined temperature and humidity, and after the particles are sprayed with the wall material. For this microencapsulation method different types of coaters like top spray, bottom spray, and tangential spray can be used [90,92].

Among the most used wall materials are gums, lipids, proteins, carbohydrates, dextrins, emulsifiers, cellulose and starch derivates [30,115].

The advantages of the fluidized bed coating method are controllable temperature and particle size distribution, low cost and slow-release profiles. Thus, the process has a low encapsulation efficiency, of maximum 50%, is time-consuming, has a predisposition to form agglomerations, and heat-sensitive materials can be easily depredated during freeze-drying [29,109,111].

- **Extrusion**

The extrusion method was first patented by Swisher in 1957 and ever since was widely used, especially in the food industry. Another common name for this method is melt injection because, for this process, the solution for encapsulation is passed through a nozzle into a gelling environment and on a small scale, can be used a syringe to load the solution [26,29,104,113].

This type of microencapsulation involves the extrusion of a liquid mixture, which contains the core and the wall materials, through an orifice and formation of droplets at the discharge point of the nozzle. The obtained droplets are dried and solidified as microparticles through physical (e.g. cooling or heating) or chemical (e.g. gelation) processes [26,104].

The final size of the droplets varies according to the nozzle diameter, feed flow rate, air flow rate or electric filed [104].

Extrusion technique can be performed by different methods, such as simple dripping, electrostatic extrusion, jet cutting, spinning disk atomization, coaxial airflow, and vibrating jet/nozzle. The main difference between these methods is the droplet formation mechanism that depends on factors like surface tension, impulse, or frictional forces [26,102,104,111].

The most common wall materials for extrusion used, single or in combinations, are maltodextrin, alginates, glucose, glucose syrup, sucrose, and glycerine [29,113].

The advantages of extrusion are the use of gentle solvents, mild temperatures or pH conditions are required, protection against oxidation – the process can be performed in both aerobic and anaerobic conditions. The prepared particles have a spherical shape with a matrix structure and easy to achieve a burst release. However, the main limitations are the difficult scale-up, the low encapsulation efficiency and the cost [29,30,111].

- **Liposome entrapment**

Liposomes are single or multi-layered lipid vesicles, which involve complete entrapment of an aqueous phase within a phospholipid-based membrane by microfluidisation or colloid mill. The phospholipid membrane has an amphiphilic character because its structure includes both hydrophilic and hydrophobic head groups. Thereby, the polar end can bind to water-soluble particles and the non-polar end to the fat-soluble particles [26,102,111,113].

Liposomes have been used in the pharmaceutical industry to achieve targeted delivery of vaccines, hormones, probiotics, vitamins, enzymes, or paramagnetic contrast agents (for cancer cell detection by MRI). Another common application is for the cosmetics industry, where liposomes are used for the stabilization of skin nutrients. Recently, this encapsulation method started to be used for the food industry, too, because the liposomes are non-toxic, biodegradable, and biocompatible materials [26,30,109].

The main advantage of the liposome entrapment method is the capacity of releasing the active core at a controlled rate, at the target site and at the desired time. As well the method allows the core material to maintain a high-water activity, which is very beneficial for core materials, such as enzymes or probiotics. However, the process is complicated to scale-up, implies a high cost, the efficiency is lower than 50%, and the stability of the microparticles is easily affected by oxidation, hydrolysis, pH or heat [30,113].

Table 2.14 presents a brief description of some microencapsulation methods by highlighting the main encapsulation process steps, the size and morphology of the microparticles, and the most relevant advantages and disadvantages of every technique.

All the methods presented are characterized by various advantages and disadvantages, so it must be analysed very well which of them fits best, ensuring that the chosen method is easy to implement both at the laboratory and industrial levels, the way of working to be simple, and the whole process to be fast, reproducible and with a low price [27,100].

The number of microencapsulation methods is still growing with time because there is a high demand for improved methods or new features that can be used for more materials and applications.

Table 2. 14. Examples of microencapsulation methods and their characteristics (adapted from [26,29,94,100,102,121])

Methods	Process steps	Particle size (µm)	Particle morphology	Advantages	Disadvantages
Interfacial polymerization	1. dissolution of a monomer and dispersion of another monomer in two immiscible liquids. 2. production of a thin interfacial polymer film through the reaction from the interface between both solutions.	0.5 – 1000	-	Easy scale-up Fast process High EE	Difficult control and handling High quantity of residual solvent
Coacervation (phase separation)	1. formation of three-immiscible chemical phases 2. deposition of the coating 3. solidification of the coating	20 – 200 (simple) 5 – 200 (complex)	reservoir core-shell multiwall	Suitable for heat-sensitive core materials Spherical, multinucleate particles Controlled release Good stability EE = 40-90%	A complex mechanism, difficult scale-up Sensitive to environmental conditions Use of organic solvents High cost
Emulsification (single or double)	For single emulsions: 1. dispersion of the core into an organic solvent with the wall material 2. emulsification in the water or oil, which contains an emulsion stabilizer 3. evaporation of the organic solvent under stirring, which leads to the formation of the microparticles. For double emulsions: the emulsification phase will be repeated.	1 – 500	matrix core-shell multiwall multicore	Easy scale-up Small spherical particles Narrow size particles distribution Burst release from matrix particles	Process difficult to control Intermediate to high cost Low EE High quantity of residual solvent
Supercritical fluid	1. preparation of dispersion with the active core in supercritical fluid 2. release of the fluid to precipitate the shell on the core	10 – 400	matrix core-shell	No need to use organic solvents, high temperatures or mechanical stress EE = 20-100%	Process difficult to control The tendency of agglomerations of the microparticles Precipitate morphology can be difficult to control and predict
Co-crystallization	1. preparation of supersaturated sucrose solution 2. addition of the core in the supersaturated solution 3. emission of substantial heat after solution reaches the sucrose crystallization temperature	-	-	Liquid core materials can be converted into dry powders without any additional drying process	Heat sensitive core materials can be degraded during the process

Table 2.14. (Continued)

Methods	Process steps	Particle size (μm)	Particle morphology	Advantages	Disadvantages
Inclusion complexation	1. mixing of the compounds 2. complexes formation by mixing, grinding, or spray-drying	0.001-0.01	reservoir	Low to medium cost	-
Spray drying	1. preparation and homogenisation of the dispersion 2. atomization of the feed dispersion 3. dehydration of the atomized particles	10 – 300	matrix	Easy handling and scale-up Reproducible and high production rates Wide choice of encapsulating agents Hydrophilic and hydrophobic core materials Spherical particles with good stability Low cost (continuous batch)	Relatively high operating temperatures Considerable losses (material can stick to the walls of the drying chamber) Difficult to control the particle size Temperature-sensitive cores get degraded Non-uniform particles with a tendency to aggregate Moderate yields for small batches
Spray cooling/chilling	1. preparation and homogenisation of the dispersion 2. atomization of the feed dispersion	20 – 300	matrix	Applicable for sensitive core materials to high temperatures and water-soluble compounds EE = 10-100% Low cost (batch/continuous)	Difficult to control the particle size Moderate yields for small batches Insoluble microparticles in water Fast release Special handling and storage conditions
Lyophilization (freeze-drying)	1. mixing the core with a coating solution 2. freeze-drying of the solution 3. grinding (optional)	1 – 1.000	matrix	Good resistance to oxidation	Energy and time-consuming process Intermediate to high cost
Fluidized bed coating	1. preparation of the coating solution 2. fluidization of core particles 3. coating of core particles	5 – 5.000	core shell multiwall multicore	Controllable particle size distribution Controllable temperature Low cost Slow release	EE = 5-50% Temperature sensitive cores can be degraded Time consuming process
Extrusion	1. preparation of the molten coating solution 2. dispersion of the core into the molten polymer 3. cooling or passing of core-coat mixture through dehydrating liquid 4. drying	1 – 10.000	matrix core shell multiwall multicore	Gentle/no organic solvents, extreme temperatures or pH conditions are not required Performed in both aerobic and anaerobic conditions Spherical particles Burst release from matrix particles	EE = 20-50% Difficult to scale-up Intermediate to high cost
Liposome entrapment	1. microfluidization 2. ultrasonication 3. reverse-phase evaporation	10 – 1.000	phospholipid bilayers	Control release	EE = 5-50% High cost

2.3.4. Encapsulating agents

A physical barrier is formed by the encapsulating agent to protect the microencapsulated core; therefore, the efficiency of the process and microparticles' stability depends on the chosen encapsulating agent. Before selecting a particular encapsulating agent, the following criteria must be taken into consideration:

- physicochemical properties of the materials composing the microparticle: the core (porosity, solubility) and the encapsulation agent (viscosity, mechanical properties).
- compatibility between working materials (the encapsulating agent cannot react with the core and needs to be insoluble in it).
- protection against various factors from the external environment which can degrade the core or affect the stability of the microparticle.
- capacity seal and hold the core materials within the microparticle during storage or processing.
- desired final size and morphology of the microparticles.
- release of the core under specific conditions.
- availability and economic factors (production cost).
- how the product will be handled and processed [29,90,100,122,123].

Regarding the physicochemical properties of the wall material, it is very important to verify the following characteristics: molecular weight, glass transition temperature, solubility, diffusivity, and film formation and emulsifier capacity. All these characteristics are beneficial to understand the compatibility of the encapsulating agent with the core, and the desired properties of the microparticles, like stability and release.

The selection of the encapsulating agent and the microencapsulation method used for the preparation of the microparticles is interdependent; thus, the material must be compatible with the working principle of the technique and the used equipment.

Each type of encapsulating agent has specific characteristics that for different applications can be assimilated as advantages or disadvantages; therefore, it is recommended the use of more encapsulating agents. The formulations that combine several materials are known as composite agents and can fulfil more of the criteria mentioned above than one single material [27,87,124].

There is a wide variety of available materials that can be used for microencapsulation as encapsulating materials. Although, when it comes to materials suitable for applications in the

pharmaceutical and food industry, the encapsulating agents must have film-forming and emulsifying properties, be biodegradable, be resistant to the gastrointestinal tract, have a low viscosity at high solids contents, and exhibit low hygroscopicity. Particularly for the food industry, the cost must be very low, so the final food product can be affordable for as many consumers as possible [104].

From the health and safety point of view, another condition that these materials must fulfil is the "generally recognized as safe" - GRAS certification and approval of local agencies like Food and Drugs Administration – FDA, from America. However, the regulations for food products are much stricter than those for pharmaceutic and even cosmetic products. Therefore, some materials can be accepted only for active encapsulation with medical applications and incompatible with foods. The regulations are also different, and the market of encapsulating agents varies from one continent to another [104,125].

The materials used as protective wall materials are polymers of natural, semi-synthetic or synthetic nature. These polymers can be water-soluble or insoluble.

The natural polymers suitable for microencapsulation are compounds classified as proteins, lipids, or carbohydrates, with a plant, microbial/animal or marine origin. They are characterized by biocompatibility, biodegradability, non-toxicity, high availability, but they have the disadvantage of variable composition.

Some synthetic polymers used are aliphatic polyesters or modified celluloses. They have very low biocompatibility but can be easily formulated with optimized features. And the semi-synthetic ones gather characteristics from both natural and synthetic materials, which can be a good alternative in some situations.

The following sections will present some of the most representative natural encapsulating agents suitable for different applications.

- **Natural polymer-based coating materials**

The natural polymers are identified as biopolymers and characterized by abundance in nature, which allows the economic advantage of having a low cost. Other important features for the

environment and the applications in the food and pharmaceutical industries are non-toxicity, biocompatibility, and biodegradability.

For microencapsulation processes, the biopolymers can be used in their original form from nature, or their composition can be improved to avoid variations.

The natural coating materials suitable for encapsulation are divided into three big groups of carbohydrates, proteins, and lipids derivatives; and their origin can be of plants, microbial/animals or marine. Table 2.15 presents some of the most representative natural polymers for every group.

Table 2. 15. Natural polymers used in microencapsulation as coating materials.

Origin	Carbohydrates	Proteins	Lipids
Plant	Starch and derivatives Cellulose and derivatives Plant exudates: ・Arabic gum ・Karaya gum ・Mesquite gum Plant extracts: ・Galactomannans ・Soluble soybean	Gluten Pea Soy Zein	Fatty acids/alcohols Glycerides Phospholipids Waxes
Microbial/animal	Chitosan and derivatives Gellan Dextran Xanthan	Gelatin Collagen Caseins Whey Keratin	Fatty acids/alcohols Glycerides Phospholipids (shellac) Waxes
Marine	Alginate Carrageenan	-	-

NOTE: adapted from B. N. Estevinho and F. Rocha – *Application of Biopolymers in Microencapsulation Processes* [114].

1. Carbohydrates

According to the number of saccharides groups found in their structure, the biomolecules of carbohydrates (saccharides) are divided into monosaccharides, disaccharides, oligosaccharides, and polysaccharides. However, of the four types of carbohydrates, only the polysaccharides are suitable for the formation of delivery systems because of their massive molecular structure that helps entrap and protect active core materials [104,119].

The polysaccharides are natural polymers with long chains of monosaccharides bound together by glycosidic linkages and represent more than 90% of carbohydrates. Due to their abundance, the polysaccharides are found in a wide variety of vegetal (e.g. starch, cellulose, arabic gum, and pectin), microbial (e.g. dextran, xanthan, and cyclodextrin), animal (e.g. chitosan), and even marine (e.g. alginate and carrageenan) sources [104,114,119,126].

Polysaccharides are biodegradable, biocompatible, safe, and sometimes inexpensive materials. Their capacity to protect the encapsulated core from the gastrointestinal tract's harsh conditions and release the core in the colon make the polysaccharides appropriate even for food and pharmaceutical products.

Like other natural compounds, the polysaccharides present variations of the structures that determine variations of solubility, digestibility, emulsification, and water retention capacity. However, this problem can be overcome because these polymers have a high potential to be modified and obtain new polymeric formulations with desired properties [119,127].

Another limitation of polysaccharides is represented by their hydrophilic nature that will determine poor protection against water vapour [126].

Plant

Starch represents one of the most abundant polysaccharides of vegetal origin, and it is a α-D-glucose polymer linked with glycosidic bonds. Its general structure is $(C_6H_{10}O_5)_n$, and it includes two main components: linear chains of amylose in the proportion of 20 – 30% and branched chains of amylopectin in a higher ratio of 70 – 80% [114,119,128].

Natural starch is a white powder, biodegradable, biocompatible, digestible, and predominantly hydrophilic (insoluble in cold water). Furthermore, starch has a low cost, and before use does not require intensive purification processes like other biopolymers. These characteristics make starch a good option for incorporating food products, although, in the case of encapsulation, hydrophobic active materials limit its use [104,119,126].

Starch-based biopolymers are usually selected because they are tasteless, odorless, transparent, and have low permeability to gases, which are good advantages for food and pharmaceutical products [126,128].

In its natural form, starch does not have emulsifying properties, therefore, modified starches are produced to improve functionality and to extend the commercial applicability. Chemical, biochemical, physical and/or enzymatic processes are used to obtain different modified starches. The most common examples are cross-linked, oxidized, acetylated, hydropropylated and partially hydrolysed molecules of starch [104,128].

The chemically modified starches have superior emulsification properties and are used with success especially for the encapsulation of volatile flavours by spray-drying. The cross-linked starches have a higher temperature and shear stability, and the ones partly hydrolysed confer protection against heat and oxidation. On the other hand, the oxidized biopolymeric starches are characterized by enhanced film formation capacity, adhesivity and drug release functionality. The acetylated starches present improved hydrophobicity, decreased swelling ability and increased resistance to enzymatic hydrolysis [104].

A significant number of modified starches result from the hydrolysis process. The resulting derivatives of starch, known as dextrins, are differentiated by their degree of hydrolysis, which is assigned to the dextrose equivalent value (DE). The derivatives with a DE value lower than 20 are known as maltodextrins; those with the value between 20 – 100 are corn syrups, while those with the DE 100 are glucose (dextrose) [104,128].

A particular case of a starch-based biopolymer is represented by maltodextrins, as they are used extensively for encapsulation purposes. Maltodextrins are creamy-white hygroscopic powders, tasteless or with a mildly sweet taste, easily digestible, and high water solubility (~70 %).

By using maltodextrins as wall materials good delivery systems for both food and pharmaceutical materials were achieved. These biopolymers can protect active core ingredients from oxidative loss and thermal degradation and, as well, have good stability properties. Commonly used for the microencapsulation process is maltodextrin DE 10 as it offers an optimum functionality in terms of solubility and moisture content.

To obtain enhanced emulsification properties, to reduce the oxygen permeability of the wall matrix, improve bioactive retention and good control release profiles, the maltodextrins are mixed with other biopolymers, such as gums, alginate, pectins or whey proteins [104,128].

Cellulose represents the main structural component from the cell walls of plants, and it is the most abundant polysaccharide found in nature. This biopolymer is a β-D-glucose with the chain units linked by β-(1→4)-glycosidic bonds [104,119,128].

The natural form of cellulose has a substantial size, and it is insoluble in water and other common solvents; therefore, its use for food and pharmaceutical applications is limited. However, it is possible to prepare modified celluloses by chemical, biochemical or physical processes [104,119].

The most widely used modified celluloses are methylcellulose (MC), carboxymethylcellulose (CMC), hydroxypropyl cellulose (HPC), hydroxypropyl-methylcellulose (HPMC), ethyl cellulose (EC), ethyl methylcellulose (EMC), cellulose ethers (CE), and cellulose acetate. Methylcellulose is characterized by high solubility, excellent film-forming properties and efficient oxygen and lipid barrier. Carboxymethylcellulose is a water-soluble, biocompatible, and biodegradable biopolymer used for drug encapsulation and binding, stabilizing thickening, and tabletting filler of active ingredients. Hydroxypropyl cellulose it is a water-soluble modified starch with good filming properties, suitable for encapsulating oil and fats. [104,114,119,128]

The **plant exudates and extracts** are widely used for food and pharmaceutical applications as they are non-toxic, biodegradable, biocompatible, and, therefore, safe for human consumption. These biopolymers are macromolecular substances with complex structures.

The plants exudates are commonly known as gums and the most representative biopolymers are arabic gum, karaya gum, mesquite gum, and tragacanth gum. Furthermore, among the most used biopolymers from plant extracts are pectin, galactomannans, and soluble soybean [128].

Arabic gum is also called acacia gum as it is composed of a dried exudate from the stems and branches of *Acacia senegal* and *Acacia seyal* trees. The arabic gum biopolymer is produced only in few locations like Senegal, Sudan and Somaliland, where these types of trees are grown commercially [128].

Its complex structure is a branched-chain, and the backbone is composed of β-(1,3) linked D-galactopyranosyl units. The side chains are formed of two up to five β-(1,3) linked D-galactopyranosyl units, joined to the main chain by 1,6-linkages [128].

Gum arabic is a neutral or slightly acidic substance without odour, or colour. Highly soluble in water, this biopolymer can dissolve in both hot and cold water up to 50 wt%. As well, arabic gum is characterized by low viscosity, excellent emulsifier properties, and can create strong protection layers for oil droplets [114,128].

For microencapsulation, arabic gum is compatible with other plant hydrocolloids, carbohydrates, proteins, and modified starches and have shown very good results especially with spray-drying and coacervation methods.

Due to its versatile properties, arabic gum is considered one of the most used encapsulating agents for the food industry, where it can fulfil functions of stabilizer, emulsifier, thickener, and flavour fixative. However, the use of arabic gum is sometimes limited by its low availability, which implies a high acquisition cost to droplets [114,128].

Pectin is a linear anionic polysaccharide extracted from the cell walls of higher plants from Europe and Latin America. In fruits, pectin is found in variable amounts and qualities, and the extraction occurs from the peel and juice of citrus or apple pulp [104,114,128].

Pectin biopolymers are high molar mass hetero-polysaccharides that include at least 65 wt% of α-(1,4) linked D-galacturonic acid-based units. The difference between pectins is given by the degree of esterification (DE) or the number of methoxy groups. Thus, high methoxyl pectins (HMP) if DE > 50 and low methoxyl pectins (LMP) when DE < 50 are distinguished. HMP presents gelling capacity in acidic conditions and with concentrations of sugar, while LMP can form gels only in the presence of cations (e.g. ions) [104,128].

Its properties recommend pectin as a valuable encapsulating agent for both food and drug delivery. Although insoluble in organic solvents, pectin is soluble in water, has low viscosity compared to other plant gums, and is most stable at pH 3-4. A controlled release can be easily achieved because this biopolymer is not digested in the upper gastrointestinal tract by gastric or intestinal enzymes. Therefore, it is poorly soluble in these conditions. Although, pectin is wholly absorbed in the colon and digested by the colon microflora [104,128].

Microbial and animal biopolymers

The biopolymers produced biotechnologically by microbial, or animal synthesis have novel functional properties. The most used microbial polymers for encapsulation are xanthan, gellan, and dextran, and for animal origin, chitosan and its derivates.

Xanthan is a high molecular mass anionic polyelectrolyte that occurs as a mixed salt of sodium, potassium, and calcium. The backbone of xanthan includes β-(1,4)-D-glucopyranosyl units, and every second unit has a trisaccharide side chain linked at the C-3 position, one D-glucuronosyl unit between two d-mannosyl units.

The bacterial species that produces xanthan by anaerobic fermentation process in a glucose medium is *Xanthomonas campestris bacterium*.

Xanthan is only soluble in cold water and is considered to be mainly non-gelling [128].

Chitosan is a linear cationic polysaccharide that consists of β-(1,4)-linked D-glucosamine and N-acetyl-D-glucosamine. The primary source of chitosan is represented by chitin, which is found in the exoskeletons of arthropods, such as crustaceans, insects, or molluscs. Chitin is the second most abundant source of biopolymers; therefore, chitin is considered a valuable renewable natural material after cellulose.

Chitosan results from alkaline deacetylation of chitin, and the composition of chitosan may vary according to the degree of acetylation (DA) and the molecular mass. For chitosan, DA value is between 40 – 98% and presents the fraction of acetyl-glucosamine units [27,104,106,119].

Chitosan is characterized by nontoxicity, biodegradability, biocompatibility, bio-functionality, film-forming capacity, antimicrobial and antioxidant activity. All these features make chitosan an excellent material suitable for encapsulation and delivery of bioactive compounds.

The amino acids from the chitosan structure are water-insoluble, positive charged and only soluble at pH < 6. Therefore, chitosan must be dissolved in an aqueous solution with one up to 3% of acetic acid.

Another solution for making chitosan water-soluble is to modify its structure using chemical processes to introduce hydrophilic functional groups, such as carboxymethyl, dihydroxy ethyl, phosphoryl or sulfuryl. Some examples of derived chitosan biopolymers with enhanced

solubility are carboxymethyl chitosan, dicarboxymethyl chitosan, N-sulfuryl chitosan and 5-methyl pyrrolidinone chitosan [27,106].

The presence of amino acids is important also because it gives chitosan the cationic character and offers the possibility of using crosslinking agents to form stable matrix products with enhanced release properties. The crosslinking agents include at least two reactive functional groups facilitating the formation of bridges between polymeric chains. The most common crosslinking agents used with chitosan are dialdehydes like glyoxal and glutaraldehyde. However, these materials limit chitosan applications, especially those for food products, due to their toxicity [27].

Well tolerated crosslinkers produce ionically crosslinked microparticles or hydrogels; since they are evaluated as biocompatible, their network is non-permanent and includes reversible links. Compared to covalently crosslinked chitosan, the ionic ones present a higher swelling sensitivity to pH changes, extending their potential applications.

Marine extracts

Seaweed represents an abundant and almost unlimited source of biopolymers. Carrageenan and alginates are the most used marine extracts, suitable as wall materials in the encapsulation process [128].

Alginate is a family of linear anionic polysaccharides, and it consists of 1,4-linked α-L-guluronic acid (G) and β-D-mannuronic acid (M) residues. Commercially alginates are produced from brown algae by aqueous alkaline extraction [104,106,128].

The alginate biopolymers are hydrophilic, non-toxic, biodegradable, biocompatible and have a low price. The most common types of alginates used for microencapsulation are sodium alginate and calcium alginate because they are easy to produce, are non-toxic and biocompatible, and their acquisition price is relatively low [104].

The limitations of alginate as an encapsulating agent are the low encapsulation efficiency due to leaching during preparation and the rapid dissolution that determines a burst release in the intestinal pH [119].

Sodium alginate has bioadhesive, gelation and pH-responsive properties, which favours its use for drug delivery. Hydrogels can be formed with sodium alginate in the presence of multivalent cations like Ca^{2+}, Zn^{2+}, Ba^{2+} or Fe^{3+}. Alginate beads are produced by directly dripping the solution with sodium alginate into calcium chloride solution ($CaCl_2$). The obtained particles have a spherical aspect, and their size depends on the solution concentration and the dimensions of the initial extruded droplet [106,119].

The mixture of alginates with other biopolymers can enhance the encapsulation efficiency and the sustained release of sensitive compounds, such as vitamins or antioxidants. The addition of chitosan to alginate will improve the stability and the release because alginate dissolves fast in intestinal pH or the presence of sodium ion, but chitosan is insoluble in these conditions [104,119].

Carrageenan is an algal extract and belongs to a family of linear high molar mass sulphated polysaccharides. The biopolymeric chains include alternating (1,3)-linked β-D-galactopyranosyl and (1,4)-linked α-D-galactopyranosyl units. There are three types of carrageenan commercially available, namely k-(kappa), ι-(iota), and λ-(lambda).

Carrageenan has pseudoplastic gel formation capacity, and in the food industry is used as a thickener.

The source of carrageenan is represented by red seaweeds – *Rhodophycae*. Variations of carrageenan appear because of the differences caused by the types of seaweeds and by the processing and blending conditions [128].

2. Proteins

The natural proteins used as wall materials are macromolecules composed of long linear chains of amino acids linked by peptide bonds. The composition of protein polymers includes up to 20 amino acids that determine a wide variety of sequences and, thus, different sizes, structures, and functions [114,126,128,129].

Proteins are characterized by film-forming, gelation, foaming emulsification, and water capacity. Also, proteins are GRAS materials and have high nutritional value and are often used

for food and pharmaceutical applications. Proteins are insoluble in acidic conditions; however, they can dissolve easily at alkaline pH. Proteins can also be modified physical, chemical, and enzymatically to obtain protective materials with specific properties. The films produced by proteins are brittle; however, the addition of certain plasticizers improves the viscoelasticity of the films. Some common bioactive microencapsulated with proteins are fats, oils, fatty acids and flavours [104,126].

According to their nature, the protein-based wall materials are divided into two groups: proteins obtained from animal products and proteins obtained from plant products [104,129].

Plant-based proteins

Different plant sources or their waste products are used to obtain plant-based proteins. Compared to the animal proteins, the vegetal ones are considered less allergenic, less expensive, and have excellent hydrophobic character. Therefore, for these reasons, plant-based proteins present more interest [104,129].

Gluten can be found in significant amounts in wheat, rye, and barley and includes a mixture of two protein groups: gliadin and glutenin. The encapsulating agents prepared with gluten present a shiny surface and have good resistance to water under certain conditions. Although gluten is insoluble in water, it can be purified by removing the starch from its composition. To improve the film properties of gluten can be used specific crosslinking agents and thermal treatments. A widespread application for gluten is represented by the bakery products [126,128].

Peas are an economical source of starch, fiber and proteins. The pea protein isolate is extracted from pea seeds and represents about 20 – 30%. In their structure can be found 65 – 85% globulins, and the rest includes albumins and glutelins. The main proteins from glubulins are legumin, vicilin and convicilin. Pea isolates form good encapsulating agents and packing as they are characterized by surface activity, structure-forming and emulsifier properties, hydrophilic character and stability to high temperature [114,129].

Soy is an important staple food product cultivated worldwide due to its nutritional value and low price. The composition of soybeans includes around 40% of protein, from which 50 up to 90% are glycinin and conglycin. The isolates of soy protein used for human consumption are

obtained from defatted soy flour. Soy protein isolate is insoluble in water and has remarkable physicochemical properties. It also exhibits surface activity, structure and gel formation, emulsifying, surfactant, fat absorption, water binding, and oxidation resistance properties that makes them suitable for encapsulation delivery system. Soy proteins present some limitations, such as losing their functionality in the case of excessive denaturation and aggregation, and their solubility is strongly dependent on pH, heat treatment, or the presence of compounds like salts. [104,114,126,129]

Zein is extracted from corn, one of the most important cereals. In the composition of zein are found four major proteins, namely α, β, γ, and δ-zein. Zein owns some good characteristics for its use as packaging systems, such as biodegradability, biocompatibility, and self-assembly capacity. Regarding the solubility of zein, this protein is soluble in ethanol and insoluble in water. Due to its properties, zein can be used for pharmaceutical and food applications [104,126,129].

Animal-based proteins

The animal-based proteins are obtained from organs, such as muscles, secretions of animals, fish or insects, eggs and milk [129].

Collagen represents the most used raw material for packaging from the class of biopolymers. It is a hydrophilic protein and is formed of three helical polypeptide chains. However, the helical structure brings the disadvantage of complex degradation; therefore, it is necessary to make a pre-treatment. The protein films produced by collagen are excellent barriers in the absence of humidity because, with the increase of moisture, the protection offered by collagen is decreasing [126,129].

Gelatine is a denatured protein of animal origin obtained from collagen through partial acid or alkaline hydrolysis or enzymatic or thermal degradation. The commercial gelatine forms are gelatine A, extracted from porcine, bovine and fish skin (acid hydrolysis), and type B, from porcine, bovine and fish skin bones (alkaline hydrolysis). Gelatins present important characteristics for encapsulation, such as biocompatibility, biodegradability, non-toxicity, water retention, film formation, emulsifying properties and anti-carcinogenicity. Another interesting particularity of gelatine is that melt when heated and solidify when cooled. The

most significant limitation of gelation is represented by the high solubility in water, which determines the fast release of active compounds in aqueous solutions.

The films produced by gelatine are transparent or slightly yellow, almost tasteless, and odorless, oxygen impermeable, and thermoreversible, with a similar melting point to the human body temperature, making these films suitable for pharmaceutical products. The food industry uses gelatine with predilection because of its relatively low price and its capacity to form hydrocolloids [104,114,126,128,129].

Caseins are the most predominant phosphoproteins found in milk and are produced from skim milk. These milk proteins include α_{s1}-casein, α_{s2}-casein, β-casein, and κ-casein, in the proportion 4:1:4:1and they differ in their net charge, hydrophilicity, and metal binding. The specific properties of caseins, emulsifying characteristics, low viscosity in solutions, slight flavor and high nutritional value recommend them as delivery system for encapsulation. Also, caseins present high heat stability, and they do not coagulate because of heat. Casein delivery systems are preferred for hydrophobic compounds, especially from the food industry[104,114,126,128,129].

Whey is a by-product of cheese obtained after the coagulation of caseins and includes four proteins α-lactalbumin, β-lactoglobulin, immunoglobulins, and serum albumin. From the manufacture process of whey, the following products are isolated: whey powders, whey protein concentrate, whey protein isolate, lactalbumin, and some whey protein fractions. Whey proteins are globular proteins that have the particularity of being soluble in their native forms in the ionic environment of milk, almost independent of pH value. However, whey becomes insoluble in two different situations at its isoelectric point, at pH around 5, and at temperatures higher than 70 °C, when it forms thermally irreversible gels. These proteins have good surface activity, low viscosity, emulsifying properties, and capacity to form hydrogels. Whey protein films are usually without flavor and can be transparent or translucent. Due to their properties and nutritional value, whey proteins are used for many food applications [104,114,126,128,129].

3. Lipids

Lipids are insoluble in water and include a variety of products found in nature like fats, oils, waxes, and phospholipids. Comparing to other classes of encapsulating agents, lipids have

excellent emulsifying capacity and film formation, present good stability and as well, are less toxic. Therefore, due to their properties, lipids are common encapsulating materials used for pharmaceutical products [104,128,129].

Fatty acids are divided into two classes of saturated and unsaturated acids with variable length, and fatty alcohols are characterized by a hydroxyl group that replaces a carboxylic one. The production of fatty acids usually involves the hydrolysis of triglycerides of naturally occurring fats and oils. Through reduction of fatty acids are obtained fatty alcohols.

The short aliphatic chain fatty acids are miscible in water, but with the increase of the chain length the water-solubility decreases. The fatty acids tend to auto-oxidize at room temperature. The fatty alcohols are characterized by emulsifying properties and can behave as non-ionic surfactants [128].

Glycerides include triglyceride, diglyceride, and monoglyceride, which are compounds with three, two, or only one fatty acid chains, covalently bonded to a glycerol molecule by ester linkages. The main component found in animal fats and vegetable oils is triglyceride. The other two glycerides, mono- and diglycerides are obtained in the same way is triglyceride; however, these two can also be prepared by synthesis. All glycerides present hydrophobic character, and only di- and monoglycerides have emulsifying properties [128].

Waxes are defined as esters of fatty acids isolated from both animal and plant products. The most common waxes used as wall materials are Beeswax, Carnauba, and Candellia wax [128].

Beeswax is obtained from the secretion of young honeybees that construct the honeycomb. The main components of beeswax are triacontylpalmitate (~75%), triacontylcerotinate (~10%), and paraffin (~15%). Its colour can range from white to brownish and its specific melting point is around 62–64°C. Beeswax can be used with most other types of waxes and oils, fatty acids, glycerides, and hydrocarbons [128].

Carnauba wax is produced by the leaves of palm trees especially from Brazil, and it is one of the hardest natural waxes. It is characterized by a melting point of 78–85°C and is compatible with the same materials as beeswax [128].

Candellila wax is extracted from the leaves of the Candelilla shrub, which can be found in the northern Mexico. This wax is less hard than carnauba wax. Its colour may vary from light

brown to light yellow. Candelilla wax is soluble in wide variety of organic solvents, and melts at a temperature of about 67–79°C. As well, this wax presents a high compatibility with other materials such as all vegetable and animal waxes, fatty acids, a large variety of natural and synthetic resins, glycerides, and hydrocarbons in some proportions [128].

Phospholipids are present in all animal and plant cells, however, commercially, are extracted from the egg yolk and soybean oil, or from fat milk globular membrane. In their composition phospholipids include two long chain of fatty acids. Lecithin is the most abundant phospholipid, and its base is choline. Phospholipids have amphiphilic character, and therefore are good emulsifying and dispersing agents. In mixture with water, phospholipids aggregate or self-assemble into well-organized structures and bilayers. If during the mixing process it is applied energy, the bilayers will produce liposomes, in which the aqueous interior is separated by one or more phospholipid bilayers from the aqueous exterior. According to the production method, the size of the liposomes may vary. One limitation of liposomes is their stability that after a period of time is lost, since they are not thermodynamically stable [128].

2.3.5. Controlled release function

One of the most important microencapsulation features is represented by the controlled release function as it improves the effectiveness and applicability of products, reduces the use of high doses, and facilitates the delivery of sensitive compounds [130,131].

Controlled release is defined as a process by which the core material is released under the influence of a stimulus, in a particular place, after a certain period and with a specific speed [27,29,132].

The study and production of delivery systems for controlled release imply a multidisciplinary scientific approach and contribute to the development of new food, pharmaceutical, health care or cosmetic products.

Controlled release is classified as delayed if the aim of the process is to delay the release of the core until the right place and/or time and sustained if the aim is to control the release rate [130].

Microencapsulation is a promising technology suitable for developing controlled release systems and already used to produce microparticles for the delivery of both drugs and food ingredients.

Compared to the conventional dosage forms, which usually implies macromolecules as carriers, the delivery systems with microparticles offer a series of advantages, such as:

- effective protection of the encapsulated active agent against degradation.
- reduced toxicity.
- an easy administration (compared to traditional forms).
- the possibility to accurately control the release rate of the incorporated core over specific periods.
- targeted and pre-programmed drug release, which match the therapeutic needs of the patient.
- separation of ingredients to avoid interactions and ensure compatibility (reduced reactivity).
- more homogenous distribution in the physiological environment.
- improved stability, especially for pharmaceutical products [123,133].

As the microparticles are wholly isolated from the external environment, they can remain safely shielded over time until the release of the core material is triggered. The protection offered by the encapsulating agent must ensure structural rigidity and to keep the microparticles stable up

to the proper time of the core release. Therefore, the features of the encapsulating agent can easily influence the release process.

Using microencapsulation, the reactivity with the external environment can be avoided, and the release of the encapsulated product can be managed in a controlled way that facilitates a reduced transfer rate from the core to the environment. As a result, the final product is easier to handle, the smell and the taste are masked, and the losses caused by poor stability are avoided.

The encapsulated materials and their interactions determine the main factors affecting the release rates; therefore, the release process can be influenced by:

- core material: morphology, size, physicochemical properties.
- covering parameters: density, crystallinity, solubility, pre-treatments, the addition of cross-linking agents.
- microparticles characteristics: size, morphology, wall thickness, number of coating layers.
- external environment factors: temperature, pH, pressure, moisture.

Different mechanisms can determine the release and delivery of the core material, and the mechanisms are classified according to the physicochemical phenomena associated. The major release mechanisms are described below:

Diffusion controlled system

The release trough diffusion is the most common mechanism, and it implies the infiltration of the wall material with the dissolution fluid. The diffusion mechanism can be divided into the reservoir and matrix system.

For the reservoir system, the core slowly diffuses through the polymeric wall. In the matrix system, the release burst through the pores of the polymer as the core is homogenously scattered in the polymeric wall. In the reservoir system, the release rate depends on the properties of the core and wall material (permeability, size, thickness), and in the case of a matrix system, the release rate is influenced by the core diffusion trough the coating [130,131].

Degradation controlled system

Release by degradation happens only if the wall materials (lipids or proteins) can be degraded by enzymes, such as proteases or lipases [130,131].

Solvent controlled system

The solvent activated release is triggered by swelling or osmotic pressure. The swelling phenomena occur when the polymer matrix and the medium are thermodynamically compatible, so the polymer swells and absorbs the fluid from the fluid, like in the case of hydrogels. A concentration variation between the core found inside and outside the membrane determines osmotic pressure, leading to a solvent flow to the inside of the microparticles and the expulsion of the saturated solution [130,131].

pH controlled system

The release of the active core can be made at a specific pH value that changes the solubility of the wall material [130,131].

Temperature controlled system

A change of temperature determines the release of the core. The release can be thermally activated when the wall material collapses because of increasing the temperature, or thermo-sensitive if it is registered a fluctuation of temperature [130,131].

Pressure controlled system

When pressure is applied to the surface of microparticles, therefore on the wall material, the release of the core is activated [130,131].

For the evaluation of each release mechanism, several models can be used, as follows:

1. statistical methods – exploratory data analysis method with repeated measures design, multivariant approach (MANOVA).
2. model dependent methods – zero order, first order, Higuchi, Korsmeyer-Peppas, Hixson-Crowell, Hopfenberg, Baker-Lonsdale, Gompartz, and Weibull models.
3. model-independent methods – difference factor (f1), similarity factor (f2) [114,134–136].

The next section will analyse some of the model-dependent methods and their mathematical models that describe the dissolution profiles.

The mathematical modelling is used to understand the release phenomena of the active core materials, to optimize and increase the efficiency of the microparticles. The experimental data must be adjusted to different kinetic models, and the evaluation of the fitting degree can determine which model describes the best release of the bioactive ingredient.

The main objectives of the mathematical models are:

- Design of new drug delivery systems.
- Prediction of the drug release rate form and drug diffusion behaviour without excessive experiments.
- Optimization of the release kinetics.
- Prediction of the effect of design parameters such as shape, size, and composition on the overall drug release rate.
- Accurate prediction of drug release profile, with the aim of improving overall therapeutic efficacy and safety of the drug [137].

The Zero-order model is applicable for the dissolution of compounds that do not disaggregate, and the release occurs slowly with a constant release rate. In this model, the drug release rate is independent of its concentration. The main equation of the zero-order model is:

$$Q_t = Q_0 + K_0 \cdot t \qquad \textbf{(2.1.)}$$

In this equation, Q_t defines the amount of active compound released or dissolved, Q_0 represents the initial amount of drug from the solution (and in most cases equals to zero), K_0 is the zero-order release constant, and t is the time. The plot representation of a zero-order model includes the cumulative amount of released drug (%) *versus* time [134,136,138,139].

Zero-order is considered an ideal model of release for pharmaceutical products because, during the entire delivery time, the drug level in the blood would remain constant.

Some examples of pharmaceutical products that require the zero-order model for the release of active compounds are transdermal systems, matrix tablets with low soluble drugs, coated forms or osmotic systems, such as antibiotics, heart and pressure medicines, pain control and antidepressants [134,138,139].

The first-order model describes the release from systems in which the release rate is dependent on the concentration. The equation used for the first-order model is:

$$Q_t = Q_0 \cdot e^{-K_1 \cdot t} \qquad \textbf{(2.2.)}$$

Where Q_t defines the amount of active compound released at time *t*, and K_1 is the first-order release constant. The experimental data of first-order model is plotted as log cumulative (%) of drug remaining *versus* time.

The first-order model can be used for different applications, such as pharmaceutical products that contain water-soluble drugs in porous matrices [136,137,139].

Higuchi model dates from 1961 when Higuchi proposed a complex mathematical model that describes the release from insoluble matrix systems. Several hypotheses underlie this model, such as: (1) the initial drug concentration from the matrix is much higher than the drug solubility, (2) the drug diffusion takes place only in one dimension (the edge effect must be negligible), (3) the drug particles are much smaller than the system thickness, (4) matrix swelling and dissolution are negligible, (5) the drug diffusivity is constant, and (6) perfect sink conditions are always attained in the release environment [136,137,139].

The systems that follow the Higuchi model describe the release as a process dependent on the square root of time, based on Fickian diffusion. The equation used for the Higuchi model is:

$$\boldsymbol{Q_t = K_H \cdot \sqrt{t}} \qquad \textbf{(2.3.)}$$

Q_t is the amount of active compound released at time *t*, and K_H is the Higuchi constant, which reflects the design variables of the system. The Higuchi model plot includes the cumulative drug released (%) *versus* the square root of the time.

Higuchi model can be applied for some transdermal system and matrix system of tablets with water-soluble drugs [136,137,139].

The Baker-Lonsdale model was developed in 1974 from the Higuchi model and characterizes the release of drugs from a spherical matrix. The equation related to this model is:

$$\boldsymbol{f_1 = \frac{3}{2}\left[1 - \left(1 - \frac{Q_t}{Q_\infty}\right)^{2/3}\right] - \frac{Q_t}{Q_\infty} = k \cdot t} \qquad \textbf{(2.4.)}$$

In this complex equation, Q_t represents the amount of drug released at time *t*, Q_∞ the amount of drug released at an infinite time, and *k* is the release constant that corresponds to the slope. The slope of the plot of f_1 with respect to time gives the release constant *k*.

The mathematical model of Baker-Lonsdale is used to linearize release data from formulations of microcapsules or microspheres [136,138,139].

Korsmeyer – Peppas model dates from the year 1983, and is used to describe the release of drugs from polymeric systems. The specific equation of this model is presented below:

$$Q_t / Q_\infty = K_K \cdot t^n \qquad \textbf{(2.5.)}$$

In this equation, Q_t / Q_∞ is the fraction of released drug at time t, K_K is the Korsmeyer – Peppas constant, and n is the release exponent. The plot for Korsmeyer – Peppas model contains the log cumulative (%) *versus* log time. To validate this model first, 60% of the data must fit the release profile [135,137,139].

The numerical value of the n exponent offers structural and geometric information about the released form and defines the release mechanism. The interpretation of the release exponent, *n*, is presented in Table 2.16.

Table 2. 16. The release exponent, n, from Korsmeyer – Peppas model [27,138].

***n* - release exponent**	**Form**	**Transport mechanism**	**Rate as a function of time**
< 0.5	Film	Fickian diffusion	$t^{-0.5}$
0.5 < *n* < 1.0		Anomalous transport	t^{n-1}
1.0		Case-II transport	Zero order release
> 1.0		Super case-II transport	t^{n-1}
< 0.45	Cylinder	Fickian diffusion	$t^{-0.55}$
0.45 < *n* < 0.89		Anomalous transport	t^{n-1}
0.89		Case-II transport	Zero order release
> 0.89		Super case-II transport	t^{n-1}
< 0.43	Sphere	Fickian diffusion	$t^{-0.57}$
0.43 < *n* < 0.85		Anomalous transport	t^{n-1}
0.85		Case-II transport	Zero order release
> 0.85		Super case-II transport	t^{n-1}

If the n exponent of the Korsmeyer-Peppas equation has a lower value than 0.43, it means that the system works under Fickian diffusion: case I transport). However, if 0.43 < n < 0.85 the transport mechanism is anomalous, and the diffusion will determine a swelling release. In the case of n = 0.85 the transport is case II, and for higher values than 0.85 it is the super case II transport.

Hixson-Crowell model is known since 1931 when Hixson and Crowell acknowledged that the regular particle area is proportional with the cubic root of its volume. The derived equation that describes the model is:

$$\boldsymbol{Q_0^{\frac{1}{3}} - Q_t^{\frac{1}{3}} = K_S \cdot t} \quad \textbf{(2.6.)}$$

Q_0 is the initial amount of the drug in the pharmaceutical dosage form, Qt is the amount of the drug in the pharmaceutical dosage form at time t, and K_S is the Hixson-Crowell constant that incorporates the surface-volume relation. The plot for this model is made between the cube root of drug (%) remaining in the matrix *versus* time.

The Hixson-Crowell model is applicable for pharmaceutical dosages like tablets, where the dissolution occurs in parallel planes with the drug surface if the tablet dimensions diminish proportionally, in such a way that the initial geometrical form keeps constant all the time [135–137,139].

Weibull model is suitable for dissolution processes, like different pharmaceutical dosage forms. The main equation of the model is described below:

$$\boldsymbol{Q_t = Q_\infty \left[1 - e^{-\left(\frac{t-t_0}{\tau_d}\right)^{\beta}}\right]} \quad \textbf{(2.7.)}$$

Where Qt is the amount of drug released at time t, and Q∞ the amount of drug released at an infinite time. The lag-time of the dissolution is t_0 (min) and normally equals zero. β defines the shape parameter of the curve, and τd is the time (min) when 63.2% of the drug has been released. Therefore, for β = 1, the curve has an exponential profile, for β > 1 sigmoidal with a turning point, and for β < 1, the release shows a steeper increase than in the case of β = 1. It results from the previous equation, being *M= Qt/ Q∞*:

$$\boldsymbol{ln\,(ln\,(\frac{1}{(1-M)})) = Z + \beta \cdot ln\,(t)} \quad \textbf{(2.8.)}$$

$$\boldsymbol{\tau_d = e^{(Z/-\beta)}} \quad \textbf{(2.9.)}$$

The plot of the Weibull model includes ln(ln(1/(1-M))) *versus* time. This model is beneficial for the comparison of different release profiles from matrix-type microparticles [138,139].

Hopfenberg model is used to compare the drug release from the surface of eroding polymers, as the surface area remains constant during the degradation process. This model characterizes the release from spheres, slabs and cylinders displaying heterogeneous erosion [138,139].

The equation that describes the Hopfenberg model is:

$$\frac{Q_t}{Q_\infty} = 1 - \left[1 - \frac{k_0 \cdot t}{C_0 \cdot a_0}\right]^n \quad \textbf{(2.10.)}$$

In this equation, Q_t is the amount of drug released at time t, and Q_∞ is the total amount of drug dissolved when the pharmaceutical dosage form is exhausted; therefore, the Q_t/Q_∞ is the fraction of dissolved drug. k_0 represents the erosion rate constant, C_0 is the initial concentration of drug in the matrix, and a_0 defines the initial radius for a sphere or cylinder or the half-thickness for a slab. The factor n can be 1, 2 or 3 for the case of a slab, cylinder, and sphere, respectively [138,139].

This model was readapted in 1998 by El-Arini and Leuenberger, and the equation was changed to accommodate the lag time (l) at the beginning of the drug release from pharmaceutical form, as follows [138,139]:

$$\frac{Q_t}{Q_\infty} = 1 - [1 - k_1 \cdot t \cdot (t - l)]^n \quad \textbf{(2.11.)}$$

In the readapted equation, k_1 equals $k_0/(C_0 \cdot a_0)$. This release system presumes that the rate-limiting step of drug release is the erosion of the matrix itself and that time-dependent diffusional resistances internal or external to eroding matrix do not influence it. Hopfenberg model is suitable for oily spheres using data from the composite profile and displays a site-specific biphasic release kinetics [136,138,139].

Gompertz model is an exponential model that describes in vitro dissolution profiles:

$$X(t) = X_{max} \cdot exp\left[-\alpha \cdot e^{\beta \cdot log\, t}\right] \quad \textbf{(2.12.)}$$

For this equation, X(t) represents the percentage of the dissolved drug at time t, and X_{max} is the maximum dissolution. The element α is the undissolved proportion at time t, described as site or scale parameter, and β is the dissolution rate per unit of time defined as shape parameter [135,136,139].

The plot release profile for this model presents a sharp increase at the beginning and after converges slowly to the asymptotic maximal dissolution. The Gompertz model is usually selected for comparative studies of drugs that have good stability and intermediate release profiles [135,136,139].

2.4. Spray-drying method

Microencapsulation by spray-drying method is a unit operation of atomization, commonly used in various industries such as food, pharmaceutical and cosmetic fields. During the spray-drying process, the feed is rapidly dried with hot gas and transformed into a powder [29,98,110,140].

The feed can be a solution, an emulsion or a dispersion and includes the mixture of the polymer and active compounds. The hot gas represents the heating medium which is usually air. It can be used nitrogen too, instead of air, if the feed liquid is a flammable solvent (ethanol, acetone) or the product is oxygen sensitive. However, nitrogen increases the operational cost of spray-drying and the tendency is to avoid it also because of environmental and safety reasons [102,141].

The drying temperature can get until 220 °C, and the exposure time of the feed solution is only of few milliseconds, therefore, the temperature inside the microparticles, should not be higher than 80 °C [142].

In 1930 were produced by spray-drying the first microparticles suitable for food industry, namely flavour microparticles with arabic gum as an encapsulating agent. Since then, spray-drying has been used successfully to encapsulate food ingredients, and over time it became the most widely used microencapsulation method for food products. Among the bioactive compounds already encapsulated with the spray-drying technique are enzymes, vitamins, polyphenols, lipids, probiotics and flavours [98,113,140].

After spray-drying was implemented on the industrial level, many perishable food products were preserved by simply removing their water content and converting them into stable instant food powders. The practice of water removal can help ensure microbiological stability, avoid the risk of chemical/biological degradation, reduce storage capacity and cost, and it helps develop instant soluble products [98].

Some common examples are dairy products (e.g. skim milk, whole cream milk or whey powders), fruit and vegetable powders, and protein products (e.g. egg, enzymes, or dairy powders).

Spray-drying is characterized as a simple, rapid, flexible, and low-cost method that produces stable microparticles. Other outstanding advantages of spray-drying are ease of use of the equipment, increased product stability, reduced product volume and weight which determines a reduced storage capacity too, and ease of handling of the final product.

The spray-drying is a continuous process that includes four consecutive stages: preparation of the feed solution, atomization, droplet formation and recovery of the powder microparticles [98,140].

The first stage is the preparation of the feed solution or dispersion, and this stage is influenced by the nature and properties of the core material and the encapsulating agent. First, the encapsulating agent is dissolved in the selected solvent, using heating when necessary. After, the core material is simply dissolved in the solution that contains the encapsulating agent if the core is water-soluble. Otherwise, it must be used an oil-in-water emulsion for oil-soluble core materials [140].

Furthermore, to facilitate the encapsulation process, the wall materials used for spray-drying should be characterized by good film-forming and emulsifying properties, high solubility in water, low viscosity for high levels of solids, low hygroscopicity, and low cost. Also, the encapsulating agent should confer protection over time and controlled release for the core material. The most common agents are proteins and carbohydrates, and when one single material does not meet the conditions previously mentioned, mixtures of materials are recommended [26,140].

The second stage of the spray-drying is represented by the atomization process, in which an atomizer transforms the liquid feed into microdroplets. The atomizer systems can be centrifugal disk atomizer, pneumatic and pressure nozzles. The first type, the centrifugal disk atomizer is used in the case of high-capacity processes because it is a flexible system, easy to handle which requires low maintenance. The second type, the pneumatic nozzle is best suitable for small-size processes since it is less efficient, and the high use of compressed air increases the operating costs. And the last type, the pressure nozzle is recommended for dry high-viscosity solutions [98,140,141].

The function of the atomizer is to confer uniformity and homogeneity of the spray, helping obtain a suitable size distribution for the powder microparticles. Therefore, during the atomization stage, a high heat-transfer surface must be obtained between the dry air and the droplet to optimize heat and mass transfers. In emulsions, the selection of a good atomizer is more complicated since some emulsions have high viscosity. Another particular case is the solid core materials because they can easily cause a blockage to the atomizer system due to large particle size or too high viscosity [98,140].

The third stage of the spray-drying process is the droplet formation, which occurs at the contact of the liquid droplets with the hot air that determines the evaporation of the solvent.

As the droplets get in contact with the hot air it starts the drying process which can work in co-current or counter-current according to the atomizer type. For co-current drying, the feed solution is sprayed in the same direction as the flow of hot air through the equipment, and this type of drying is usually applied for ingredients used in foods and nutraceuticals.

However, for counter-current the feed is sprayed in the opposite direction of the flow of hot air, which makes the dry product to be exposed to high temperatures, and in the case of thermosensitive products this type of drying is not recommended. As well the energy consumption is much higher in counter-current than in co-current [98,140].

The hot air used for drying reaches temperatures from 110 till 220 °C (inlet temperature), and with such high temperature the evaporation occurs almost instantly. Usually, the temperature reached by the dried particles is of 50 – 80 °C (outlet temperature), which limits thermal degradation for most core materials.

When the liquid phase (droplets) and the gas phase (hot air) get in contact, it is established a balance between the temperature and the vapour partial pressure. Thus, the heat transfer takes place out from air towards the product due to temperature difference. However, the water transfer is carried out in the opposite direction because of the vapour pressure difference.

During drying, heat is transferred to the droplets and this helps vaporize the water from the wall material, moreover, it avoids the heat exposure to the core material. At this point the temperature of the droplets increases until it reaches a constant value. Then the evaporation is carried out at constant values of temperature and water vapour partial pressure. As the droplet water content reaches a critical value, a dry crust is formed on the droplet surface, and when the particle temperature is the same to the air, the drying process is considered complete [98,140].

Parameters like feed concentration and temperature, flow rate, inlet and outlet temperatures can easily affect the efficiency of the encapsulation process.

The concentration and the temperature can modify the viscosity and the fluidity of the feed, which in turn affects its fluidity and capacity to be homogeneously sprayed. With the increase of feed temperature, the droplet size decreases, although too high temperatures can degrade heat-sensitive bioactive compounds.

The optimization of the feed rate has a significant influence on sprayed droplets because through atomization must be ensured a certain drying level for all feed solution.

The difference between the inlet and outlet temperatures is directly proportional to the microcapsule drying rate and the final water content.

A lower inlet temperature provokes a low evaporation rate, which causes the formation of microparticles with high density membranes, high water content, poor fluidity, and tendency of agglomeration. As well, a higher inlet temperature is not beneficial because causes excessive evaporation and cracks. Premature release or loss of encapsulated cores are triggered by the cracks that appear on the surface of microparticles.

The value of the outlet temperature can be controlled by the feed rate, after the inlet temperature is set up.

The fourth stage of the spray-drying process is the recovery of the microencapsulated powder. First the dried microparticles are redirected from the drying chamber to a cyclone by the air stream and only after the microparticles are collected [29].

The way of recovering the powder differs according to the type of spray drier. Therefore, the powder can be separated from drying air using a cyclone or the dense particles are collected from the bottom of the drying chamber while the fine ones pass through a cyclone to be separated from the humid air. As well, some equipments have extra features such as bag-filters, to remove the finest powder, and chemical scrubbers, for the remaining powder.

In the end of the encapsulation, the collected powder must be stored until further use. To ensure storage stability it is important to take into consideration the materials used as encapsulating agents, the experimental parameters of the equipment and the storage environment because all of them can affect the stability of the microparticles. For the storage environment must be considered factors like temperature, humidity, and light. In most cases, the best results in terms of stability were observed in the case of low temperature and water activity, and absence of light[140].

The obtained microparticles from a spray-drying process are matrix type, have spherical aspect, can be compact or hollow and can present some degree of shrinkage. The characteristics of the dried particles are dependent on several factors like the feed composition, drying temperature conditions, the water and gas content. Furthermore, the size of the microparticles can be controlled if the spray-dryer has integrated a fluidised bed drier.

In Figure 2.9. the schematic representation of a common spray-dryer equipment is presented. The main elements that compose the equipment are a feed reservoir, a feed pump, an atomizer with the nozzle, a heater, a drying gas supplier, a drying chamber, a cyclone, a collecting vessel, a filter and an aspirator [110,143].

First the intake air is heated (1) and when it reaches the proper drying temperature the feed solution starts to be pumped up to the drying chamber with the help of a peristaltic pump (2). Next, the atomization process begins, and the solution is sprayed through the nozzle system (3) and directed to the drying chamber. When the liquid feed gets in contact with the hot air, the dried droplets start to form (4). After, the droplets dry very fast (5) and are pushed in the colleting vessel from where will be later collected (6) [143].

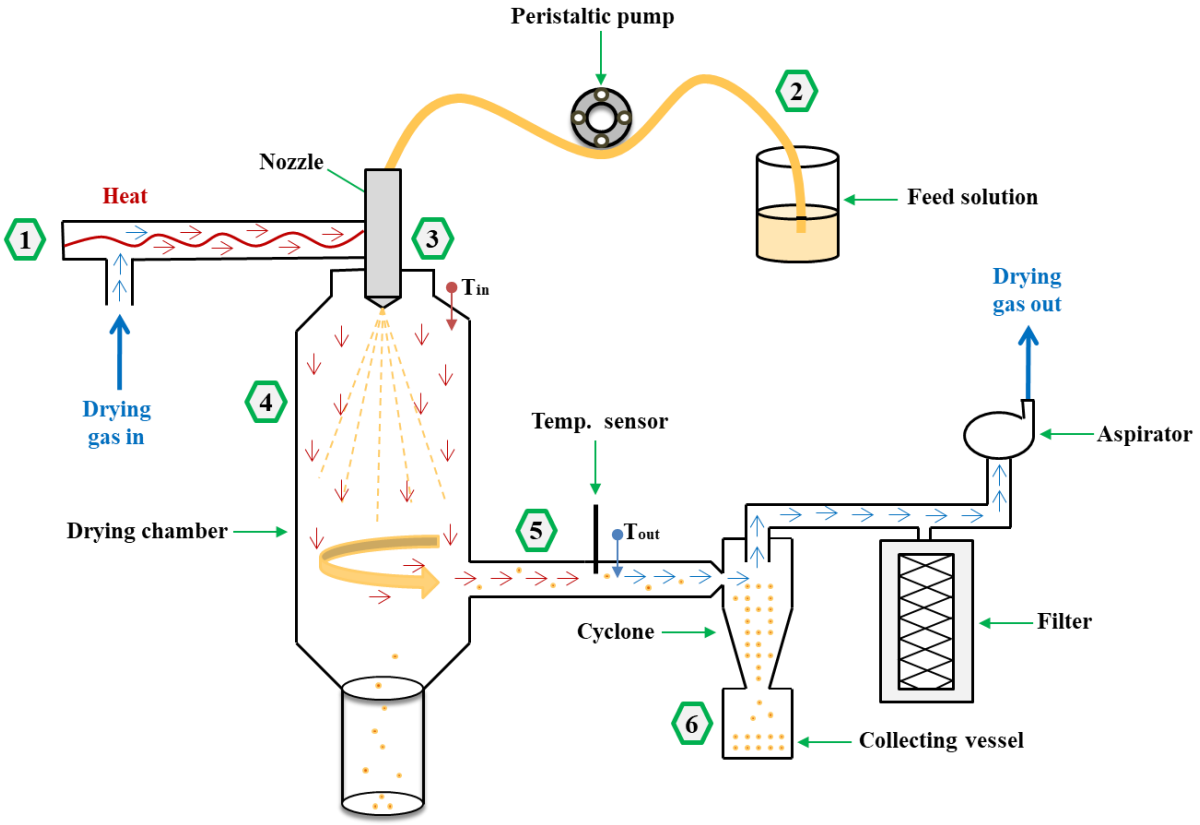

Figure 2. 9. Schematic representation of the spray-drying equipment and how the encapsulation occurs: (1) – intake air is heated, (2) – feed solution is pumped up, (3) – atomization process, (4) – droplet formation, (5) – droplet drying, and (6) – microparticles collecting.

Spray-drying is preferred instead of other microencapsulation techniques because it is considered a quick, reproducible, and cost-effective process. The microencapsulation process

occurs in a continuous batch that ensures high production rates and determines the low production cost [143].

The overall reduced cost of spray-drying technology is a significant advantage, especially for the food industry. Food products must have affordable prices for as many consumers as possible, which is not mandatory for the pharmaceutical industry [30].

The process is flexible because it can be adapted easily to other processing equipment commonly used for encapsulation.

In terms of materials, spray-drying can use different types of encapsulating agents, and the core can be both hydrophilic and hydrophobic.

As well the equipment is easy to scale-up and facilitates the handling and storage of the final powder. Obtained microparticles have a spherical shape with a diameter up to 300 μm, present good stability, and reach up to 90% of encapsulation efficiency [111].

Micron-size products in powder form are easier to incorporate in further formulations, can facilitate the targeted transport of sensitive compounds, and therefore, have more application possibilities [110].

However, spray-drying presents several disadvantages that can restrain the encapsulation process. One of the most significant drawbacks is represented by the relatively high operating temperatures, limiting the use of temperature-sensitive core materials. Furthermore, the spray-drying method registers moderate values of the product yield, as there are considerable losses caused by the material that remains stuck to the walls of the equipment. And another challenge is to control the size of the obtained microparticles that usually are non-uniform and tend to agglomerate [26,111].

The main parameters of the spray-drying equipment that should be maintained at an optimal level are gas flow rate of feed solution, the inlet and outlet temperature. And the optimal conditions for a spray-drying process are in fact "a compromise" between high air temperature, a high content of dissolved product in the feed solution and a soft pulverization flow that can avoid undesirable properties on the final products like expansion or cracks.

The performance of the spray-drying process can be evaluated by the determination of two percentual parameters, as follows, the product yield and the encapsulation efficiency.

The product yield (PY) measures the percentual ratio between the output and the input of materials and its mathematical equation is presented below:

$$\mathbf{PY}\ (\%) = \frac{(\mathbf{amount\ of\ recovered\ powder})}{(\mathbf{initial\ amount\ of\ solid\ materials})} \cdot \mathbf{100} \qquad \mathbf{(2.13.)}$$

The amount of recovered powder is represented by the mass of microparticles obtained after the microencapsulation process, and the initial amount of solid materials is the sum of the mass of the encapsulating agent and the mass of the core.

The encapsulation efficiency (EE) is defined as the percentual ratio between the amount of encapsulated material and the amount of released active material (Equation 2.14). To determine the amount of encapsulated material the difference between total material released and the amount of free material must be done.

$$\mathbf{EE}\ (\%) = \frac{(\mathbf{total\ encapsulated\ material\ released} - \mathbf{"free"\ encapsulated\ material})}{(\mathbf{total\ encapsulated\ material\ released})} \cdot \mathbf{100} \qquad \mathbf{(2.14.)}$$

During the microencapsulation process not all core material will get inside the microparticles, because part of the core mass remains “unentrapped” or at the surface of the microparticles. Therefore, to quantify the free core material the amount of encapsulated core right after the dispersion of the microparticles in the solvent must be measured.

2.5. An overview of the microencapsulation of B vitamins for pharmaceutical and food applications

The complex of B vitamins is a group of eight dietary essential micronutrients which includes vitamins B1, B2, B3, B5, B6, B7, B9 and B12. Each of these vitamins performs different physiological functions focused on growth, reproduction, and metabolism.

As part of everyday life, a regular intake of B vitamins must be ensured either through food diet or nutrient supplementation. However, in certain situations the specific intake, known as RDA (presented in Table 2.1.) cannot be achieved and thus vitamin deficiencies occur. Most common situations that trigger B vitamins deficiencies are digestive diseases that hinder vitamin assimilation, and improper food diets that lack variety of nutritious products or which are not sufficient in terms of quantity.

Low or improper vitamin deposits/stocks must be corrected as fast as possible to avoid further health complications. B vitamin deficiency treatment includes a new diet based on products enriched with vitamins, vitamin supplements, and medical treatment. Consequently, there is high need of pharmaceutical and food products with vitamin content which can be used for prevention and treatment as well.

Although, the use of B vitamins is limited because of their high reactivity to ambient conditions such as temperature, light, humidity, oxygen, which can easily provoke their degradation and loss of functional properties. Withal, the degradation and loss of stability of B vitamins may occur at any time during storage time, product manufacturing, or digestion process, therefore a solution to overcome low stability is emerged. As well, these micronutrients cannot be always provided from natural sources, therefore most of industrial applications favours the use of synthesized B vitamins.

The limitations of B vitamins can be overcome by the implementation of a system to preserve sensitive compounds. In this regard, microencapsulation technique has been commonly used to deliver and incorporate vitamins into different products.

Microencapsulation is described as the technology that permits the formation of protective physical carriers for various types of compounds, including the B vitamins, which by encapsulation are transformed into functional bioactive compounds.

Vitamins from B complex were encapsulated over the time for two reasons: first to ensure protection and stability, and second to sustain the release of the vitamins under controlled conditions.

Microencapsulation has been noted as a tool for the delivery of compounds often considered technically sensitive or unfeasible. Applying microencapsulation for vitamins brings several valuable benefits: increased stability and protection from external factors (temperature, oxygen, light), easy handling and storage, ensured organoleptic properties, improved bioavailability, and protection of reactions with other compounds.

Furthermore, another proof comes from a significant number of scientific publications available on Scopus data base regarding microencapsulation. Were considered all available studies published until 31st of December 2020.

Until 2020 was observed an exponential growth of published papers about microencapsulation in general and microencapsulation by the spray-drying method, as presented in Figure 2.10. The first two publications about microencapsulation are from the year 1960, and 989 publications were reached in the year 2020. The microencapsulation by spray-drying first paper appeared only in 1977, and in 2020, 452 publications are registered.

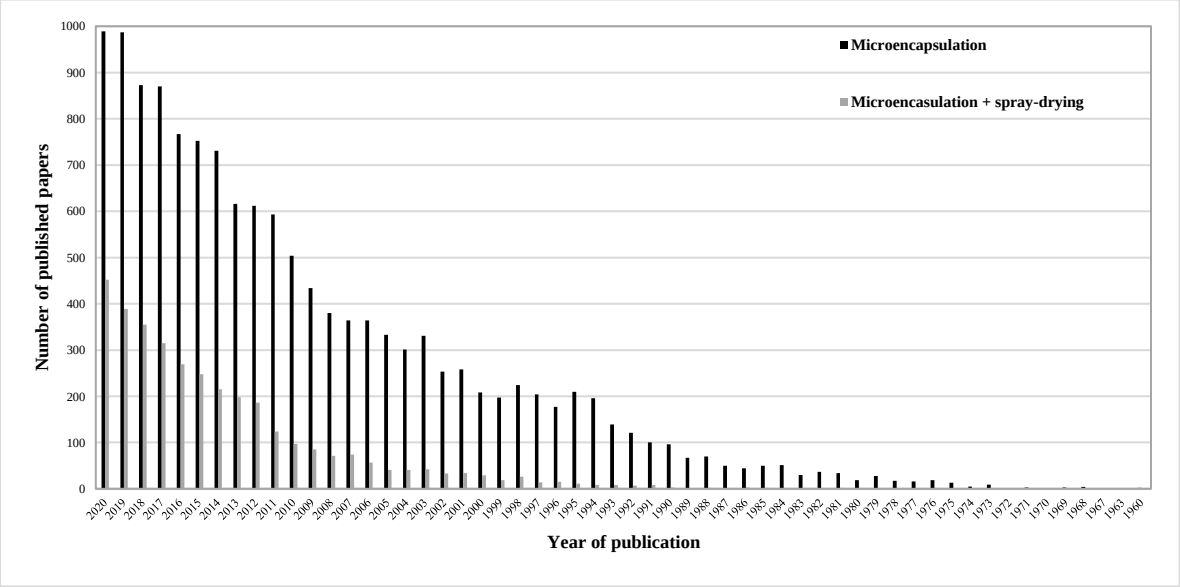

Figure 2. 10. Published papers on the database www.scopus.com, with the following terms “microencapsulation” and “microencapsulation + spray-drying” relative to the year of publication.

In Figure 2.11, it is shown that the occurrence of the publications about microencapsulation of vitamins is also exponential in time. The first work about the microencapsulation of vitamins was registered in the year 1971, and 20 years later, in 1991 appeared a work done using the spray-drying method. For the year 2020 a much higher number of publications can be observed, namely 160 for general microencapsulation of vitamins and 114 for the papers in which spray-drying method was chosen.

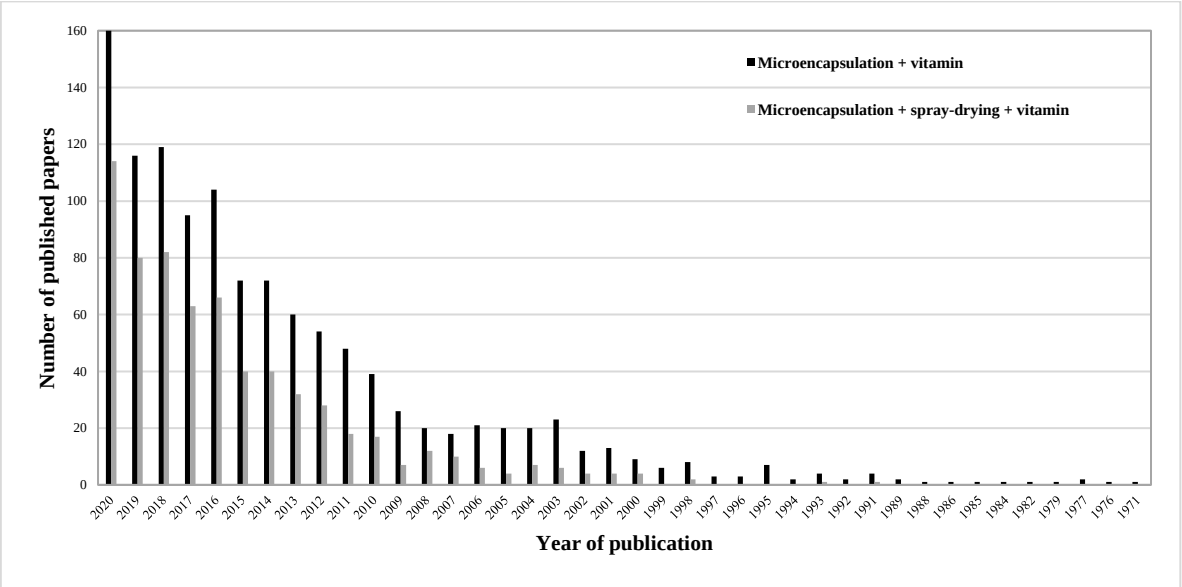

Figure 2. 11. Published papers with the following terms "microencapsulation + vitamin" and "microencapsulation + spray-drying + vitamin" relative to the year of publication, according to the database www.scopus.com

This subchapter includes an overview on microencapsulation of B vitamins, and a case study for the studies in which was used the spray-drying method. Thus, the evolution of publications related to microencapsulation of vitamins in general and microencapsulation of vitamins using the spray-drying method was analysed to understand the need of studying the current subject.

Recently, the demand on products with vitamin content has considerably increased due to multiple benefits of the application of these products in varieties of food and pharmaceutical formulations. Hence, it is important to know how the microencapsulation and its processing factors influence the powder properties and how to optimize the suitable ranges of processing factors.

Only a few literature reviews on microencapsulation of vitamins are available, in which are discussed the most important achievements and as well the problems encountered during such

types of work that can limit the encapsulation process. However, these works focus on all vitamins, not only on the B vitamins.

Although there are no specific reviews focusing on B vitamins, a number of 39 studies performed in the last 22 years (1999 – 2021) was found, in which B vitamins were used as core materials for microencapsulation, as presented in the next table:

Table 2. 17. Number of studies in which B vitamins were selected as core materials for different microencapsulation methods.

B vitamin	B1	B2	B3	B5	B6	B7	B9	B12
No. of studies	4	13	8	-	2	-	2	12

As can be observed in Table 2.17, vitamins B2, B3 and B12 were used in more studies, vitamins B1, B6 and B9 appear in fewer studies, and vitamins B5 and B7 could not be found in any study.

The overall amount of work regarding B vitamins is still very low and taking into consideration the fact that some B vitamins were not studied even once it is clear that there is a gap in the literature of microencapsulation and the scientists should focus more on B complex. Thus, this field remains new and with many challenges, especially regarding the stability of these compounds.

Below, in Tables 2.19 – 24, all these studies are presented, by summarizing the materials used for encapsulation, the selected method, the main outcomes of the study and the possible final application.

Table 2. 18. The incidence of the microencapsulation techniques.

Number of microencapsulation processes		
chemical	**physico-chemical**	**physico-mechanical**
1	14	25

From Table 2.18 it is shown the tendency of using mostly only physico-chemical and physico-mechanical microencapsulation processes for the microencapsulation of B vitamins, since only in one study a chemical process was preferred, namely interfacial polymerization [144]. The study of Nordgreen et al. studied the production of nutrient delivery microcapsules for the diets of fish (marine larvae and suspension feeders). Using interfacial polymerization method and cross-linked proteins as delivery systems some micronutrients (thiamin mononitrate, vitamin C, vitamin E, vitamin A, iron and zinc) were encapsulated.
Regarding the methods of microencapsulation that use physico-chemical processes, it was observed that most of the authors selected different types of emulsification for their work and only for one study the co-crystallization method.

The most significant number of studies is represented by the physico-mechanical process since 24 out of 40 used methods like spray-drying, freeze-drying and fluidized bed coating.

All the methods presented are characterized by various advantages and disadvantages, so it must be analysed very well which of them fits best, ensuring that the chosen method is easy to implement both at the laboratory and industrial levels, the way of working is simple, and the whole process is fast, reproducible and with a low price [27,100].

The number of microencapsulation methods is still growing with time because there is a high demand for improved methods or new features that can be used for more materials and applications.

Table 2. 19. Microencapsulation studies reported for vitamin B1, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S1	VB1 (thiamine hydrochloride)	Cellulose acetate phthalate (CAP) Eudragit® RS PO	Emulsion solvent evaporation with a continuos oil-phase	• Non-poros microspheres with a mean size of 600 μm. • In vitro release of VB1: - very fast, 80% in less than 30 min and 90% in one hour, from CAP microspehres in a solution of 0.1M HCl. - extremely slow, 7% after 7 h, from CAP microspehres in a solution of ethanol. - prolonged for over 6 h, from Eudragit® RS PO microspehres in a solution of 0.1M HCl in the first 2 h and phosphate buffer (pH 6.8) after 2 h.	Pharmaceutical industry	(Silva and Ferreira, 1999) [145]
S2	VB1 (thiamine hydrochloride)	Emulsifier: In the inner aqueous phase: Polyglycerol polyrunoleate (PGPR) In the outer interface: 1. Water-whey protein isolate-xanthan gum blend 2. Water-whey protein isolate-locust bean gum blend	Water-in-oil-in-water multiple emulsions (W/O/W)	• Improved stability and less polydispersity in droplet size. • Efficient entrapment of VB1 at pH≤2, with a loss of less than 18% after three weeks of storage at room temperature. At neutral pH the loss is of 100%.	Food industry Pharmaceutical industry	(Benichou et al., 2002) [146]
S3	VB1	Cross-linked protein	Interfacial polymerization	• The results suggest that cross-linked protein capsules are not suitable for the delivery of water-soluble nutrients like VB1, since it was registered a loss higher than 90%.	Veterinary industry	(Nordgreen et al., 2008) [144]
S4	VB1 (thiamine hydrochloride) VB6 (pyridoxine hydrochloride)	Ferulic acid-grafted chitosan	Spray-drying	• The product yield ranged between 63.58 and 65.12 %. • The encapsulation efficiency was of 91 ± 2.31 for VB1 and 8 3± 3.17 for VB6. • The microspheres had a compact smooth surface without crackers or pores and a size of 3.2 – 6.5 μm. • The controlled release studies showed a very slow release of both vitamins: after 2h was registered a realase of 24 ± 2.45 % and after 100h a release of 87 ± 3.14%. • Preliminary anti-inflammatory activity for the vitamin loaded microspheres was demonstrated.	Food industry	(Chatterjee et al., 2016) [147]

Table 2. 20. Microencapsulation studies reported for vitamin B2, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S5	VB2 (sodium salt of riboflavin-5´-monophosphate)	Kerosene oil phase Span 80 (sorbitan monooleate) Tween 20 (polyoxyethylene sorbitan monolaurate)	Water-in-oil-in-water multiple emulsions (W/O/W)	• The initiale encapsulation yield was of 92 – 98% and after heating at 80 °C for 20 min the yield value decreased till 85 – 87%. • The release of VB2 followed first order kinetics at pH 2 – 10.	Food industry	(Owusu et al., 1992) [148]
S6	VB2 (riboflavin sodium phosphate)	Noncrosslinked gelatin Crosslinked gelatin with glutharaldehyde and formaldehyde	Emulsification solvent extraction	• The microencapsulation efficiency was of 95%.	Pharmaceutical industry	(Ugwoke et al., 1997) [149]
S7	VB2 (riboflavine)	Calcium-pectinate beads Calcium-pectinate-alginate beads	Freeze-drying	• Complete release was reached in less than 10 hours for calcium-pectinate-alginate beads and only 50% in the same time for calcium-pectinate beads.	Pharmaceutical industry	(Talukder and Fassihi, 2004) [150]
S8	VB2 (riboflavine)	Uncoated barium alginate beads Ethyl cellulose coated barium alginate beads	Emulsification	• Controlled release studies of VB2 were performed in simulated gastric fluid at pH 1.2 for 0-2 h and after in simulated intestinal fluid at pH 6.8 for 2 - 48h at human body temperature 37 °C. • The fastest release was observed from the uncoated barium alginate beads at pH 1.2.	Pharmaceutical industry	(Bajpai and Sharma, 2005) [151]
S9	VB2 (riboflavine)	Gum arabic β-carotene with gum arabic	Spray-drying	• Both types of microparticles proved to be efficient and protect VB2 against photodegradation in milk, avoiding nutritional losses.	Food industry	(Boiero et al., 2014) [152]
S10	VB2 (riboflavine)	Alginate montmorillonite (MMT) clay crosslinked with calcium and barium	Alginate beads	• The produce alginate beads loaded with VB2 had an increased encapsulation efficiency and slowed release due to the influence of the addition of MMT. • The Ca-alginate beads increased the efficiency from 49.9 till 93.7% after the formulation was improved with MMT and for Ba-alginate from 55.0 till up to 98.1%. • The release of VB2 from both alginate beads follow the kinetics of Ritger and Peppas models and the formulations have spherical aspect, but in the case of Ca-alginate can be observed deeper cracks and fractures.	Pharmaceutical industry	(Kaygusuz et al., 2015) [153]
S11	VB2 (riboflavine)	Whey protein hydrogels	Entrapment in hydrogels (microbeads)	• The release of VB2 from the microbeads fitted a pseudo first-order kinetic model and after one hour was released 48% of VB2.	Food industry	(O`Neill et al., 2015) [154]

Table 2.20. (continued)

	Core material	Wall material	Method	Main outcomes	Application	Reference
S12	VB2 (riboflavine)	Polylactic acid Polylactic co-glycolic acid	Oil in water emulsion solvent evaporation	• The product yield reached higher value than 90% and the encapsulation efficiency was 85%. • The release of VB2 registered a burst after 12h and continue to release gradually for over 3 days. • The kinetics of the release follows zero order.	Pharmaceutical industry	(El-hay et al., 2016) [155]
S13	VB2 (riboflavine)	Interpenetrated polymer network (IPN) poly(N-isopropylacrylamide-co-AAc) hydrogel	Hydrogel swelling	• The controlled release of VB2 from IPN poly (NIPAAm -co-AAc) hydrogel was influenced by pH and temperature. • The release of VB2 was faster when the temperature was higher and the pH more acidic. Maximum release was registered at 52 °C and pH 7 (91.47%) and at 45 °C (77.97%).	Pharmaceutical industry	(Brahima et al., 2017) [156]
S14	VB2 (riboflavine)	Galactomannan biopolymer (with and without surfactant F127)	Spray – drying	• The encapsulation efficiency ranged in the interval 87.14 – 88.53%. • The controlled release studied were made at pH 1.2 and pH 6.8. • The microparticles showed antifungal potencial against *Trichophyton rubrum*.	Food industry Pharmaceutical industry	(S.S. de Farias et al., 2018) [157]
S15	VB2 (riboflavine)	Whey protein (tuned via desolvation and crosslinking)	Spray – drying	• The desolvating method improved the crosslinked WPI-riboflavin microparticles. • The most important advantages of ethanol, as desolvating agent are the reduction of drying temperature, increasing the encapsulation efficiency and the potencial for food industry applications, also as a low-cost method.	Food industry Nutraceutical industry	(Ye et al., 2019) [158]
S16	VB2 (riboflavin 5´-monophosphate sodium salt dydrate – RMSD) VB3 (nicotinic acid – NA) VB3 (nicotinamide – NAM)	Triple shellac coatings: 1. Inner shellac coating 2. Subcoating of citric acid or sodium bicarbonate/Lycoat 3. Outer shellac coating	Fluidized bed (prepared by a coater with bottom spray)	• The release from the shellac microcapsules differed according to the acidic (citric acid) or alcalin (sodium bicarbonate) subcoating. • The dissolution from simple shellac microcapsules with RMSD and NA was very fast and in the case of NAM the release was delayed. • In the case of microcapsules with a subcoating it was observed that in the case of citric acid the premature release was counteracted. As for sodium bicarbonate formulation the release was enhanced by better shellac swelling.	Food industry Pharmaceutical industry	(Theismann et al., 2019) [159]
S17	VB2 (riboflavine)	Na-alginate Chitosan (cross – linkend with glutaraldehyde)	Emulsification ionic-gel	• The concentration of the Na-alginate and chitosan influenced the final value of the encapsulation efficiency. • The highest encapsulation efficiency of 55.34% was obtained for 3% of Na-alginate and of 55.70% for 2% of chitosan. This was considered the optimum point, because for higher values of concentrations the efficiency decreased, and until those values was registered a decrease of the efficiency.	Food industry Pharmaceutical industry	(Danarto et al., 2020) [160]

Table 2. 21. Microencapsulation studies reported for vitamin B3, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S18	VB3 (nicotinamide adenine dinucleotide – NAD)	Halloysite clay (alumino-silicate clay)	Microcylinder (air dry)	• For the preparation of halloysite microcylinders was necessary to add polyvinylpyrrolidone to the initial solution of NAD. • Were produced 2 types of cylinders: with he average length of 17.1 μm from premium halloysite and with a smaller length of only4 μm from `G` halloysite.	-	(Price et al., 2001) [161]
S19	VB3 (nicotinic acid and nicotinamide)	Starch gel	Spray-drying	• The microparticles with 2% of nicotinic acid have the capacity to prevent the colour deterioration of haemoglobin concentrate during 7 weeks of storage at room temperature. • The microparticles with 2.5% nicotinamide showed also good stability, but lower than the first type of microparticles. Although th red colourant had higher capacity for nicotinamide when pH was 4.5 or 7.5.	Food industry	(Saguer et al., 2003) [162]
S20	VB3 (niancin)	Ethyl cellulose	Water-in-oil-in-oil double emulsion solvent diffusion method (W/O/O)	• The product yield of the process was of 85% and the entrapment efficiencty of 72%. • The produced microspheres were spherical, free flowing with the particle size ranging between 405 and 560 μm. • In vitro tests were performed in distilled water at 37 ° for 10h and the release fit best Higuchi model characterized by diffusion control.	Pharmaceutical industry	(Maravajhala et al., 2009) [163]
S21	VB3 (nicotinamide)	Hydroxypropyl methylcellulose (HPMC E5)	Spray-drying – a novel method using ternary solid dispersion (TSD)	• Ther obtained particles were amorphous, with spherical form and smooth surface. • After 3 months the particles were still stable.	Pharmaceutical industry	(Paidi et al., 2015) [164]
S22	VB3 (nicotinic acid)	Whey protein isolate	Spray – drying	• The product yield of the process showed a value of 56.0 ± 2.7% and an efficiency of 95.2 ± 0.7%. • The microparticles are spheroidal and have a small size of around 5 μm. • The release of VB3 from whey protein microcapsules is very fast and it requires only 2 min.	Food industry	(Panyoyai et al., 2016) [165]
S23	VB3 (nicotinamide)	Supercritical CO_2 – antisolvent	Co-crystallization using supercritical CO_2 antisolvent micronization method	• Were produced for the first time 2:1 co-crystals of the anti-inflammatory drug diflunisal and VB3. • The products obtained were crystalline with a needle form and uniform width, but variable length.	Pharmaceutical industry	(Cuadra et al., 2016) [166]

Table 2.21. (continued)

Core material		Wall material	Method	Main outcomes	Application	Reference
S24	VB3 (nicotinic acid – NA) VB3 (nicotinamide – NAM)	Triple shellac coatings: 4. Inner shellac coating 5. Subcoating of sodium bicarbonate for NA and citric acid for NAM Outer shellac coating	Fluidized bed (prepared by a: coater with bottom spray for NA microcapsules and with a granulator for NAM microcapsules)	• Were produced NA and NAM delayed-release microcapsules targeting the ileocolonic region, with potential for prediabetes and type 2 diabetes therapies. • The in vitro studies showed that the microcapsules are stable at pH 1.4, 4.5 and 6.8. The release was triggered only at pH 7.4 in the ileocolonic region.	Pharmaceutical industry	(Fangmann et al., 2018) [167]
S16	VB2 (riboflavin 5´-monophosphate sodium salt dydrate – RMSD) VB3 (nicotinic acid – NA) VB3 (nicotinamide – NAM)	Triple shellac coatings: 6. Inner shellac coating 7. Subcoating of citric acid or sodium bicarbonate/Lycoat 8. Outer shellac coating	Fluidized bed (prepared by a coater with bottom spray)	• The release from the shellac microcapsules differed according to the acidic (citric acid) or alcalin (sodium bicarbonate) subcoating. • The dissolution from simple shellac microcapsules with RMSD and NA was very fast and in the case of NAM the release was delayed. • In the case of microcapsules with a subcoating it was observed that in the case of citric acid the premature release was counteracted. As for sodium bicarbonate formulation the release was enhanced by better shellac swelling.	Food industry Pharmaceutical industry	(Theismann et al., 2019) [159]

Table 2. 22. Microencapsulation studies reported for vitamin B6, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S4	VB1 (thiamine hydrochloride) VB6 (pyridoxine hydrochloride)	Ferulic acid-grafted chitosan	Spray-drying	• The product yield ranged between 63.58 and 65.12 %. • The encapsulation efficiency was of 91 ± 2.31 for VB1 and 8 3± 3.17 for VB6. • The microspheres had a compact smooth surface without crackes or pores and a size of 3.2 – 6.5 μm. • The release-controlled release studies showed a very slow release of both vitamins: after 2h was registered a realase of 24 ± 2.45 % and after 100h a release of 87 ± 3.14%. • Preliminary anti-inflammatory activity for the vitamin loaded microspheres was demonstrated.	Food industry	(Chatterjee et al., 2016) [147]
S25	VB6	C12-15 alcohol benzoate (AB) Lauryl PEG polydimethylsiloxyethyl dimethicone (KF-6038) Tween 80	Water-in-oil-in-water multiple emulsions ($W_1/O/W_2$)	• For this study was evaluated the stability of $W_1/O/W_2$ multiple emulstions and the encapsulation efficiency through an electrochemical approach. • For a loading of VB6 of 1.5 wt % the efficiency was of 86.9 ± 0.59%, but after 2 weeks it decreased to 72.2 ± 0.43%	-	(Chen et al., 2020) [168]

Table 2. 23. Microencapsulation studies reported for vitamin B9, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S26	VB9 (folic acid) VB9 (5-methyltetrahydrofolic acid, 5-MTHF)	A combination of pectin (P) and sodium alginate (A): 1. P60:A40 2. P70:A30 3. P80:A20	Spray-drying	• As core material was preferred 5-MTHF, a form of VB9, instead of folic acid because it is less likely to mask the symptoms of VB12 deficiency. • Spray-drying was chosen to protect 5-MTHF since the thermal degradation studies showed a very high loss of almost 70%, compared to just 17% for folic acid. • After microencapsulation the stability of 5-MTHF was improved. • The formulation P80:A20 proved to be the best encapsulating agent and led to a loading efficiency of 60% VB9.	Food industry	(Shrestha et al., 2012) [169]
S27	VB9 (folic acid)	Indian horse chestnut starch (SF) β-cyclodextrin (BF)	Spray-drying	• The encapsulation efficiency for the microparticles with SF was of 57.29% and for BF 76.10%. However, the product yield was almost similar for both formulations, 50.29% for BF and 53.15% for SF. • The release of VB9 from both types of microparticles was similar, and VB9 was released in considerable amounts in the lower part of gastric intestine tract.	Food industry	(Ahmad et al., 2017) [170]
S28	VB9 (folic acid)	Arabic gum Sodium alginate Modified chitosan Pectin Modified starch	Spray-drying	• The product yield of the process was of and the encapsulation process of almost 100% for all formulations with one exception for modified starch. • All microparticles showed a spherical aspect and a regular shape, besides modified starch that had an irregular shape. • Release studies proved a fast release for modified starch, modified chitosan and sodium alginate; and a slow release for the rest of the microparticles. The release of VB9 was achieved in a time interval of few minutes till less than 2 hours. • Weibull kinetic model fitted the best the release profiles.	Food industry	(Estevinho et al., 2020) [171]

Table 2. 24. Microencapsulation studies reported for vitamin B12, presented in chronological order of publication.

Core material		Wall material	Method	Main outcomes	Application	Reference
S29	VB12 (cyanocobalamin)	Microcrystalline cellulose (MC) Dextran microspheres (DM) Hydroxypropyl cellulose (HP) Croscarmallose (CC) Crospovidone (CP)	Spray-drying Freeze-drying	• With this study it was proved that spray-drying is a very good alternative to the conventional freeze-drying process for the production of nasal powders. • The in vivo bioavailability tests done on rabbits proved showed the best results for MC with a value of 25% absolute bioavailabity, followed by CP with 14% and DM with 7%.	Pharmaceutical industry	(Garcia-Arieta et al., 2001) [172]
S30	VB12	Calcium alginate beads	Freeze-drying	• The resulted calcium alginate beads loade with VB12 had a spherical shape with porous surface, a diameter of 0.81 ± 0.01 mm and a moisture content of 21%. • VB12 was released very fast, as 60% ($t_{60\%}$) in just 30 sec, from the calcium alginate beads.	Pharmaceutical industry	(Abubakr et al., 2009) [173]
S31	VB12	Poly(vinyl alcohol)/poly(acrylic acid) hydrogel	Emulsion polymerization	• For the production of VB12 loaded microcapsules was a three-step interfacial emulsion polymerization technique and for the production of VB12 microspheres a one-step emulsion polymerization process. • Were obtained spherical with smooth surface microcapsules and less spherical shaped microspheres with rough surface. • The release of VB12 was faster from the microcapsules than from the microspheres.	-	(Yun and Kim, 2009) [174]
S32	VB12	Calcium alginate	Inkjet (piezoelectric drop-on-demand inkjet technology)	• Calcium alginate hydrogel microcapsules were produced with a narrow size distribution of 50 – 70 μm. • It was proved that the shape of the microcapsules can be influenced by increasing the viscosity of the solution, resulting in microcapsules with elongated, spherical or flattened aspect.	-	(Dohnal and Stepanek, 2010) [175]
S33	VB12	Thiolated and unmodified polymer poly(acrylic acid)-cysteine (PAA-cys)	Spray-drying	• The produced microparticles had a mean diameter of 1 to 3 μm for unmodified PAA-cys formulations and of 2.452 ± 2.26 μm for the thiolated formulations. • 95% of VB12 was released in 3h fom modified formulations and 98% from the unmodified ones.	Pharmaceutical industry	(Sarti et al., 2012) [176]

Table 2.24. (continued)

Core material		Wall material	Method	Main outcomes	Application	Reference
S34	VB12	Eudragit® NE (a neutral copolymer based on ethyl acrylate and ethyl mathacrylate)	Microfluidic jet spray-drying	• Different formulations of microparticles were produced by adding lactose and silica nanoparticles as additives. • Simple formulation, without additives, reached an efficiency of 95% and the produced microcapsules had a bowl like shape with a size of aprox. 100 μm. • The formulations with lactose varied the efficiency between 94 – 99%, the particles were more spherical but with a smaller size of 70 – 86 μm. • The efficiency of the formulations with silica was of 95%, the particles had increased shape deformation and the size was around 95 μm. • In terms of release the simple microparticles had the slowest release of VB12 of 40% in 32h. Contrary the formulations with additives were faster: with addition of lactose all VB12 was released in 2h and with silica 90% of VB12 in half hour.	Pharmaceutical industry	(Liu et al., 2013) [177]
S35	VB12	Cross-linked albumin	Water-in-oil emulsion (W/O) With/without poly(vinyl)alcohol - PVA	• The loading efficiency of the microparticles was of 78.4 ± 1.5%. • The average diameter of the microparticles was of 1643 μm. • The best results in terms of size, homogeneity and sphericity were obtained for the formulation of microparticles produced at 15 min of stirring without PVA.	Pharmaceutical industry	(Sitta et al., 2014) [178]
S36	VB12 (cyanocobalamin)	*N*,*N*-dimethylacrylamide-modified Arabic gum (AGm-DMAAm)	Hydrogel synthesis	• The release profiles of VB12 from AGm-DMAAm hydrogel fit the first order kinetic model. • The temperature affected the release process and were observed differences in the partition activity (α) and the constant of release rate (k_R) when the temperature was changed with 10 °.	Pharmaceutical industry Agriculture industry	(Bossoni et al., 2014) [179]
S37	VB12 (cyanocobalamin) Vitamin C (ascorbic acid)	Chitosan Modified chitosan Sodium alginate	Spray-drying	• The product yield for both formulations was around 45% and the mean diameter of the microparticles was of approx. 3 μm. • The microparticles with chitosan as wall material presented a rough surface and those with modified chitosan and sodium alginate had a very smooth surface. • The presence of vitamins C and B12 was confirmed by a UV-method. • The release from chitosan microparticles was made in 120 min, while from sodium alginate in 15 min and from modified chitosan in only 10 min.	Food industry Pharmaceutical industry	(Estevinho et al., 2016) [32]

Table 2.24. (continued)

Core material		Wall material	Method	Main outcomes	Application	Reference
S38	VB12 (cyanocobalamin)	Soy lecithin and vegetal fat	Spray-chilling	• The solid lipid microparticles (SLM) loaded with VB12 produced by this method improved the stability of the vitamin. • The product yield and the encapsulation efficiency registered high values of 80.7 – 99.7% and 76.7 – 101.1%. • For the SLM loaded with VB12 was observed a spherical shape with smooth surface and the size in the range of 13.28 and 26.99 μm. • The stability of the formulations was ensured for stored SLM over 120 days. • The release of VB12 varied from few minutes till up to 180min.	Food industry	(Mazzocato et al., 2019) [180]
S39	VB12 (cyanocobalamin)	Cyanobacterial extracellular polymer (100%) Cyanobacterial extracellular polymer + arabic gum (50% + 50%)	Spray-drying	• The microparticles loaded with Vb12 had a spherical shape and a rough surface. • The size of the microparticles produced with cyanobacterial extracellular polymer had a mean diameter of 8 μm and the ones in combination with arabic gum presented a smaller diameter. • The release of VB12 was done in less than 3 h for the microparticles with cyanobacterial extracellular polymer and follow the kinetics of first order model. • Meanwhile the second formulation released the vitamin in around 30 min and the release profile fits the best to Weibull model.	Food industry	(Estevinho et al., 2019) [181]
S40	VB12 (cyanocobalamin) + Vitamin D3 (cholcalciferol)	Optimized combination between: Gum acacia (GA) Hi-Cap® 100 (HC) Maltodextrin (MD)	Spray-drying	• After the experimental design for the wall material, it was concluded that the best physico-functional properties are for 38:60:2 ratio of GA, HC and MD. • With this formulation were obtained the best values for the entrapment efficiency, total encapsulation efficiency, thermal and storage stability. • The in-vivo tests showed that both vitamins have slowe release and VB12 is absorbed after 4 h and VD3 after 8 h.	Food industry	(Bajaj et al., 2021) [182]

2.5.1. A case study: spray-drying method applied for B vitamins

In the recent years, the development of food and pharmaceutical products that include B vitamins formulations has emerged. The biggest challenges to obtain these kinds of products are the capacity to obtain qualitative products, with therapeutic benefits and a fair low price that can make the final products available on as many markets as possible. However, the sensitive nature of B vitamins to external factors limits more this process.

To facilitate the preservation of B vitamins and their incorporation into products, the spray-drying method can be used, which has the capacity to convert solutions into dried powders.

Spray-drying is a microencapsulation method and a versatile delivery system used worldwide for various applications in pharmaceutical, food and cosmetic industrial sectors. Besides vitamins, the most common encapsulated bioactive compounds are enzymes, probiotics, microorganisms, polyphenols, antioxidants, colourants, flavours, essential oils, and minerals.

In the last years, the demand for B vitamin content products has considerably increased due to their multiple benefits on health, and increased consumers demand on nutritious products. Therefore, for a better understanding of how spray-drying is used for such products, it is mandatory to analyse the current studies on this topic.

The aim of this case study was to present a literature review with the works that reported the use of spray-drying to produce microparticles with B vitamins as core materials. In the previous subchapters were described some general aspects about spray-drying method, therefore this part will sum up the available information on B vitamins.

Similarities and drawbacks between studies are underlined for a better understanding of how spray-drying can be further improved not only for B vitamins, but for similar sensitive bioactive compounds too.

As presented in the previous section of the chapter (2.5.), in the literature were found 40 studies of microencapsulation that used different methods to microencapsulate B vitamins. However, from all studies a preference for spray-drying method was observed, which was identified in 16 studies, the equivalent to ~ 40% from all studies.

The distribution of the B vitamins in the studies is presented in Table 2.25. The vitamin that appears in most works is vitamin B12 with 6 studies, followed by vitamins B2, B3 and B9 with

3 studies each, vitamins B1 and B6 with only one study, and vitamins B5 and B7 without any study yet.

Table 2. 25. The number of studies in which B vitamins were selected as core materials for a spray-drying process.

B vitamin	B1	B2	B3	B5	B6	B7	B9	B12
No. of studies	1	3	3	-	1	-	3	6

In Table 2.26, the materials used for the preparation of microparticles, the type of spray-dryer equipment and the main experimental conditions can be found. More information about the main outcomes and applications of each study is presented in the previous Subchapter 2.5. where all microencapsulation studies about B vitamins, regardless the used method, are analysed.

All 16 studies were developed in the last 20 years, between 2001 and 2021, and since this number is low, a gap in the literature for this topic can be identified.

In terms of encapsulation agents, a wide variety of carbohydrates materials used alone or in combinations with other agents is observed.

Regarding the spray-dryer equipment, a preference for Mini Spray-Dryer Büchi B-290, used in 6 studies, can be observed. As well, the Mini Spray-Dryer Büchi B-191 appears in 3 studies, and the remaining studies use other specific equipment for this method.

The specific experimental parameters for the spray-drying process are inlet temperature, outlet temperature, feed flow rate, air flow rate, aspiration, and nozzle. However, not all studies present in detail the experimental set-up. As can be observed in Table 2.26, the inlet temperature ranged between 110 and 180 °C, the outlet between 50 and 100 °C, pressure varied from 2 to 6 bar, and the feed flow rate from 1.5 till 900 ml/min.

Table 2. 26. Experimental parameters used for the microencapsulation of B vitamins through the spray-drying method.

	Core material	Wall material	Equipment	Parameters	Reference
1.	VB1 (thiamine hydrochloride) VB6 (pyridoxine hydrochloride)	Ferulic acid-grafted chitosan	-	Inlet temp. 140 °C Outlet temp. 77 °C Feed flow rate 10 mL/min	(Chatterjee et al., 2016) [147]
2.	VB2 (riboflavine)	Gum arabic β-carotene with gum arabic	Labplant SD-04 Labplant UK Ltd., Huddersfield, 123 UK	Inlet temp. 170 °C Outlet temp. 110 °C Nozzle 0.7 mm Feed flow rate 30 mL/min Pressure 5 bar	(Boiero et al., 2014) [152]
3.	VB2 (riboflavine)	Galactomannan biopolymer (with and without surfactant F127)	Mini Spray-Dryer Büchi B-290 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 130°C Outlet temp. 100 °C Pump flow 10%	(S.S. de Farias et al., 2018) [157]
4.	VB2 (riboflavine)	Whey protein (tuned via desolvation and crosslinking)	A triple nozzles micro-fluidic-jet spray dryer (MFJSD) (Nantong Dong Concept New Material Technology Ltd., China)	Inlet temp. 180°C Outlet temp. 80 °C Nozzle 100 μm (microfluidic aerossol)	(Ye et al., 2019) [158]
5.	VB3 (nicotinic acid and nicotinamide)	Starch gel	Labplant SD-05 Labplant UK Ltd., Huddersfield, UK	Inlet temp. 140 °C Outlet temp. 80 °C Feed flow rate 900 mL/h Pressure 5 bar	(Saguer et al., 2003) [162]
6.	VB3 (nicotinamide)	Hydroxypropyl methylcellulose (HPMC E5)	Mini Spray-Dryer Büchi B-191 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 110 – 120 °C Outlet temp. 65 – 70 °C Aspiration 80 – 100% Feed flow rate 10 – 20%	(Paidi et al., 2015) [164]
7.	VB3 (nicotinic acid)	Whey protein isolate	Lab Plant Spray Dryer SD Basic FT30MKIII (Keison products, Chelmsford, Essex, UK)	Inlet temp. 120 °C Outlet temp. 75 °C Nozzle 0.5 mm Feed flow rate 8.5 mL/min Pressure 2.5 bar	(Panyoyai et al., 2016) [165]
8.	VB9 (folic acid and 5-methyltetrahydrofolic acid, 5-MTHF)	A combination of pectin (P) and sodium alginate (A): 1. P60:A40 2. P70:A30 3. P80:A20	Saurin, model SL20, Australia	Inlet temp. 150 °C Outlet temp. 50 °C Twin nozzle	(Shrestha et al., 2012) [169]
9.	VB9 (folic acid)	Horse chestnut starch β-cyclodextrin	Mini Spray-Dryer Büchi B-290 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 130 °C Outlet temp. 80 °C Air flow rate 140 l/h	(Ahmad et al., 2017) [170]
10.	VB9 (folic acid)	Arabic gum Sodium alginate Modified chitosan Pectin Modified starch	Mini Spray-Dryer Büchi B-290 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 120 °C Outlet temp. 58 °C Nozzle 0.5 mm Feed flow rate 4 mL/min (15%) Aspiration 32 m^3/h (80%) Pressure 6 bar	(Estevinho et al., 2020) [171]

Table 2.26. (continued)

	Core material	Wall material	Equipment	Parameters	Reference
11.	VB12 (cyanocobalamin)	Microcrystalline cellulose (MC) Dextran microspheres (DM) Hydroxypropyl cellulose (HP) Croscarmallose (CC) Crospovidone (CP)	Mini Spray-Dryer Büchi B-191 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 150 °C Feed flow rate 11 mL/min Air flow rate 800 l/h Aspiration vacuum 35 mbar	(Garcia-Arieta et al., 2001)[172]
12.	VB12	Thiolated and unmodified polymer poly(acrylic acid)-cysteine (PAA-cys)	Mini Spray-Dryer Büchi B-191 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 150 °C Outlet temp. 95 °C Nozzle 0.7 mm Aspiration 100% Feed flow rate 1.5 mL/min (4%) Pressure 5 bar	(Sarti et al., 2012) [176]
13.	VB12	Eudragit® NE (a neutral copolymer based on ethyl acrylate and ethyl mathacrylate)	Micro-fluidic jet spray-dryer	Inlet temp. 146 °C Outlet temp. 83 °C	(Liu et al., 2013) [177]
14.	VB12 (cyanocobalamin) Vitamin C (ascorbic acid)	Chitosan Modified chitosan Sodium alginate	Mini Spray-Dryer Büchi B-290 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 120 °C Outlet temp. 65 °C Nozzle 0.5 mm Feed flow rate 4 mL/min (15%) Aspiration 32 m^3/h (80%) Pressure 6 bar	(Estevinho et al., 2016) [32]
15.	VB12 (cyanocobalamin)	Cyanobacterial extracellular polymer (100%) Cyanobacterial extracellular polymer + arabic gum (50% + 50%)	Mini Spray-Dryer Büchi B-290 (Büchi Laboratorius-Technik, Flawil, Switzerland)	Inlet temp. 120 °C Outlet temp. 85 °C Nozzle 0.5 mm Feed flow rate 4 mL/min (15%) Aspiration 32 m^3/h (80%) Pressure 6 bar	(Estevinho et al., 2019) [181]
16.	VB12 (cyanocobalamin) + VD3 (cholcalciferol)	Optimized combination between: Gum acacia (CA) Hi-Cap® 100 (HC) Maltodextrin (MD)	Spray Mate JICL LSD-48 mini spray dryer (Jay Instruments and Systems Pvt. Ltd., Navi Mumbai, India)	Inlet temp. 140 ± 2 °C Outlet temp. 60 ± 2 °C Nozzle 0.5 mm Pressure 2 bar	(Bajaj et al., 2021) [182]

Regarding the final application of each study a majority can be observed for food applications, although some studies are suitable for pharmaceutical industry too, and just a few for both food and pharma sectors.

Vitamins B1 and B6 appear in only one study, in which Chatterjee et al. developed vitamin loaded microspheres with a phenolic acid-grafted chitosan derivative as wall material [147]. Through their activity, these two water-soluble vitamins bring essential health benefits such as anti-nociceptive and anti-inflammatory effects for vitamin B1, and proper function of metabolism, cardiovascular and immunity systems for vitamin B6 [1,2,4]. However, both vitamins

are thermally unstable and for their protection the authors proposed microencapsulation by spray-drying as protection system [147].

The microencapsulation process proved to be efficient, as the product yield varied from 63.58 till 65.12%, and the encapsulation efficiency was of 91 ± 2.31% for vitamin B1 and 83 ± 3.17% for vitamin B6. The microspheres were compact with smooth surfaces, without no cracks or pores and mean diameter of 4.5 and 4.8 μm [147].

In vitro controlled release studies made by the authors were done in acidic medium and proved to have a sustained release, since after 2 h was registered a release of 24 ± 2.45% of the vitamins, and only after 100 h a release of 87 ± 3.14%. As well, Chatterjee et al. performed in vivo test to prove that these microspheres have anti-inflammatory activity in carrageenan induced paw edema of albino rats. Considering the overall results, the authors affirmed that these microspheres with vitamins B1 and B6 are suitable for food and nutraceutical industries as dietary supplement or ingredient in functional foods [147].

For the next three studies, **vitamin B2** was selected to prepare microparticles with potential applications for food and pharmaceutical industries [152,157,158]. Vitamin B2 plays an important role for human health and it is known to be involved in energy metabolism and to keep visions and skin in good parameters [1,2,4]. Since vitamin B2 is easily affected by the exposure to light, the authors Boiero et al., S.S. de Farias et al., and Ye et al. aimed to solve this stability problem and to preserve or even enhance the functional properties of vitamin B2 [152,157,158].

For the first study Boiero et al. used formulations of arabic gum with and without β-carotene to confer protection for vitamin B2 against the photo-degradation of whole-milk. As any other staple food, milk plays an important role in human nutrition therefore its nutrients must be kept active and stable. It is known that the oxidative stability of milk can be affected by the presence of vitamin B2, which absorbs the environmental light and generates electronically excited states of flavin, a form of vitamin B2 [152].

Both formulations prepared by Boiero et al. with arabic gum as encapsulating agent had a benefic effect on vitamin B2, as it was registered a decrease of the apparent first-order rate constant of vitamin B2 photo-degradation of 26% for the microparticles prepared without β-carotene, and of 30% for those with β-carotene [152].

With the second study about vitamin B2, S.S. de Farias et al. aimed to produce formulations of vitamin B2 with a simple biopolymer – galactomannan, and with the biopolymer and the

surfactant F127 in two concentrations 0.10% and 0.15%. Different physico-chemical analysis were performed on the obtained microparticles to appreciate the microencapsulation process [157].

The biopolymeric protection was successful for all formulations, fact proved by the high values of encapsulation efficiency that ranged between 87.14 and 88.53%. The viscometric tests showed that the increase of the surfactant F127 concentration determined the decrease of viscosity, which is useful for a greater flow of the solution with the microparticles, helps release the core faster, and makes the microparticles suitable for application in injectable drugs. Another important result of this study is the antifungal activity test, which showed that the simple formulation of biopolymer-vitamin is enough for the fungal growth inhibition of *T. rubrum*. Regarding the release profiles, the authors obtained similar profiles for all formulations and in both basic and acid mediums [157].

With their work S.S. de Farias et al. confirmed that the use of surfactant is not making a significant difference and all obtained microparticles loaded with vitamin B2 are proper for the incorporation in drugs or food matrices [157].

In the last study about vitamin B2, Ye et al. suggested a simple methodology to improve the microparticles of whey protein isolate loaded with vitamin B2. Thus, using desolvation and cross-linking was possible to improve gastric digestibility, release sites and release profiles for the spray-dried microparticles [158].

As desolvating agent was selected ethanol, which led to decreasing the drying temperature, increasing the efficiency of the process and facilitation of vitamin B2 – whey protein isolate complexes formation. Both desolvation and cross-linking methods changed the morphology of the microparticles, and in terms of moisture content was observed a decrease for the case of desolvation and an increase for cross-linking. To determine the best conditions, the content of ethanol and calcium ions varied from 0 to 50% v/v and from 0 to 2 mM, respectively. Ye et al. noted that the best results obtained were for the sample with a content of 30% v/v ethanol and no calcium crosslinking because in less than 30 min peptic digestion was achieved. As well, the samples with more than 30% v/v ethanol with 1 and 2 mM Ca^{2+} showed excellent gastric resistance and intestinal release [158].

Vitamin B3 is essential for neurological, brain and nervous systems and it is characterized as the most stable compound from B complex [1,2,4]. In three different studies, Saguer et al., Paidi

et al., and Panyoyai et al. selected spray-drying method as a delivery system for vitamin B3 [162,164,165].

Saguer et al. presents an interesting approach for two types of vitamin B3, nicotinic acid and nicotinamide, which were used as colour stabilizers for spray-dried porcine red blood cells with starch gel as wall material. Blood haemoglobin is an important source of red colourant for food industry and also has nutritional value because can help treat iron deficiency. However, blood haemoglobin needs a colour-fixing compound capable to protect it. In this study Saguer et al. proposed the use of vitamin B3 as it is known to form complexes with myoglobin which presents many similarities with haemoglobin. The formulations that included 2% (w/v) of nicotinic acid prevented the colour deterioration during the spray-drying process and during storage time. Similar results were observed in the case of nicotinamide with concentrations higher than 2.5% (w/v) [162].

In the research of Paidi et al. was used a novel spray-dried ternary solid dispersion (TSD) composed of hydrophilic polymer, hydroxypropyl methylcellulose – HPMC E5 and a hydrotropic agent, vitamin B3. The developed TSD were amorphous and had a spherical shape [164].

Panyoyai et al. studied spray-dried microparticles of vitamin B3. Whey protein was chosen as encapsulating agent because is a biopolymer suitable for food industry and has the capacity to control the release of small molecules. The spray-drying process was evaluated by product yield (56.0 ± 2.7%) and encapsulation efficiency (95.2 ± 0.7%). The obtained microparticles had spherical aspect and a relative small size of 5 μm. Release profiles showed a very fast process that finished in only 2 minutes [165].

Vitamin B9 is important for red blood cells, DNA synthesis, mental and emotional health. However, the task of preparing stable delivery systems for vitamin B9 is very difficult to accomplish because of its high sensitivity to external factors like light, temperature, pH and oxygen [1,2,4]. In their studies, the authors Shrestha et al., Ahmad at el., and Estevinho et al. managed to obtain different types of microparticles loaded with vitamin B9 [169–171].

Shrestha et al. proposed a comparative study between two forms of vitamin B9, namely folic acid and 5-methyltetrahydrofolic acid (5-MTHF). Folic acid has been already used in food fortification because its bioavailability in natural products is very low, and supplementation became a necessity. 5-MTHF was not used yet in fortification, since is less bioavailable and degrades easily during food processing operations. Even if 5-MTHF presents these limitations

the authors propose this alternative approach because this particular form of vitamin B9 is less likely to mask the symptoms of B12 deficiency in older populations comparing to folic acid form [169].

This work started with the study of thermal stability of both types of vitamin B9 during boiling and autoclaving at various time intervals and in different liquid model food matrices like milk, soymilk, starch–water and water. And the results showed a severe thermal degradation of around 70% for 5-MTHF and only 17% for folic acid [169].

Therefore Shrestha et al. developed microparticles loaded with these compounds. Combinations with pectin (P) and sodium alginate (A) in different proportions P60:A40, P70:A30, P80:A20 were selected as protective wall materials. The overall stability of 5-MTHF improved after microencapsulation and the best results were obtained for P80:A20 as the loading efficiency reached 60% [169].

For the last part of the study were compared 5-MTHF in its free form and microparticles with 5-MTHF during extrusion process, and was observed that retention for the free form was of 65.3–83.2% and for the encapsulated form of 84–94.5%, proving a better stability [169].

Ahmad at el. microencapsulated vitamin B9 with Indian horse chestnut starch (SF) and with β-cyclodextrin (BF) using spray-drying to further analyse the protection of the microparticles in simulated gastric conditions. The product yield showed very close values of 50.29% for BF and 53.15% for SF, and in terms of encapsulation efficiency was observed a higher difference between formulations:76.10% for BF and 57.29% for SF [170].

The release of VB9 was studied during in-vitro digestion and the release percentage for the mouth, gastric and intestinal conditions were considered. Both BF and SF microparticles presented alike release behaviour and in the first 5 minutes just a low amount of VB9 was released, namely 11.9% from BF and 15.3% from SF. Later after 4 hours of release in the intestinal conditions was reached 84% of release for BF and 92.1% for SF [170].

This study proved that Indian chestnut starch can be used as a low-cost alternative encapsulating agent for functional food ingredients and should be explored more in future works [170].

Estevinho et al. present a preliminary evaluation of vitamin B9 microparticles prepared with biopolymeric materials. The aim of the work was to characterize and compare the different types of formulations prepared and to study the release of the vitamin. The selection of

encapsulating agents included arabic gum, sodium alginate, modified chitosan, pectin and modified starch [171].

The product yield of this encapsulation process ranged in the interval 13 – 50% and the encapsulation efficiency was of almost 100% for all formulations, besides the one with modified starch. Regarding their morphology, all microparticles presented spherical aspect and a regular shape, with one exception of modified starch that had an irregular shape[171].

Considering the results from in vitro tests the obtained microparticles loaded with vitamin B9 are suitable for different food applications. The release profiles registered a fast release for modified starch, modified chitosan, and sodium alginate; and a slow release for the rest of the microparticles. The results of the release were used also to determine the best kinetic model applicable and Weibull model proved to be the best to fit the release profiles[171].

Vitamin B12 is involved in red blood cell formation, regulates the DNA, prevents birth defects, and helps the nervous and gastrointestinal systems. As well, vitamin B12, the most complex vitamin in terms of chemical structure from the family of B vitamins, and in terms of stability, is predisposed to degradation when exposed to light [1,2,4].

Surprisingly, vitamin B12 appears in the highest number of studies that produced microparticles loaded with B vitamins by a spray-drying method, namely six different works of Garcia-Arieta et al., Sarti et al., Liu et al, Estevinho et al. (1), Estevinho et al. (2) and Bajaj et al. [32,172,176,177,181,182].

Garcia-Arieta et al. proposed the microencapsulation of vitamin B12 by spray-drying as an alternative to the conventional freeze-drying process used to produce nasal powders. Microcrystalline cellulose (MC), dextran microspheres (DM), hydroxypropyl cellulose (HP), croscarmallose (CC) and crospovidone (CP) are the five wall materials with high water absorption ability tested along this study. In vivo studies were performed on rabbits and the best results were observed for MC with a value of 25% absolute bioavailabity, followed by CP with 14% and DM with 7% [172].

Sarti et al. aimed to produce a mucoadhesive and permeation-enhancing delivery system for vitamin B12 by the means of spray-drying method and thiolated polymer poly(acrylic acid)-cysteine (PAA-cys) as wall material. Different microparticles were prepared, with the diameter of 1 to 3 μm for unmodified PAA-cys formulations and of 2.452 ± 2.26 μm for the thiolated

formulations. The release studies confirmed after 3h that 95% of VB12 from modified formulations and 98% from the unmodified ones were released [176].

Liu et al. present a different approach for the microencapsulation of vitamin B12 than the classic spray-drying process. For this research was used a personalized microfluidic jet spray drying method that used an aerosol nozzle capable to handle different precursors and to prepare uniform, non-agglomerated microparticles with controllable particle size. As encapsulating agent was used Eudragit® NE, which is a neutral copolymer based on ethyl acrylate and ethyl methacrylate [177].

During the study, different formulations of microparticles were prepared by adding lactose and silica nanoparticles as additives. It was observed that the simple formulation, without additives, reached an efficiency of 95% and the microparticles had a bowl like shape with a size of around 100 μm. In the case of the formulations with lactose the efficiency was even higher up to 99%, the particles were more spherical but had a smaller size of 70 – 86 μm. For the formulations with silica, the particles presented deformations and the size was around 95 μm, the efficiency being 95% [177].

The release from the simple microparticles registered a very slow release of 40% of vitamin B12 in 32h, however the formulations with additives were very fast: with the addition of lactose all vitamin was released in 2h and with silica 90% in only half hour [177].

Estevinho et al. (1) prepared microparticles suitable for food products with vitamin B12 and vitamin C and three biopolymers, namely chitosan, modified chitosan, and sodium alginate. This approach was used for the first time by the authors, and they obtained for both vitamins a product yield of around 45%. The mean diameter of the microparticles was of around 3 μm. Chitosan microparticles had a rough surface and the ones with the modified form of chitosan and sodium alginate a smooth surface. Using a UV-method the authors confirmed the presence of the vitamins inside the microparticles. For the release tests, it was necessary 120 min for a complete release from chitosan microparticles, although from sodium alginate only15 min and from modified chitosan only 10 min [32]

Vitamin B12 was microencapsulated by Estevinho et al. (2) in a different study in which as encapsulating agents were used cyanobacterial extracellular polymer simple formulations and in combination with arabic gum. The same configuration of the previous study was selected, but in terms of product yield, the values were much lower, fact that proves the high influence of the materials selected as encapsulating agents. For the simple formulations the product yield

was of only 4%, therefore a different agent with the purpose of improving the process was added. With the new formulation that included cyanobacterial extracellular polymer with arabic gum (50% + 50%) a product yield of 18.8% was obtained [181] .

In terms of morphology microparticles with spherical shape and rough surface and in terms of size a mean diameter of 8 μm for simple formulations and a smaller diameter for those in combination with arabic gum, were obtained. The release of vitamin B12 required less than 3 h for the microparticles with cyanobacterial extracellular polymer and followed a kinetics of first order. Meanwhile the improved formulation with arabic gum released the vitamin in around 30 min and the release profile fitted the Weibull model [181].

Bajaj et al. presented a wall material optimization study to be used for the microencapsulation of vitamins B12 and vitamin D3. In order to optimize the properties of the wall material the authors worked with combinations of gum acacia (GA), Hi-Cap® 100 (HC), and maltodextrin (MD). The best physico-functional properties were obtained for 38:60:2 ratio of GA, HC and MD and with this formulation were achieved the best values for the entrapment efficiency, total encapsulation efficiency, thermal and storage stability. The in-vivo tests proved a slow release for both vitamins since for the absorption of vitamin B12 are necessary 4 hours and for vitamin D3 8 hours [182].

One of the most challenging aspects regarding B vitamins is their incorporation into products like fortified food products, nutraceutical supplements, or medicines. Such products are sometimes difficult to manufacture because of the sensitive nature of vitamins prone to degradation.

This review helps understand the current knowledge on spray drying technique applied to different B vitamins, current trends of spray drying to overcome the limitations of conventional spray drying, and how this microencapsulation process can be optimized in future works to increase the vitamins applicability.

However, this collection of studies show that spray-drying method can produce micro delivery systems that are designed to protect, transport, and control release rate of B vitamins to increase their bioavailability.

Although the option of encapsulating B vitamins by a spray-drying method attracted the interests of scientists over time, only few works were done. To obtain certain quality of the microparticles, it is necessary to find out the optimum conditions for spray drying process.

Therefore, more research is needed for the optimization of spray drying process and as well, it is necessary to study the encapsulation with this technique of the rest of vitamins that do not appear in any study, namely vitamins B5 and B7.

Another important task for the future is to explore the possibility of using more than one B vitamin as core material and as well to study combinations of biopolymeric wall materials, which can also help increase the final applicability.

Chapter 3

Materials and methods

In the third chapter the used materials and the experimental methods applied for every study of this research are described in detail. The information provided in this part of the thesis is useful to understand the methodological approaches used to produce microparticles with B vitamins content.

3.1. Reagents

All reagents required to perform the experimental part were of analytical grade purity. The main reagents used to produce microparticles are divided into two big groups: materials to be encapsulated (Table 3.1) and encapsulating agents (Table 3.2). Below the selected compounds are presented, including information like product name, purity, supplier, and specifications for identification, as follows:

Table 3. 1. Encapsulated materials.

Encapsulated material	Product details
1. vitamin B1	thiamine hydrochloride, ≥99% Sigma Aldrich, Germany (T4625, CAS-No. 67-03-8).
2. vitamin B2	riboflavin from Eremothecium ashbyii, ≥98% Sigma Aldrich, China (R4500-256, Lot #WXBC3912V, CAS-No. 83-88-5)
3. vitamin B3	nicotinamide, ≥98% Sigma Aldrich, USA (N3376-100G, Lot #SLBT5505, CAS-No.: 98-92-0).
4. vitamin B12	cyanocobalamin, ≥98% Sigma Aldrich, China (V2876, Lot # MKBQ9972V, CAS-No. 68-19-9)

Table 3. 2. Encapsulating agents.

Encapsulating agent	Product details
1. arabic gum	Fluka, Germany (30888, Lot #BCBK8649V).
2. carrageenan	Sigma Aldrich, USA (1001761179 C1013-1006, Lot #SLBH9868V).
3. chitosan	Sigma Aldrich, Germany (Cat. No. 448877).
4. maltodextrin	Sigma Aldrich, USA (1001841656 419672, Lot # MKBN6629V).
5. modified chitosan	China Easter Group (Batch no. SH20091010).
6. modified starch	Alfa Aesar GmbH&Co KG, Germany (36673, Lot D04X013).
7. pectin	Sigma Aldrich, Switzerland (101582340 76282, Lot #BCBN5335V).
8. sodium alginate	Sigma Aldrich, USA (1001503523 180947, Lot #MKBH8463V).
9. xanthan	Sigma Aldrich, USA (1001900732 G1253, Lot # MKBQ9467V).

Besides the encapsulated materials and encapsulating agents, along the experimental part, the following materials were necessary:

Table 3. 3. Other materials required for the experimental work.

Other materials	Product details
1. deionized water	collected from a water purification Millipore device.
2. ethanol 99%	as dispersant médium used during the measurements done to determine the particle size (Valente e Ribeiro, LDA).
3. acetic acid (1% (V/V))	with the viscosity of 200 mPa·s (25 °C) - for the preparation of chitosan solution 1% (w/V).
4. hydrochloric acid 37%	for the preparation of simulated gastric fluid (Sigma Aldrich, 25,814-8, CAS-No.: 7647-01-0).
5. sodium chloride	for the preparation of simulated gastric fluid (AppliChem Panreac ITW Companies, 131,659.1211, Lot 0000542745, CAS-No: 7647-14-5).

3.2. Equipment

All the weight measurements of the materials were done with the help of an analytical balance type Adam Equipment WA120 (USA).

The aqueous solutions were prepared with deionized water (DW) collected from a water purification system with a 0.45 μm filter, type MilliporeTM (Massachusetts, USA).

For the homogenization of solutions were used several types of agitators: a vortex shaker – IKA VORTEX Genius 3 (Staufen, Germany) and magnetic stirrer plates – Stuart Scientific SM20 (UK) and Scansci MS-H-Pro Digital Hotplate (Berlin, Germany).

Corrections of the pH for certain solutions were done by measurements with a pH meter.

The biopolymeric microparticles with vitamin contents were produced with a Mini Spray Dryer B-290 equipment with a standard 0.5 mm nozzle, from BÜCHI (Flavil Switzerland).

The external morphology of the microparticles was examined with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) from CEMUP by scanning electron microscopy (SEM). The size of the microparticles was determined with a Coulter-LS 230 (Miami, USA) using the laser granulometry method.

The samples for the validation of the analytical method and also for vitamins' release tests were evaluated by a spectrophotometric method with an equipment from Sarspec SPEC RES+ UV/VIS (Portugal).

3.3. Preparation of experimental solutions

The research "Development of the microencapsulation of soluble vitamins with different biopolymers by a spray drying process" gathers four studies conducted independently of each other, using the same type of equipment, but with some differences of the experimental setups.

Through the research of the first study, "*Optimization of work conditions"* several experimental conditions were analysed and to accomplish this vitamin B12 was chosen as a model vitamin for the microencapsulation process. In this work only one encapsulating agent, the biopolymer modified chitosan, was used.

For the other three studies it was decided to use more encapsulating agents, to identify similarities and differences. Thus, for second study – "*Microencapsulation of vitamin B12",* seven different biopolymers were selected, for the third study – "*Microencapsulation of vitamin B1"* nine biopolymers were prepared and for the last study: fourth study – "*Microencapsulation of vitamins B2 and B3",* six biopolymers for each vitamin.

So, for every study several feed solutions for the microencapsulation process were necessary. All these experimental solutions were prepared at room temperature with deionized water.

The steps that have been followed to obtain the feed solutions and the differences between the studies are presented below. Additional information about the concentrations of each solution and what encapsulating agents were selected for every study are presented in Table 3.4.

First, the encapsulating agent solution was prepared, and every solution was placed under stirring for 2 hours at 1200 rpm, as the homogenization takes time. Second, the solution of the vitamin to be encapsulated was prepared, and the mixing was done during 10 min with the help of the shaker. After, both solutions were joined and mixed.

The feed solution is considered ready to be pumped to the spray-dryer system after stirring on a magnetic plate, 30 min set up at a speed of 500 rpm.

For all four studies, additional feed solutions without vitamin content were prepared for the analysis of empty microcapsules.

Some of the release tests were done in simulated gastric fluid (SGF) medium. The stock solution was prepared as described in the European Pharmacopeia 7.0 (2010): 2.0 g of sodium

chloride was dissolved in 7.0 mL of hydrochloric acid and then water was added until 1000 mL. Additional corrections of the pH were made with the pH – meter until a final pH of 1.2 was reached.

Table 3. 4. Experimental solutions prepared for every study.

Study no.	Vitamin solution	Biopolymeric solution
1	vitamin B12 1% (w/V) vitamin B12 2% (w/V) vitamin B12 3% (w/V) vitamin B12 4% (w/V) vitamin B12 5% (w/V)	mod. chitosan 1% (w/V)
2	vitamin B12 2% (w/V)	arabic gum 1% (w/V) sodium alginate 1% (w/V) carrageenan 1% (w/V) maltodextrin 1% (w/V) modified starch 1% (w/V) pectin 1% (w/V) xanthan 1% (w/V)
3	vitamin B1 2% (w/V)	arabic gum 1% (w/V) sodium alginate 1% (w/V) carrageenan 1% (w/V) chitosan 1% (w/V) mod. chitosan 1% (w/V) maltodextrin 1% (w/V) modified starch 1% (w/V) pectin 1% (w/V) xanthan 1% (w/V)
4	vitamin B2 2% (w/V) and vitamin B3 2% (w/V)	arabic gum 1% (w/V) sodium alginate 1% (w/V) chitosan 1% (w/V) mod. chitosan 1% (w/V) maltodextrin 1% (w/V) pectin 1% (w/V)

3.4. Microencapsulation process

Different types of microparticles (loaded with vitamins and empty) were produced with a Mini Spray Dryer B-290 from BÜCHI, Flavil Switzerland. This laboratory-scale device is equipped with a two-fluid co-current nozzle atomization system.

The configuration used for all experiments is presented in Table 3.5.

Table 3. 5. Parameters configuration for the spray-drying process.

Experimental parameters	Configuration
Solution flow rate	4 mL/min (15%)
Airflow rate	32 m3/h (80%)
Inlet temperature (T_{in})	120 °C
Air pressure	6 bars
Nozzle	5 mm

The outlet temperature is the only parameter that varied during the spray-drying process and the values registered during every study are presented in Table 3.6. The value of this parameter can be set up from the beginning of the process because it is the result of the whole microencapsulation process (selected values of the other parameters and the properties of the feed solution).

Table 3. 6. Outlet temperature registered during every study.

Study no.	Outlet temperature (T_{out})
1	53 – 58 °C (VB12)
2	56 – 67 °C (VB12)
3	50 – 67 °C (VB1)
4	63 – 72 °C (VB2) 60 – 68 °C (VB3)
empty microparticles	60 – 68 °C

The experimental conditions were selected based on the optimized experimental conditions of previous works of the authors and other similar studies [27,32,87,122].

The powder samples collected from the main equipment at the end of every experiment were kept covered in aluminium foil and stored in the fridge at 4 °C until further analysis, to avoid degradation processes.

3.4.1. The product yield of the spray-drying process

The performance of the spray-drying process was evaluated by the determination of the product yield, using Equation 2.13., presented in Subchapter 2.4.

3.5. Characterization of microparticles

The characterization of the microparticles followed two types of analysis: surface morphology and particle size distribution.

3.5.1. Scanning electron microscopy evaluation

Scanning electron microscopy (SEM) was used to observe the physical appearance of the microcapsules. The analysis was performed with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) at Centro de Materiais da Universidade do Porto (CEMUP).

The sample preparation involved 2 operations: first – fixing each powder sample on a brass stub using double-sided adhesive tape, and second – coating with a thin layer of gold in vacuum and electrically conductive, in a Jeol JFC 100 equipment.

3.5.2. Laser granulometry analysis

The microparticles were characterized in terms of number and volume average size using laser granulometry technique and a Coulter-LS 230 (Miami, USA) equipment.

Each sample was previously ultrasound irradiated. Ethanol was used as a dispersant solution to prevent the possible aggregation of the microparticles. All readings were the result of three tests average of 30 seconds runs each.

3.6. Controlled release studies

Controlled release studies were performed for two related reasons: first to demonstrate the presence of vitamins inside the microparticles and second, to evaluate the release profile.

The release profiles are important tools because their evaluation offers a series of valuable information like:

- encapsulation efficiency.
- the influence of experimental parameters
- the time necessary for complete release.
- kinetic models that can describe the release process.
- stability of the microparticles overtime.

These studies were carried out with a spectrophotometer from Sarspec SPEC RES+ UV/VIS (Portugal) and a LightScab software. The equipment was customized with an external device for selecting the desired temperature and the intensity of the magnetic stirring for the solution analysed inside the cuvette. The systems in charge of temperature and stirring were both operated via optical fibber connectors.

The microparticles were released in two different dissolution mediums: in DW (pH = 5.6) at 20 °C and in SGF (pH = 1.2) at 37 °C. The readings were done in the UV domain at specific wavelengths values for every vitamin, as presented in Table 3.8.

For the studies: "Optimization of work conditions" (Chapter 4), "Microencapsulation of vitamin B12" (Chapter 5) and "Microencapsulation of vitamin B1" (Chapter 6), the acquisition of data was set up to register in continuous mode every 30 s for all encapsulating agents, besides for modified starch every 5 seconds. But in the case of the last study "Microencapsulation of vitamins B2 and B3" (Chapter 7) a different time interval, of 10 seconds, was selected, because it was concluded that a smaller period would offer more information.

3.6.1. Validation of the analytical method

Before proceeding to the controlled release study, it was necessary to validate the analytical method [183–185], to prove that spectrophotometry is suitable for the selected vitamins. Therefore, specific parameters were determined and are presented in Table 3.7, namely: linearity range,

equation of calibration curve, coefficient of correlation – R^2, the limit of detection (LOD), the limit of quantification (LOQ), accuracy and precision; and only specificity in Table 3.8.

To evaluate the limit of detection (LOD) and the limit of quantification (LOQ), it was necessary to calculate the standard deviation of response (σ) and the slope of the calibration curve (b), according to the following formulas: LOD = (3.3 × σ) / b and LOQ= (10 × σ)/ b.

Accuracy was expressed as the percentage of recovery (the percentage ratio between the obtained and the expected concentration of vitamin) for the last three concentrations. And the intra-day precision was also expressed for the last three concentrations.

To avoid possible interference of the vitamins with the encapsulating agents the values of their wavelengths were verified. And as it can be observed in Table 3.8., the wavelengths are different, so the specificity rule is also respected.

Table 3. 7. Parameters calculated to validate the analytical method.

		Linearity (mg/ml)	**Equation**	**R^2**	**LOD** (mg/L)	**LOQ** (mg/L)	**Accuracy** ***(%)***	**Precision**
Vitamin B12	**SGF** (37 °C)	0.0025 – 0.1	y = 16.847x + 0.0601	0.995	0.350	0.116	99.3 98.8 96.6%	0.147 1.078 0.679
Vitamin B1	**DW** (20 °C)	0.0005 – 0.01	y = 58.739x – 0.0085	0.999	0.146	0.482	94.3 96.1 98.2	0.764 0.800 0.855
	SGF (37 °C)	0.0005 – 0.01	y = 74.769x + 0.0174	0.999	0.151	0.505	102.5 101 98.1	0.218 0.253 0.208
Vitamin B2	**DW** (20 °C)	0.0005 – 0.012	y = 65.698x + 0.0372	0.996	0.317	1.055	102.197 101.850 95.910	0.880 0.228 0.679
	SGF (37 °C)	0.0005 – 0.012	y = 67.376x + 0.0176	0.997	0.301	1.003	104.340 98.957 97.059	0.356 0.312 0.389
Vitamin B3	**DW** (20 °C)	0.00125 – 0.03	y = 21.815x + 0.0045	0.999	0.310	1.032	101.574 99.901 99.040	1.694 0.280 0.236
	SGF (37 °C)	0.00125 – 0.03	y = 33.073x + 0.0236	0.999	0.555	1.849	101.251 100.553 97.569	0.311 0.318 0.430

Table 3. 8. Specificity: detected wavelengths for the encapsulated materials and agents.

The wavelength of the encapsulated materials:	
Vitamin B1	261 ± 4 nm (DW), 246 ± 1 nm (SGF)
Vitamin B2	266 ± 2 nm
Vitamin B3	266 ± 2 nm
Vitamin B12	361.4 ± 1 nm
The wavelength of the encapsulating agents:	
Gum arabic	225 ± 1 nm
Carrageenan	228 ± 3 nm
Chitosan	229 ± 1 nm
Mod. chitosan	220 ± 1 nm
Maltodextrin	227 ± 3 nm
Mod. starch	197 ± 1 nm
Sodium alginate	199 nm
Pectin	230 ± 1 nm
Xanthan	232 nm

3.6.2. Evaluation of release profiles

All release experiments required a certain amount of microparticles (Table 3.9.) placed in 3 mL of analysing liquid (DW and SGF) to be evaluated until all the vitamin content was released and the polymeric membrane was disintegrated.

Table 3. 9. The amount of microparticles used for a release experiment.

Encapsulated material	Amount of microparticles
Vitamin B1	4 mg
Vitamin B2	3 mg
Vitamin B3	5 mg
Vitamin B12	3 mg

The study “Optimization of work conditions”, Chapter 4, followed the effect of three factors: magnetic stirring (with and without agitation), analysing liquid (deionized water and SGF) and temperature (room temperature ≈ 20 °C, and human body temperature, 37 °C). Besides these three factors, during this study also the position of powder in the cuvette (on top or on the bottom of the analysing liquid) was evaluated.

Considering the results, it was decided for all further studies to place the sample on top of the analysing liquid, DW or SGF, control the temperature and adjust the intensity of magnetic stirring.

The amount of microparticles used for each experiment (mg/sample) was calculated through the mass balance of the reagents (assuming that during spray-drying process the ratio of the encapsulated material/encapsulating agent is constant). This calculation was previously optimized in another study of the authors [32].

The release process was different for every formulation because the time required for the vitamins to be released was not the same. Besides the comparison of the behaviour of different biopolymers used as encapsulating agents, it was made also a comparison between fresh and old samples. Therefore, to further evaluate the stability of the microparticles, samples were analysed again after a period of time during which the samples were stored and preserved (Table 3.10.). Release tests of old samples were repeated according to the method described for fresh samples.

Table 3. 10. Interval of time after which was done the stability evaluation for every study.

Study no.	Storage time interval
1	3 months 6 months
2	4 months
3	4 months
4	4 months

3.6.3. Encapsulation efficiency

Apart from the product yield, the success of the microencapsulation process was determined by another process factor, known as encapsulation efficiency. The calculation method is presented in Equation 2.14., presented in Subchapter 2.4.

3.6.4. Kinetic models

After the release profiles were established, the experimental data was adjusted to several kinetic models and after evaluating the fitting degree it was determined which model better describes the process of release for each vitamin. The selection of kinetic models included zero order, Higuchi, Korsmeryer-Peppas and Weibull models. The specific equations and parameters are described in Subchapter 2.3.5.

3.7. Statistical analysis of the experimental data

The statistical analysis was evaluated by the coefficient of variation (CV) and for all tests the values were lower than 10%. Each test was performed three times and the final result was calculated as the mean of the triplicates.

Chapter 4

Optimization of work conditions

The fourth chapter presents the first study made for this research that follows the optimization of work conditions using vitamin B12 as a model core material and modified chitosan as an encapsulating agent.
The study is in the format of the published paper:

***"Study of microencapsulation and controlled release of modified chitosan microparticles containing vitamin B12"**, Ioana C. Carlan, Berta N. Estevinho, Fernando Rocha, Powder Technology, 318, 162–169, 2017.*
DOI: 10.1016/j.powtec.2017.05.041.

4.1. Abstract

Vitamin B12 is a very important micronutrient essential for health (due to its functions) supplied by the diet, mostly, from animal sources. Its deficiency may cause critical health problems, whereby it is important to incorporate this vitamin in food supplements and additives. On the other hand, vitamin B12 is a sensitive compound and can be easily degrade during food process and storage. Through microencapsulation it is possible to overtake some of these limitations and improve its application in food industry. The aim of this work was to produce microparticles with vitamin B12 (1, 2, 3, 4 and 5% (w/w)) and to study its release namely in simulated gastric conditions (SGF). Modified chitosan was chosen as encapsulating agent, considering its biocompatibility and good solubility in water. The microparticles were prepared by a spray-drying technique and then characterized in terms of morphology (Scanning Electron Microscopy - SEM) and particle size (Laser Granulometry Analysis). An UV spectrophotometric method was validated, to evaluate the vitamin release and its stability. The average diameter of the particles varies with vitamin concentration, ranging from 3 to 8 μm, considering the differential volume distribution, and from 0.1 to 0.9 μm considering the differential number distribution. The product yield of the spray-drying process was around 57% for the microparticles with vitamin. The SEM images show microparticles with a regular round shape and a smooth surface. The release time increases with increasing of pH, and decreases with decreasing temperature. The Weibull kinetic model fits very well the experimental results obtained in SGF at 37 ºC. Microparticles stability was evaluated after 3 and 6 months and a small decrease in the amount of vitamin released was observed. Vitamin losses are less than 10% for 3 months and less than 20% for six months storage. So, microparticles of vitamin B12 with a good stability can be produced by spray-drying using modified chitosan.

Keywords: microencapsulation, modified chitosan, spray drying, vitamin B12, Weibull model.

4.2. Introduction

Vitamin B12, a water-soluble compound, known as the most complex vitamin in terms of chemical structure [4,35,38,40,186] is responsible for the following important life-sustaining biological functions: rapid DNA synthesis, red blood cell formation, cell division and development, and neurological functions. Also it promotes human growth and is bone marrow tissue responsible [32,38,187].

The main food sources of vitamin B12 are meat, seafood, eggs and dairy products. Vegetarians, pregnant women and people with food-bound B12 malabsorption are the three segments of population who may suffer from lack of vitamin B12 [4,52,187,188]. They are prone to develop pernicious anemia, the most common type of vitamin B12 deficiency, and should consider taking supplements of vitamin B12. The main cause is improper digestion, a disease of autoimmune origin in which the gastric mucosa suffers an atrophy process in the stomach [40,50,51,189]. Typical symptoms are: loss of appetite, weight, taste and smell, inflammation of tongue (glossitis), weakness, impotence, irritability, mild depression and memory impairment [4,50,51].

For the treatment of vitamin B12 deficiency, supplements with this vitamin are required [4,43,46,52]. So, when vitamin B12, provided from natural sources, is not enough, synthesized forms are requested for food and pharmaceutical applications. One solution to produce vitamin B12 enriched products can be by microencapsulation.

In the microencapsulation technique, a core (liquid drops, solid particles or gas compounds) is entrapped with a coating material (called encapsulating agent) [190,191]. In this way the core cannot come in contact with the external environment since a physical barrier is formed by the encapsulating agent for its protection [29,97]. Therefore, the microencapsulation of vitamin B12 brings several valuable benefits such as increased stability and protection from external factors (temperature, oxygen, light), easy handling and storage, ensured organoleptic properties, improved bioavailability and protection of reactions with other compounds [87].

Among all microencapsulation methods this research focuses on spray-drying technique to produce vitamin B12 microparticles. Spray-drying is considered to be the most used microencapsulating technique [87,192,193], especially in the food industry [194,195], since the late 1950 when flavors were microencapsulated to avoid degradation and oxidation processes. This

method is preferred over others because the process is simple, flexible and economical. The low cost and the good quality microcapsules obtained recommend spray-drying as a very good way to convert aqueous solutions into stable powders [30,87,196,197]. Another benefit of this ultra-fast drying and non-aggressive technology is that it offers unique possibilities for the design of the particles in terms of morphology [194,198].

For all these reasons the microencapsulation of vitamin B12 is an important issue. However, to the best of our knowledge, few papers have been published about the microencapsulation of the vitamin B12.

In the present work, modified chitosan was chosen as a shell material for the preparation of vitamin B12 microparticles, taking into consideration all the advantages of this natural biopolymer. Modified chitosan is obtained by carboxylation from chitosan and has the advantage of being water soluble, increasing its applicability in food and pharmaceutical processes. Chitosan is the N-deacetylated derivative of chitin, the most abundant natural amino polysaccharide, found in the exoskeleton of crustaceans (crabs and shrimp shells) and some insects [199–201]. Features like high biocompatibility and biodegradability, antimicrobial properties, ability to form films and non-toxicity [117,199,202] transformed chitosan into a very good carrier for drug controlled delivery achieved by encapsulation [117,119,132,202]. The biggest limitation of chitosan is its poor solubility in water and this was solved by changing its chemical structure. So, modified chitosan can be used in several applications in controlled drug-delivery systems like flavours [122], vitamins [32], immobilized enzymes [198,203,204], antioxidants [205] and cells [206]. In food industry it can also be used as additive, coating material, to retard microorganism growth [117,199,202,207].

For this study, modified chitosan microcapsules were obtained using a spray-dryer technique. Different microcapsules formulations were tested, namely with different amounts of vitamin B12: 1, 2, 3, 4 and 5% (w/w). The microparticles were characterized in terms of size and morphology and the release profiles were assessed by spectrophotometric analysis. The release profiles were obtained under different experimental conditions, pH (deionized water and simulated gastric fluid (SGF)), agitation (with and without agitation) and temperature (room temperature and 37 ºC). In a previous work [32] the authors have already optimized some operating conditions for the vitamin B12 microencapsulation with modified chitosan. However, important issues were not studied, such as the release profiles in simulated gastric conditions (*in vitro* studies) that are important to understand the absorption in the digestive

system. Stability studies were developed in the present work to evaluate the stability of the microencapsulated formulations and the viability of their use in food applications.

4.3. Materials and methods

Reagents

All reagents used were analytical grade.

Vitamin B12 was acquired from Sigma-Aldrich, China (product code: V2876, lot # MKBQ9972V, CAS-No. 68-19-9).

Modified chitosan was provided from China Easter Group (Dong Chen) Co. Ltd (lot SH20091010). This type of chitosan produced by carboxylation has a deacetylation degree of 96.5% and it was selected due to its properties: pharmaceutical grade and soluble in water with a viscosity of 5 mPa·s at 1%, 25 ºC.

Simulated gastric fluid was prepared using hydrochloric acid and sodium chloride. Hydrochloric acid was bought from Sigma-Aldrich (product code: 25,814-8, CAS-No.: 7647-01-0) and sodium chloride from AppliChem Panreac ITW Companies (product code: 131659.1211, lot 0000542745, CAS-No: 7647-14-5).

Preparation of solutions

All solutions were prepared at room temperature with deionized water.

Samples with different concentrations of vitamin B12 1, 2, 3, 4 and 5% (w/V) of vitamin B12 were prepared. Each sample was after mixed in the shaker for 10 minutes. A 1% (w/V) solution of modified chitosan was prepared and placed under stirring for 2 hours at a speed of 1200 rpm.

For the microencapsulation experiments, the samples of modified chitosan were mixed with the ones of vitamin B12 to obtain feed solutions for the spray-dryer. The stirring was made for 30 minutes at a speed of 500 rpm.

The simulated gastric fluid (SGF) was prepared as described in the European Pharmacopeia 7.0 (2010): 2.0 g of sodium chloride was dissolved in 7.0 mL of hydrochloric acid and then water was added until 1000 mL. Additional corrections of the pH were made to the stock solution of SGF, to obtain a final pH of 1.2, controlled by a pH-meter.

Preparation of microparticles: spray-drying process

Microcapsules of vitamin B12 were produced in a Mini Spray Dryer B-290 from BÜCHI (Flavil Switzerland), a laboratory scale device equipped with a two-fluid co-current nozzle atomization system. The feed solutions were spray-dried under the following conditions: solution and air flow rates, air pressure and inlet temperature were set at 4 mL/min (15%), 32 m^3/h (80%), 6 bar and 120 ºC, respectively. It was used a standard 0.5 mm nozzle, and the outlet temperature varied between 53 and 58 ºC.

The same operating conditions were set for all samples. This configuration was selected after some preliminary studies done to achieve the most feasible parameters of the spray-dryer equipment [32,122,207].

After the microcapsules were collected, they were kept at constant temperature (4 ºC) and protected from light, covered by aluminum foil.

Microparticles characterization

- **Scanning electron microscopy**

Scanning Electron Microscopy, SEM, was performed with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) at Centro de Materiais da Universidade do Porto (CEMUP).

The powder samples (microparticles) were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in vacuum, with a thin layer of gold in a Jeol JFC 100 equipment.

- **Laser granulometry analysis**

For the determination of microcapsules size a Coulter-LS 230 Particle Size Analyzer (Miami, USA) was used. By laser granulometry, particles were characterized by number and volume average. For each sample an average of three 60 seconds runs was set. The procedure was made after each sample was ultrasound - irradiated. Ethanol was used as dispersant solution in order to avoid the microparticles aggregation.

Controlled release profile studies

A spectrophotometric method was selected to confirm the presence of vitamin B12 in microcapsules and for the evaluation of the release profiles.

A spectrophotometer from Sarspec SPEC RES+ UV/VIS (Portugal) with Light Scan software and equipped with an external device for heating and stirring of the solution in the cuvette was used. Magnetic stirring and temperature control are operated via optical fiber connectors. For each test readings were done in the UV domain at 361.4 nm wavelength, where the absorbance is maximal, and the data acquisition was set for continuous recording every 30 seconds.

- **Validation of the analytical method**

The validation of this method was done with a calibration method that confirmed the possibility of release studies. For this, 13 standard solutions were prepared in the range of 0.0025 g/L to 0.1000 g/L at room temperature and with deionized water. The calibrations were done in triplicate with coefficients of variation smaller than 10%.

All readings were done at 361.4 nm wavelength. The calibration curve proved to be linear in the calibration range of 0.0025 to 0.1000 mg/mL and the linear regression equation was $y = 16.847x + 0.0601$ with a good correlation coefficient, $R^2 = 0.9951$.

To evaluate the limit of detection (LOD) and the limit of quantification (LOQ), it was necessary to calculate the standard deviation of response (σ) and the slope of the calibration curve (b), according to the following formulas: $LOD = (3.3 \times \sigma)/b$ and $LOQ = (10 \times \sigma)/b$. For this calibration curve LOD was estimated to be 0.0035 μg/mL and LOQ 0.0116 μg/mL.

The percentage of recovery (the percentage ratio between the obtained and the expected concentration of vitamin) was expressed by accuracy for the last 6 concentrations (107.8, 103.3, 99.9, 99.3, 98.8 and 96.6%).

- **Release studies**

Release studies required 3 mg of microparticles placed in 3 ml of analyzing liquid (deionized water and SGF) to analyze until all the vitamin content was released and the polymeric membrane was disintegrated. The position of powder in the cuvette (on top or on the bottom of the analyzing liquid) was optimized and decided to put on the top, considering the reproducibility, viability and stability of the results.

Taking into account the amount of reagents required for these experiments, the proportions of vitamin/modified chitosan, and the working parameters of spray-drying, the maximum concentration of the released vitamin was estimated through mass balance. This amount was after confirmed experimentally by the amount of vitamin released from modified chitosan microcapsules, according to the calibration curve values [32].

The optimization of work conditions followed the effect of three main factors: magnetic stirring (with and without agitation), analyzing liquid (deionized water and SGF) and temperature (room temperature ≈ 20 ºC, and human body temperature, 37 ºC).

- **Stability evaluation**

Release tests over time were made according to the method described before. The release profiles obtained (for 3 and 6 months storage) were compared with fresh samples.

4.4. Results and discussion

Production of microparticles

These assays are related to the optimization of the microencapsulation of the vitamin B12 with modified chitosan. Previous works of Estevinho et. al [204] and preliminary studies were used for the selection of the operating conditions for spray-drying experiments. Six microparticles formulations with vitamin B12 content of 0, 1, 2, 3, 4, and 5% (w/w) were produced as described in the experimental section.

The product yield of the spray drying process (quantity of powder recovered reported to the quantity of used raw materials) ranged from 56 to 58% for the microparticles with vitamin and 33% for microcapsules without vitamin.

The obtained results were considered to be good according to reported values from similar works: 41 – 56% for both vitamins C and B12 [32], 30 – 50% for spray-drying processes of galactosidase enzyme [203,204] and for a food flavour [122], and 40% for rosmarinic acid [205].

For this temperature, some particles deposition was observed on the cylinder and on the cyclone wall of spray dryer. The particles typical dimension is small (around 3 μm), and the efficiency of the cyclone to separate small particles decreases, some of them being aspirated with the air leaving the spray dryer. Also, the sample volume influences the product yield, small volumes implying higher relative losses [198]. All these factors influence the reported values of product yield. The product yield increases with the addition of vitamin. This can be attributed to the effect of molecule interaction. The vitamin addition to the polymer will create more stable particles.

Morphological characterization of microparticles

- **SEM Analysis**

The morphology of modified chitosan microparticles with and without vitamin was examined by SEM (Figure 4.1). SEM images show that modified chitosan microparticles, for any vitamin concentration, have a regular spherical shape and a smooth surface, also in agreement with a previous work [32]. In terms of size, both types of microcapsules, with or without vitamin, present a similar size. Abubakr et. al. [173] focused on other type of drying methods for vitamin B12 – loaded Ca-alginate beads revealed different type of morphology. For oven and vacuum dried

beads the particles were shrunken and with some cracks, the polymer structure network being collapsed. Furthermore, in the case of freeze-drying, the beads presented a spherical shape. Dohnal et. al [175] selected a drop-on-demand technology for incorporating vitamin B12 in microcapsules using also Ca-alginate as encapsulating agent. In this work, it was observed, in terms of morphology, that the shape changes from elongated to spherical to flattened according to the increasing of the viscosity value.

Sarti et al (2012) [176], prepared poly(acrylic acid)–cysteine microparticles containing vitamin B12 by a spray drying method and they also presented a spherical and smooth surface.

- **Laser Granulometry Analysis**

By Laser Granulometry Analysis, microparticles with an average diameter varying between 3 and 8 μm, considering the differential volume distribution, were obtained (Figure 4.2.). Considering the differential number distribution, the average diameter of the microparticles ranged from 0.1 to 0.9 μm (Figure 4.3.). The size distribution curves are unimodal. The differences between the average diameters of the microparticles obtained considering the different distributions can be associated to aggregation processes. Modified chitosan microparticles have the tendency to aggregate, fact confirmed also by SEM images. The aggregation will increase the average diameter of the microparticles when a volume distribution is considered.

On the other hand, the formation of very small particles, typical for the spray drying process, will decrease the average diameter when a number distribution is considered. The effect of increasing the vitamin concentration provokes a decrease in size of microparticles in terms of volume distribution. For the number distribution, it appears that there is not a clear tendency in the variation of size with the vitamin concentration.

Sarti et al (2012) [176] obtained, by spray drying, vitamin B12 microparticles with a mean diameter of 2.452 ± 2.26 μm using poly(acrylic acid)–cysteine as encapsulating agent. For the case of alginate beads, Abubakr et al. [173] obtained particles with a diameter of 0.68 to 0.81 mm and Rosiński et al. [208] obtained higher values, 0.25 to 2.001 mm.

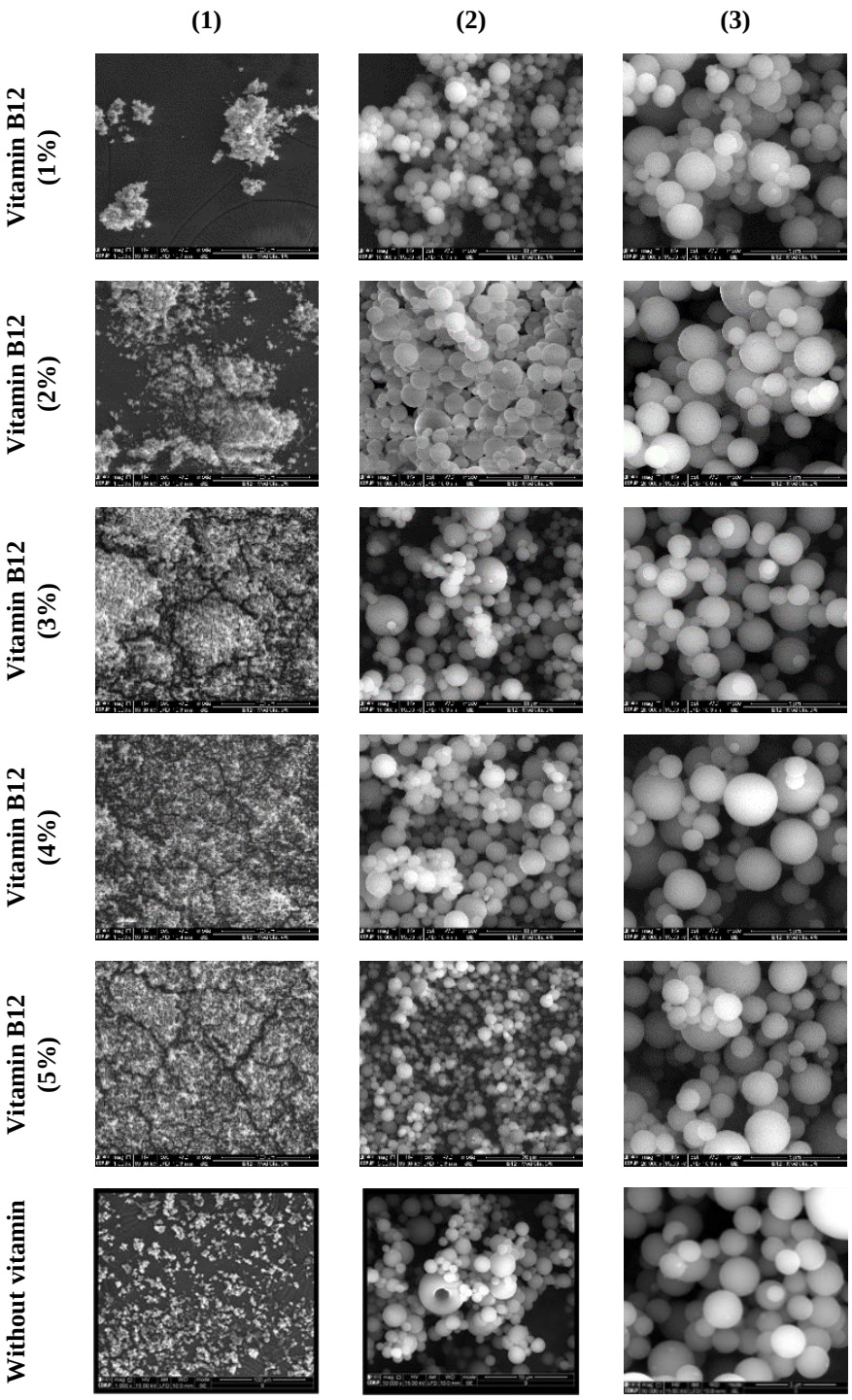

Figure 4. 1. SEM images of modified chitosan microparticles without and with vitamin B12. Magnification: (1) – 1000 times, (2) – 10000 times and (3) – 20000 times, beam intensity (HV) 10.00 kV, distance between the sample and the lens (WD) less than 12 mm.

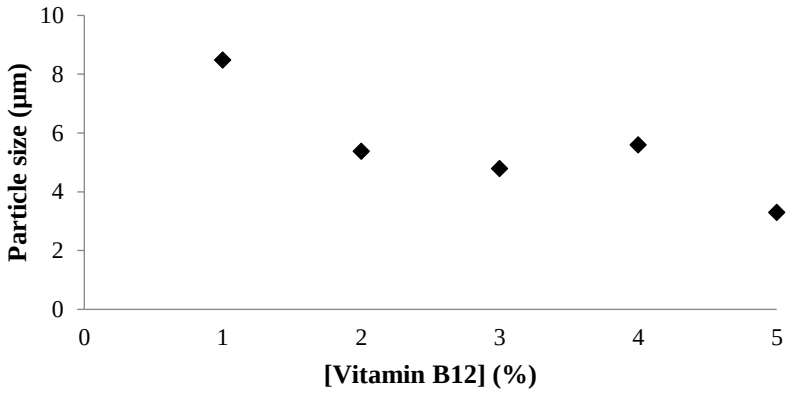

Figure 4. 2. Mean particle size in terms of volume for modified chitosan microparticles with vitamin B12 (1-5 (% w/w)).

Figure 4. 3. Mean particle size in terms of number for modified chitosan microparticles with vitamin B12 (1-5 (% w/w)).

Controlled release studies

- **Release studies**

The release assays are important to evaluate the bioavailability and the stability of the vitamin [209]. In these assays, several conditions were studied and optimized: stirring mode (static solution and stirred solution), release liquid (deionized water and SGF) and also the temperature (room temperature and human body temperature). Every test has been repeated three times and results are expressed by the mean value of the triplicates. Also, standard

deviation and coefficient of variation were calculated for all samples. The values of standard deviation are small and, to not affect the graphic quality of the figures, are not presented, the coefficients of variation are always less than 10%.

The release tests were performed in deionized water, neutral pH, and in simulated gastric solution, pH 1.2, to evaluate the bioavailability of the vitamin in the human body (in vitro). The oral route of vitamin B12 administration represents the most simple form, and with the highest compliance among users. However, the bioavailability of some compounds is limited for various reasons such as the short residence time of the dosage form at the absorption site and the poor membrane permeability [176]. Because of this it is so important to study and simulate the release profiles.

In Figure 4.4., the results of the assays performed in deionized water at room temperature with magnetic stirring are presented. In these conditions vitamin B12 concentration reaches its highest value (total release) in less than 10 min, depending on the powder concentration.

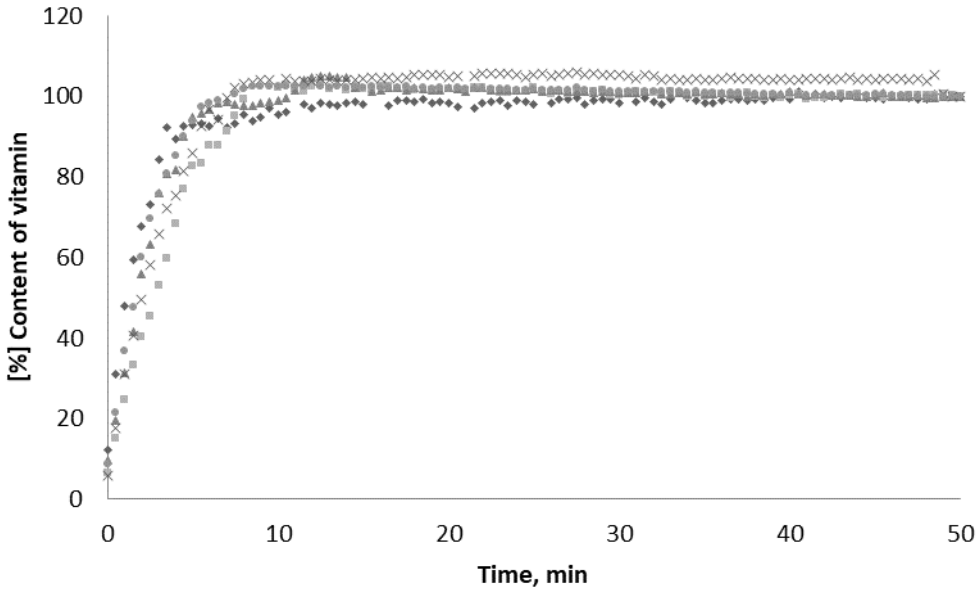

Figure 4. 4. Release profile for vitamin B12 microparticles made in deionized water at room temperature, with magnetic stirring: ♦ 1%, ■ 2%, ▲ 3%, × 4%, ● 5% (w/w) of vitamin B12.

Figure 4.5. presents the results obtained in SGF at the same conditions of previous figure. The time needed to achieve the total release was longer and increases with increasing of the concentration of vitamin B12 in powder.

Comparing Figure 4.4. with Figure 4.5., we can observe the effect of the pH in the release profile. In the first case a very fast release (10 min) is observed, while in the case of SGF experiments the release lasts approximately 10 times more. This phenomenon is related with the pH of the release solution. Modified chitosan is soluble in water (neutral pH), its solubility decreasing with the decrease of pH.

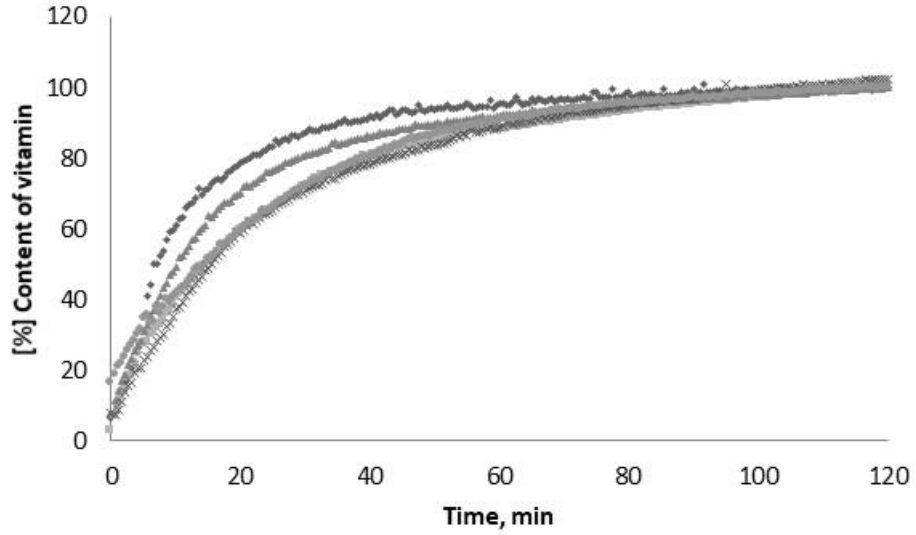

Figure 4. 5. Release profile for vitamin B12 microparticles made in SGF at room temperature, with magnetic stirring: ♦ 1%, ■ 2%, ▲ 3%, × 4%, ● 5% (w/w) of vitamin B12.

The effect of the temperature, 37 ºC, on the release in SGF is shown in Figure 4.6. It can be observed that the release is faster than for room temperature, the maximum value being around 40 min. The concentration of vitamin B12 in the powder seems to not affect the release time.

So, the presence of vitamin B12 in microparticles prepared by spray-drying process was confirmed in the release tests by UV measurements.

Sarti et al (2012) [176] tested, in in vitro studies, the release of Vitamin B12 from poly(acrylic acid)–cysteine microparticles in PBS at pH 7.2. The total amount of vitamin B12 was released within 3 h and they concluded that PAA–cysteine conjugate improves the permeation of Vitamin B12 across the intestinal mucosa [176].

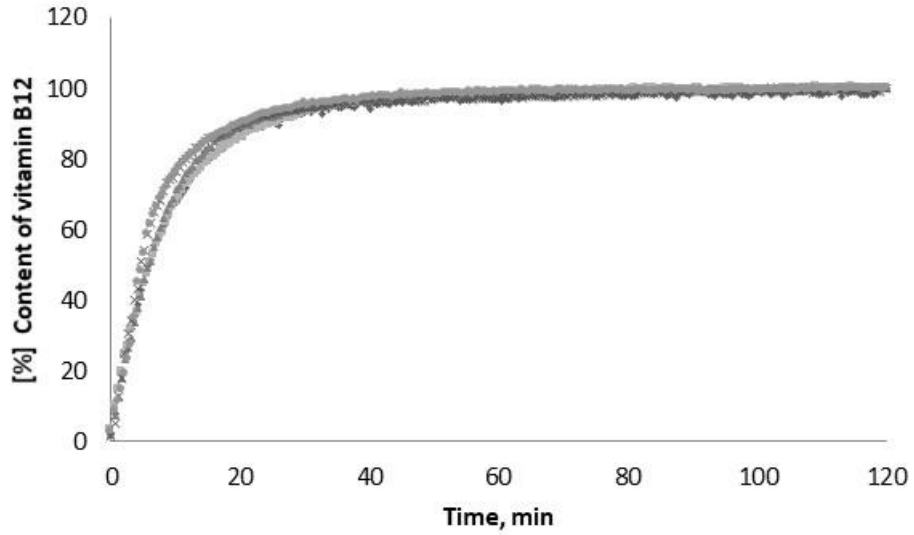

Figure 4. 6. Release profile for vitamin B12 microparticles made in SGF at human body temperature (37 °C), with magnetic stirring: ♦ 1%, ■ 2%, ▲ 3%, × 4%, ● 5% (w/w) of vitamin B12.

• **Kinetic models**

A previous work of the authors has already focused the application of different models to the release profiles of the vitamin B12 from modified chitosan microparticles in deionised water. Estevinho and Rocha (2017) [210] studied the controlled release of two vitamins (vitamin B12 and C), microencapsulated by a spray-drying process. The release equation that best adapts to the experimental results was the Weibull model. The Weibull model is more adequate to compare the release profiles of matrix type drug delivery [134,211], which is the case of the microcapsules produced by spray drying, which are normally matrix type (with the encapsulated substance distributed in the encapsulating agent), and the mechanisms of release involved are typically controlled by solvents action and by diffusion [27,212,213].

In the present study, considering that only the medium of the release of the microparticles was different of the previous one, it is expected to obtain similar results. For this reason, it was only applied the Weibull model (Equation 4.1.), for the assays with SGF at 37 ºC, and as expected with very good correlation coefficients. In Table 4.1, the parameters of Weibull model are presented.

$$\boldsymbol{M_t = M_\infty \left[1 - e^{-\left(\frac{t-t_0}{\tau_d}\right)^{\beta}}\right]} \qquad (\mathbf{4.1.})$$

β represents the shape parameter of the curve and τ_d the time (min) when 63.2% of *M* has been dissolved/released [134,210,211]. For β=1, the shape of the dissolution/release curve corresponds exactly to the shape of an exponential profile and for β<1 the shape of the curve would show a steeper increase than the one with β=1 [210].

Table 4. 1. Weibull model parameters.

Weibull model			
Microparticles	**τ_d experimental values (63.2% of the release) (min)**	**τ_d calculated (min)**	**β**
1%	8.0	7.9	0.91
2%	8.0	7.3	0.96
3%	8.0	9.1	0.93
4%	6.0	6.8	0.69
5%	6.5	7.7	0.86

• **Stability of the microparticles: three and six months storage**

Using the optimized conditions, release tests were repeated, for three and six months storage, to evaluate the stability of the microparticles. The results are shown in Figure 4.7. Analyzing Figure 7, one can conclude that the time release decreases with storage time, mainly for the 6 months storage samples. In what concerns the effect of vitamin concentration the differences are not significant.

Table 4. 2. Mass loss of vitamin B12 over time (after 3 and 6 months) in percentage for each type of microcapsules formulation: 1, 2, 3, 4 and 5% (w/w) of vitamin.

Vitamin B12 content in microcapsules (% (W/W))	**Mass loss of vitamin B12 (%) after**	
	3 months	**6 months**
1%	8.5	16.0
2%	8.5	15.0
3%	5.0	14.3
4%	2.8	9.1
5%	0.5	19.2

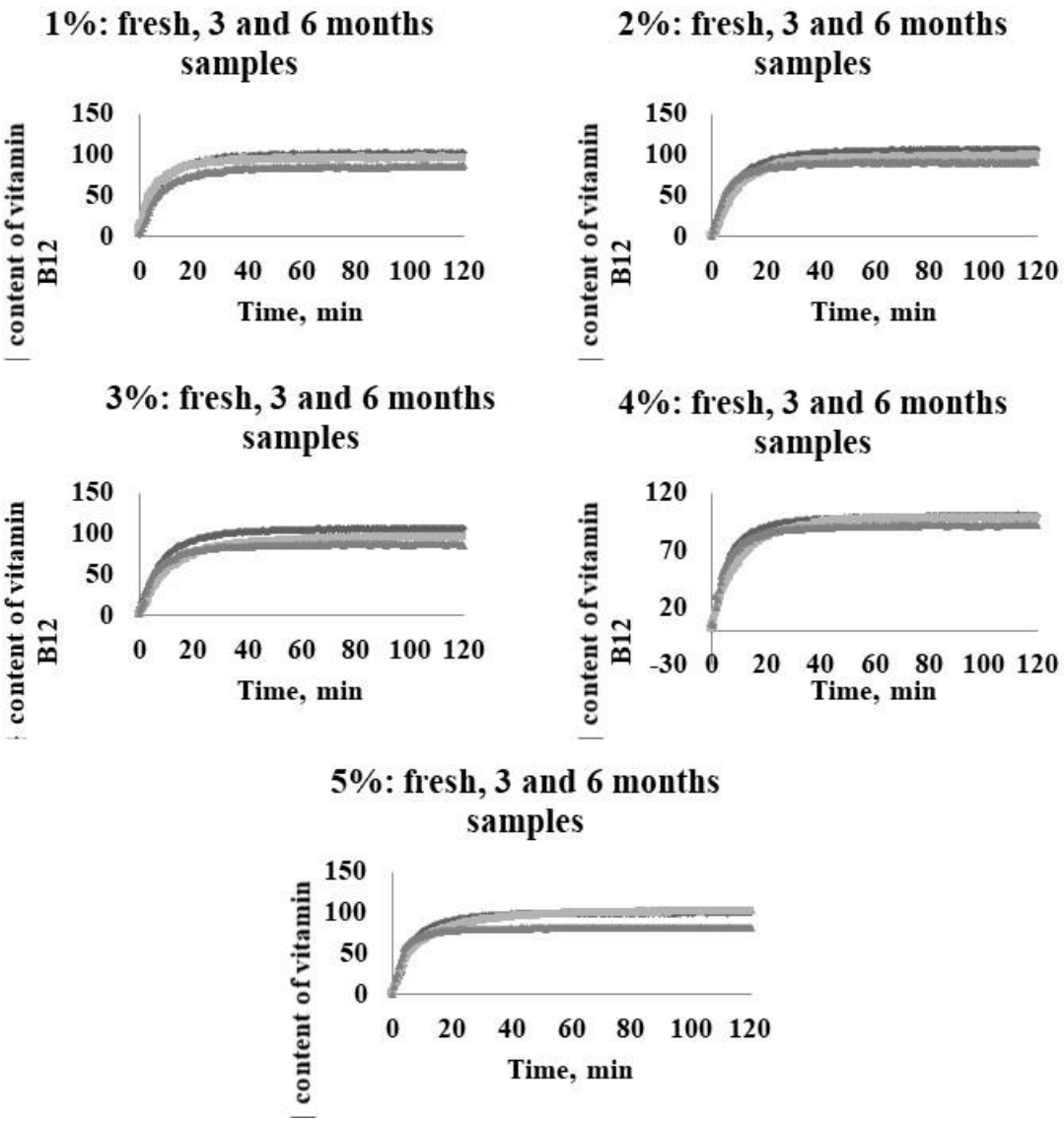

Figure 4. 7. Comparison of release profiles for SGF at 37 ºC: ♦ fresh, ■ 3 and ▲ 6 old months samples with 1%, 2%, 3%, 4% and 5% (w/w) content of vitamin.

The mass loss of vitamin B12 in terms of percentage, during the two time intervals is shown in Table 4.2. In the case of three months samples, a small vitamin loss, less than 8.5%, for all the microparticles (1-5% (w/w)), was registered. This loss decreases with the increase of vitamin concentration. A higher decrease of vitamin was observed (19.2%) in the case of 6 months storage for the microparticles containing higher amount of vitamin. These losses of vitamin are very acceptable, less than 10% for 3 months and less than 20% for six months storage.

So, it can be concluded that the microencapsulation by spray drying is a good process to prepare microparticles with vitamin B12, the stability of the vitamin being maintained over 6 months.

Through this way, the vitamins will be protected from oxidation, light, moisture and other factors during the storage time. On the other hand, a fast release associated to the modified chitosan can be an advantage to use this type of microparticles for applications in medical and food industry.

4.5. Conclusion

The aim of this study was to investigate the release of vitamin B12 from modified chitosan microparticles in a controlled way and in simulated gastric conditions. The performed tests yielded worthwhile results, proved the success of the vitamin B12 microencapsulation and gave important information about the parameters that affect the release of these vitamins from the microcapsules.

From SEM images, it can be observed that microcapsules have approximately the same aspect for all the formulations tested: spherical shape, uniform and smooth exterior surface. Results obtained by laser granulometry seem to show that particle size increases with the vitamin concentration.

Several conditions and work parameters were tested in order to select those that were considered to be feasible in terms of reproducibility and release profiles. The evaluation of vitamin B12 release through a spectrophotometric method was possible and the best results are obtained when the sample is released in SGF under continuous stirring at 37 ºC.

The release results obtained in SGF, for human body temperature, are well fitted by Weibull model.

Stability tests for three and six months samples showed good results; vitamin losses less than 10% for 3 months and less than 20% for six months storage were obtained.

Chapter 5

Microencapsulation of vitamin B12

The second study focused on the microencapsulation of vitamin B12 and is presented in the fifth chapter of the thesis.
The study is in the format of the published paper:

"Study of different encapsulating agents for the microencapsulation of vitamin B12", *Ioana C. Carlan, Berta N. Estevinho, Fernando Rocha, Environmental Engineering and Management Journal (EEMJ), 17(4), 855-864, 2018.*
DOI: 10.30638/eemj.2018.086.

5.1. Abstract

Recently, the studies about vitamin B12 increased due to the high number of people who can develop vitamin B12 deficiency, namely: vegetarians, pregnant women or with vitamin B12 malabsorption. One solution to correct the low nutritional intake of vitamin B12 can be using food supplements or pharmaceuticals, based on the vitamin B12 microencapsulation. In the present research, the vitamin B12 microencapsulation and the controlled release of fresh and 4 months' storage samples of vitamin B12 microcapsules were studied. The microcapsules were prepared using a spray-drying technique, and 7 biopolymers were used as encapsulating agents: arabic gum, sodium alginate, carrageenan, maltodextrin, modified starch, xanthan and pectin. The product yield of the spray-dryer ranged from 20 to 50 %. The microparticles were also characterized in terms of size and morphology. The vitamin B12 release profiles from microcapsules were assessed by spectrophotometric analysis, at 361.4 nm, in deionised water at 22 ºC and simulated gastric fluid at 37 ºC. This study showed that the vitamin B12 microcapsules, with good stability properties, can be produced with several encapsulating agents and proved the possibility of releasing the vitamin in different periods of time.

Keywords: Biopolymers, encapsulating agents, microencapsulation, spray drying, vitamin B12.

5.2. Introduction

Vitamins delivery systems used for health maintenance improved significantly in the last decades, due to the new food diet varieties and the challenge of treating diseases in a fast and less painful way possible. Daily administration of vitamins is mandatory [1,214] therefore it is a constant "battle" for the industry fields to produce new food and pharmaceutical products with vitamin content [26,28]. The main problem of these essential organic compounds is their stability, so a protective system to keep their properties active and to avoid any type of degradation is necessary [28].

Microencapsulation is a method widely used for drug controlled delivery and is suitable also for vitamins, since it was tested with success for many sensitive bioactives [26,28,32,87,100,203,215,216].

This research work chose vitamin B12 as a model core material to be tested by spray-drying technique with several encapsulating agents within the biopolymers class. Known also as cyanocobalamin, vitamin B12 is a water-soluble vitamin, stable in normal conditions if not exposed to direct light [1,4,187,214].

In the case of balanced diet, which includes also products of animal origin, people are rarely found to suffer from vitamin B12 deficiency, since the human body can store more than the required amount. However, vegetarians and pregnant women or during lactation are predisposed to suffer from this type of deficiency. For them and those patients who suffer from vitamin B12 malabsorption, a way to correct the level of vitamin must be found [39,46,52,187,217,218].

Spray-drying is known as the most used microencapsulation method for food industry applications. Stable powders can be obtained from a one-step process of atomization. Also because of the low cost, continuous work mode, easy handling of equipment, spray-drying process is preferred among other microencapsulation techniques [28,29,87,98,124,219].

There is a big interest in changing the synthetic materials used for microencapsulation, so biopolymers will be a good alternative [184]. The option of using biopolymers, as encapsulating agents for the spray-drying process, can bring several advantages: bioavailability, biocompatibility, stability and no toxicity [119,132]. For all these reasons, biopolymers can be considered suitable for the protection of vitamin B12, and further use of the microcapsules for applications of food and pharmaceutical industry, since the consumer is not exposed to any risks [26,194].

The aim of this study was to investigate possible shell materials suitable for vitamin B12 oral delivery systems. These biopolymers materials are carbohydrates of different origin plants: arabic gum, maltodextrin and pectin; marine: sodium alginate and carrageenan; and also microbial origin: xanthan [26,28,124]. Microparticles were prepared by spray-drying method and then characterized regarding their size and surface aspect, using laser granulometry analysis and scanning electron microscopy (SEM). Finally, release profiles were evaluated by spectrophotometric analysis: tests were performed in deionized water at ambient room temperature (22 ºC) and in simulated gastric fluid (SGF) at the human body temperature (37 ºC).

The microencapsulation of vitamin B12 can be an option to integrate the vitamin in products that belong to the class of food supplements and pharmaceuticals, like: enriched food products, instant drinks, effervescent capsules, ready to eat cereal-bars, and multivitamin compressed caps.

5.3. Materials and methods

Material

High purity and pharmaceutical grade reagents were selected for this work. Vitamin B12 was provided from Sigma-Aldrich, China (V2876, Lot # MKBQ9972V). Arabic gum from acacia tree was acquired from Fluka, Germany (30888, Lot #BCBK8649V), pectin from apples was obtained from Sigma-Aldrich, Switzerland (101582340 76282, Lot #BCBN5335V) and modified starch from Alfa Aesar GmbH&Co KG, Germany (36673, Lot D04X013). From Sigma-Aldrich, USA were purchased: sodium alginate (1001503523 180947, Lot # MKBH8463V), maltodextrin (1001841656 419672, Lot # MKBN6629V), carrageenan (1001761179 C1013 -1006, Lot # SLBH9868V) and xanthan gum from Xanthomas campestris (1001900732 G1253, Lot # MKBQ9467V).

For the formulation of the simulated gastric fluid, hydrochloric acid from Sigma-Aldrich (25,814-8) and sodium chloride from AppliChem Panreac ITW Companies (131659.1211, Lot 0000542745) were used.

Preparation of solutions

A different spray-drying feed solution was prepared for each one of the 7 encapsulating agents. Solutions with a content of 1 % (w/V) encapsulating agent (placed under stirring for 2 hours at a speed of 1200 rpm), and solutions with 2 % (w/V) of vitamin B12 (mixed in the shaker for 10 minutes) were prepared.

For the microencapsulation experiments, the encapsulating agent samples were mixed with vitamin B12 samples to obtain feed solutions for the spray-dryer. 100 mL of encapsulating agent solution were mixed with 10 mL of vitamin B12 solution. The stirring was made for 30 minutes at a speed of 500 rpm. Feed solutions without vitamin content were prepared for the analysis of empty microcapsules.

The formulation of simulated gastric fluid (SGF) followed the European Pharmacopeia 7.0 (2010). A stock solution of SGF (1 L - aqueous solution) was prepared with 2.0 g of sodium chloride and 7.0 mL of hydrochloric acid 37%. Additional pH corrections were done with a pH-meter until a final pH value of 1.2 was reached.

All solutions were prepared at room temperature with deionized water.

Spray-drying process

The microparticles (empty and with vitamin B12) were prepared by a spray-drying technique in a Mini Spray Dryer B-290 from BÜCHI (Flavil Switzerland) with a standard 0.5 mm nozzle. For the shell materials, the following 7 biopolymers were used: arabic gum, sodium alginate, carrageenan, maltodextrin, modified starch, xanthan and pectin.

Each solution was fed up under the following experimental conditions: solution and air flow rates - 4 mL/min (15%), 32 m^3/h (80%), air pressure and inlet temperature - 6 bar and 120 ºC, respectively. The outlet temperature varied between 56 and 67 ºC, in the case of vitamin B12 microcapsules, and between 60 and 68 ºC, for the empty microcapsules. The same experimental conditions were applied for all the samples and were selected based on the literature and some previous studies [27,32,87,205].

The final products were stored and kept for further tests at 4 ºC, protected from light, to avoid degradation processes.

Microparticles characterization

The characterization of microparticles followed 2 types of analysis: surface morphology and particle size distribution. The surface morphology was examined by Scanning Electron Microscopy (SEM) and was performed with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) at Centro de Materiais da Universidade do Porto (CEMUP). The powder samples (microparticles) were previously fixed on a brass stub using double-sided adhesive tape and then were made electrically conductive by coating, in vacuum, with a thin layer of gold in a Jeol JFC 100 equipment.

For the determination of particle size distribution, a Coulter-LS 230 Particle Size Analyzer (Miami, USA) was used. By laser granulometry, particles were characterized by number and volume average. For each sample an average of three 30 seconds runs was set. The procedure was made after each sample was ultrasound-irradiated, and ethanol was used as dispersant solution to avoid the microparticles aggregation.

Analytical method for controlled release profile studies

An analytical method was chosen to confirm, by spectrophotometry, the presence of vitamin B12, as an active core inside the microcapsules. The release tests were carried out in 2 types of

dissolution mediums at different temperatures: deionised water at 22 ºC and SGF at 37 ºC. The evaluation of vitamin B12 was done by measuring the absorbance with a spectrophotometer from Sarspec SPEC RES+ UV/VIS (Portugal), equipped with an external device for heating and stirring of the analyzed solution. For each test, readings were done in the UV domain at 361.4 nm wavelength and the data acquisition was set for continuous recording every 30 seconds in the case of the following encapsulating agents: arabic gum, sodium alginate, carrageenan, maltodextrin, xanthan and pectin, and every 5 seconds just for modified starch.

This method was first validated to prove the possibility of running the release studies. 13 standard solutions, in the range of 0.0025 g/L to 0.1000 g/L, were analyzed at room temperature. A linear calibration curve was obtained, with a good correlation coefficient R^2 = 0.9918. The limit of detection (LOD) was estimated to be 0.0563 µg/mL and the limit of quantification (LOQ) 0.1876 µg/mL.

For the release studies, 3 mg of microparticles were placed inside the cuvette filled with 3 ml of analyzing liquid. The quantity of vitamin B12 microcapsules was selected according to the methodology described in a previous work [32], being determined by mass balance of reagents used, assuming that during the spray-drying process the ratio of core material/encapsulating agent remained constant. Continuous stirring mode was set on for the cuvette and, depending on the type of analysis, temperature was adjusted (first round of experiments at 22 ºC, second one at 37 ºC). Tests were considered finished when all the vitamin content was released, corresponding to the stabilization in time of the value of absorbance. Samples were stored protected from light, in the fridge (4 ºC) for 4 months and then were tested as described in this section, to check the stability of the samples.

All release tests and the calibration solutions were performed in triplicate, showing coefficients of variation smaller than 10 %.

5.4. Results and discussion

Product yield

The product yield (%) of the spray drying process was determined for each system studied and was expressed as a ratio of recovered powder reported to the introduced amount of raw materials (Figure 5.1.).

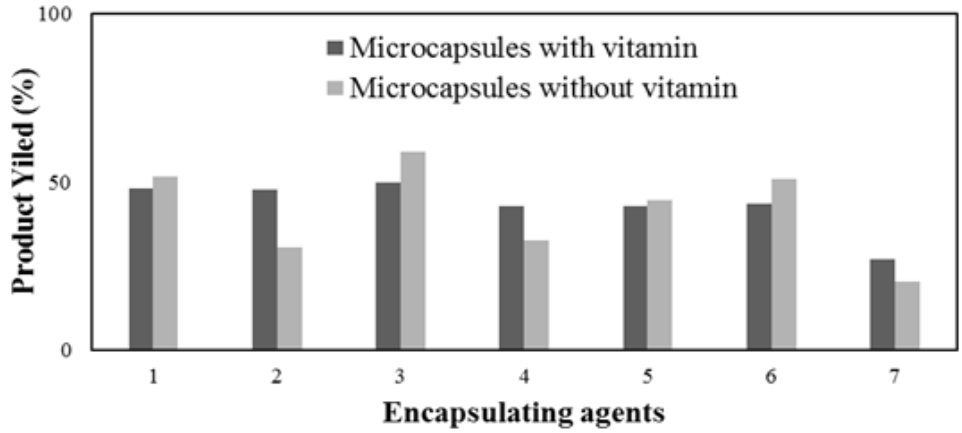

Figure 5. 1. Product yield for spray-drying process (1 – arabic gum, 2 – carrageenan, 3 – maltodextrin, 4 – modified starch, 5 – pectin, 6 – sodium alginate, 7 – xanthan).

The results obtained for both types of microparticles are between 27 and 50 % for vitamin B12 microparticles, and between 20 and 59 % for empty ones, as shown in Figure 5.1. These values are acceptable compared with previous works: 41 – 56 % for vitamins B12 and C [32] , and 30 – 50 % for food flavours [122] and enzymes [198,204]. The only encapsulating agent having a low product yield is xanthan: 27 % for microparticles with vitamin B12 and 20 % for the empty ones.

Microparticles characterization in terms of size and morphology

All microparticles, with or without vitamin B12, were characterized regarding their surface morphology and their size. The specific morphology for each biopolymer was observed in SEM images. In Figure. 5.2, microcapsules with spherical shape and smooth surface, corresponding to sodium alginate, carrageenan, maltodextrin and pectin, are presented. Microcapsules for the other 3 encapsulating agents, arabic gum, modified starch and also xanthan, presenting

spherical shape with rough surface, can be observed in Figure 5.3. The morphology found in the first group of microparticles was also obtained for vitamin B12 microparticles with modified chitosan and sodium alginate [32] and with poly (acrylic acid)-cysteine [176].

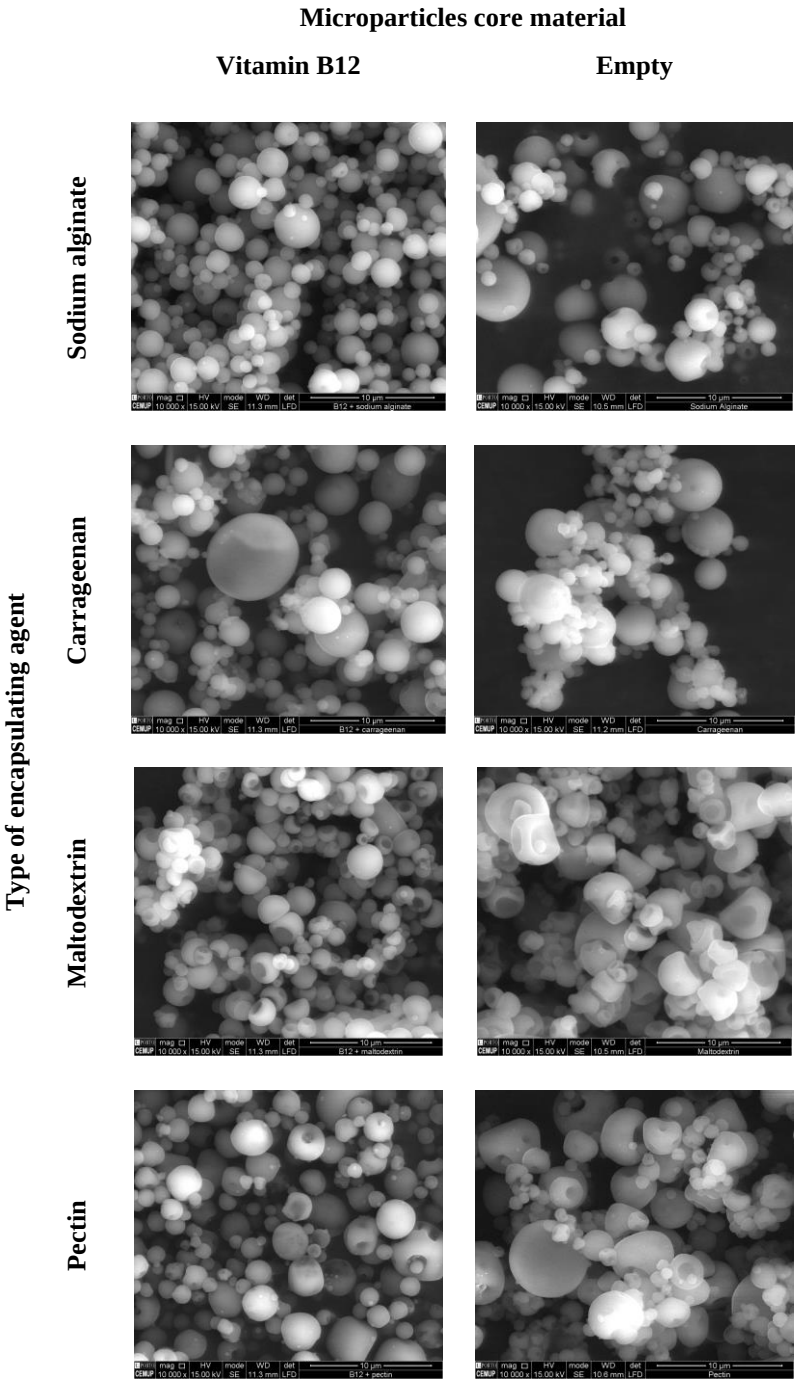

Figure 5. 2. SEM images of sodium alginate, carrageenan, maltodextrin and pectin microcapsules, with and without vitamin B12. Magnification = 10. 000 times, beam intensity (HV) 15.00 kV, distance between the sample and the lens (WD) around 10 mm

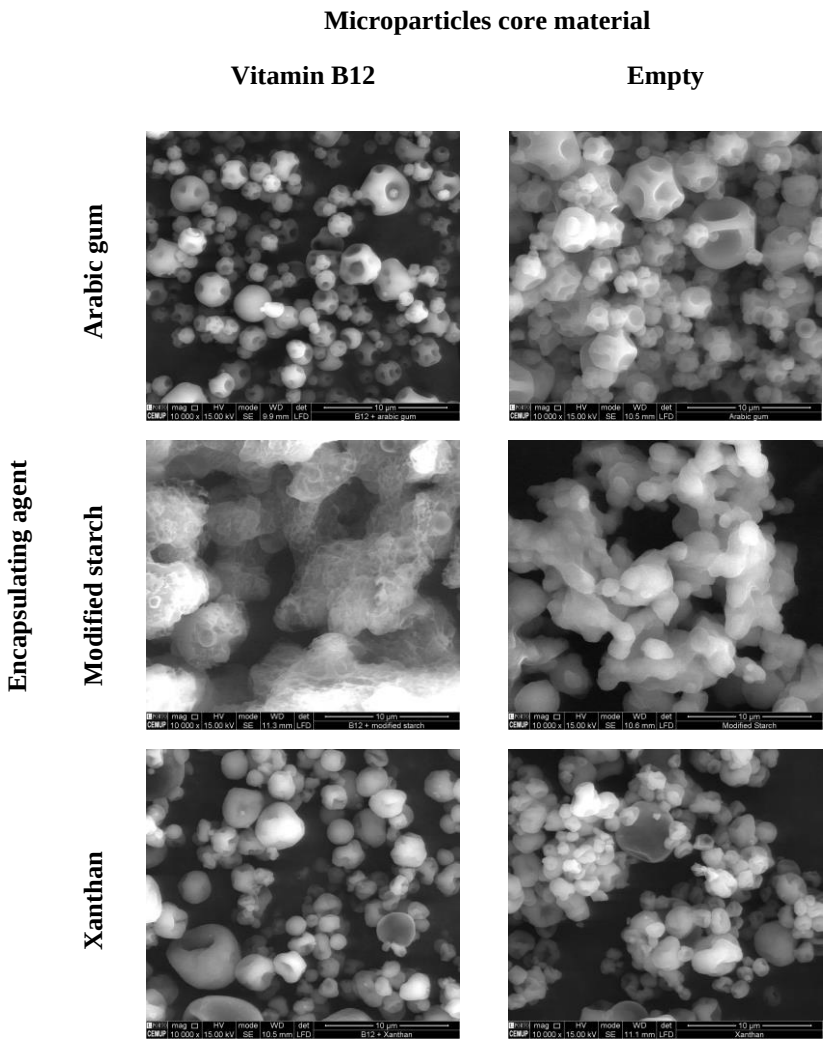

Figure 5. 3. SEM images of arabic gum, modified starch and xanthan microcapsules with and without vitamin B12. Magnification = 10. 000 times, beam intensity (HV) 15.00 kV, distance between the sample and the lens (WD) around 10 mm.

On the other hand, similar morphology to the second group of encapsulating agents was obtained with chitosan capsules [32]. Size characterization of microparticles performed by laser granulometry shows different sizes for microcapsules with and without vitamin B12 (Table 5.1).

For microparticles with active core material the average diameter according to volume distribution increases from 3.17 up to 6.67 μm in this order: arabic gum, sodium alginate, maltodextrin, carrageenan, xanthan and pectin. As for the number distribution, the value of

average diameter is maintained constant at approximately 1 μm, suggesting aggregation processes, fact highlighted also by the SEM images.

Empty microcapsules present mean sizes, for volume distribution, higher than the microcapsules with vitamin content. Regarding the number distribution of empty microcapsules, it can be observed that the behavior is the opposite; all the values are smaller than 1 μm excepting the sizes of pectin and modified starch capsules, 1.77 and 3.62, respectively. It was not possible to obtain the size distribution of the empty maltodextrin capsules.

Table 5. 1. Particle mean size by laser granulometry considering volume and number distribution for microcapsules with vitamin B12 and for empty microcapsules

		Differencial volume distribution		**Differencial number distribution**	
		Microcapsule core material			
		Vitamin B12	**Empty**	**Vitamin B12**	**Empty**
Encapsulating agent	**Arabic gum**	3.17	4.22	0.96	0.57
	Sodium alginate	3.35	10.18	0.93	0.60
	Maltodextrin	4.83	-	0.94	-
	Carrageenan	5.16	7.44	0.98	0.54
	Xanthan	6.58	7.56	0.93	0.72
	Pectin	6.67	4.66	0.99	1.77
	Modified starch	32.17	42.00	2.74	3.62

Regarding modified starch microparticles, the sizes found are much higher than for the other encapsulating agents, even in the case of empty microcapsules. As shown in the Table 5.1, for vitamin B12 capsules the obtained values are 32.17 μm for volume distribution and 3.62 μm for number distribution. SEM images for this biopolymer, Figure 5.3, show a significant particle aggregation.

Controlled release studies for fresh and 4 months samples

The controlled release profiles of vitamin B12 from biopolymers microcapsules were evaluated based on spectrophotometric measurements done at vitamin's specific wavelength: 361.4 nm. Release tests were performed using two different solvents: deionized water at room

temperature to simulate the main conditions at food industry, and SGF solution at 37 ºC to recreate human gastric conditions. Different release profiles behaviors were observed, demonstrating the effect of the type of microparticle, pH and temperature. The evaluation of the release of vitamin B12 was considered to be complete when the value of the absorbance remained stable.

Taking into account the different types of found morphologies, the release profiles are presented in two figures. In Figure 5.4, one can observe the release profiles for the following encapsulating agents: sodium alginate, carrageenan, maltodextrin and pectin. In this figure, the profiles obtained in the two solvents, water and SGF, for fresh samples, are presented. In Figure 5.5 the same is made for the other three encapsulating agents: arabic gum, modified starch and xanthan.

As can be observed in Figures. 5.4 and 5.5, microparticles tend to dissolve gradually, some of them slowly, some moderately or very fast. The microparticles of arabic gum, carrageenan, maltodextrin and modified starch present fast releases, very fast in the case of modified starch, and similar profiles. In all these cases, the release profiles in water and in SGF are almost equal and the release is completed in less of 5 min. The type of release is provoked by the type of encapsulating agent used, the sensibility of the biopolymer to the change of the pH, and the type of interactions between the encapsulating agent and vitamin that are established. In these specific cases the release profile had the same behaviour in water and in SGF for each encapsulating agent used. So, for these specific cases the type of solvent is irrelevant. Regarding sodium alginate microparticles the release is moderate, less than 15 min in both solvents, and one observes a different profile in the release in water, this release being also slower.

In the case of pectin microparticles, the release is completed in less than 70 min in water, faster in SGF, but the release profile is similar for the two solvents. Xanthan showed to be the most sensitive polymer to pH and temperature changes. The release is slower, and the effect of the solvent is pronounced: the release changes from 1 h in SCF to about 15 h in water, and the profile is different.

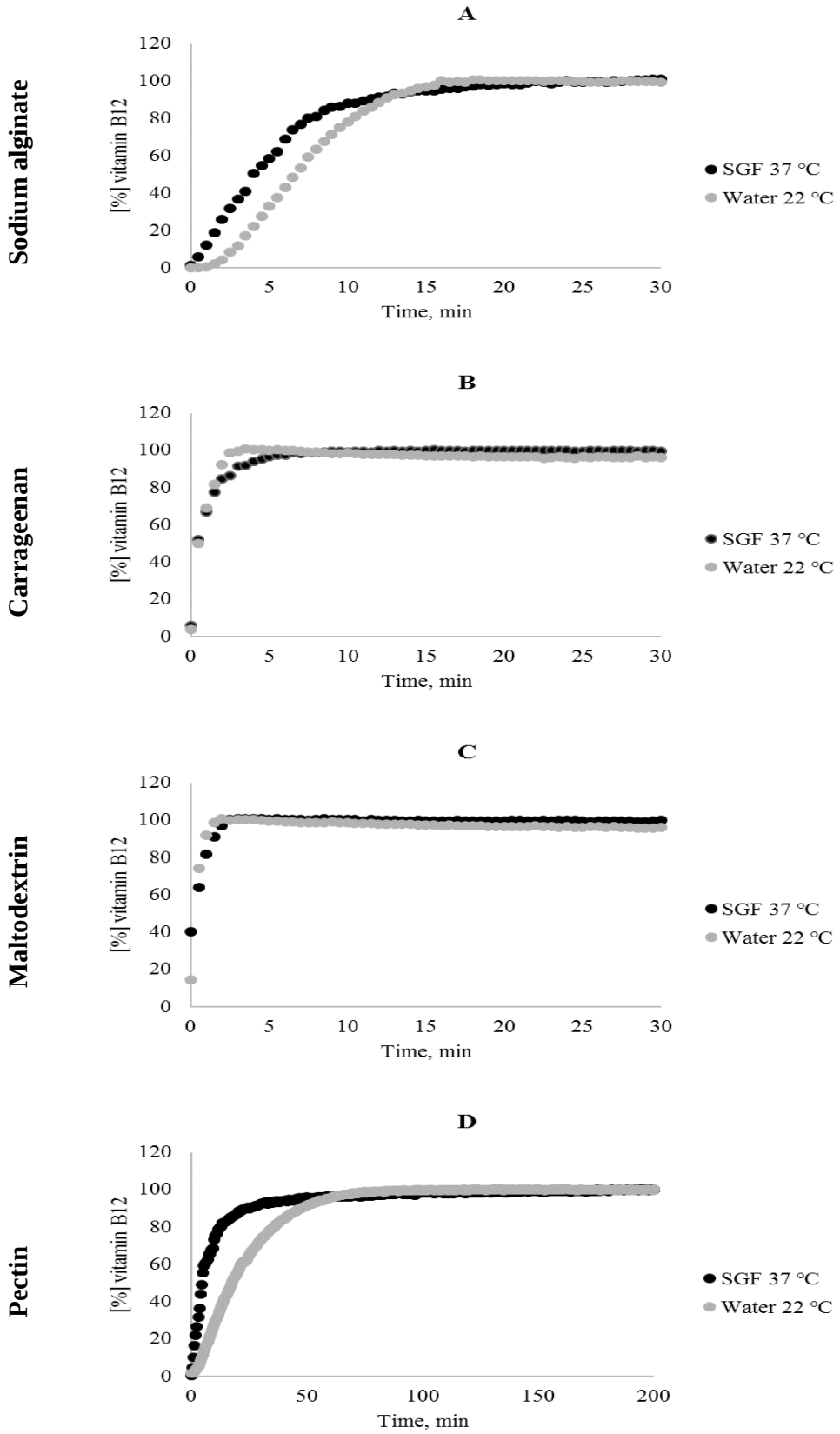

Figure 5. 4. Vitamin B12 release profile performed in: SGF at 37 ºC and deionized water at 22 ºC from microcapsules of (A) – sodium alginate, (B) – carrageenan, (C) – maltodextrin and (D) – pectin.

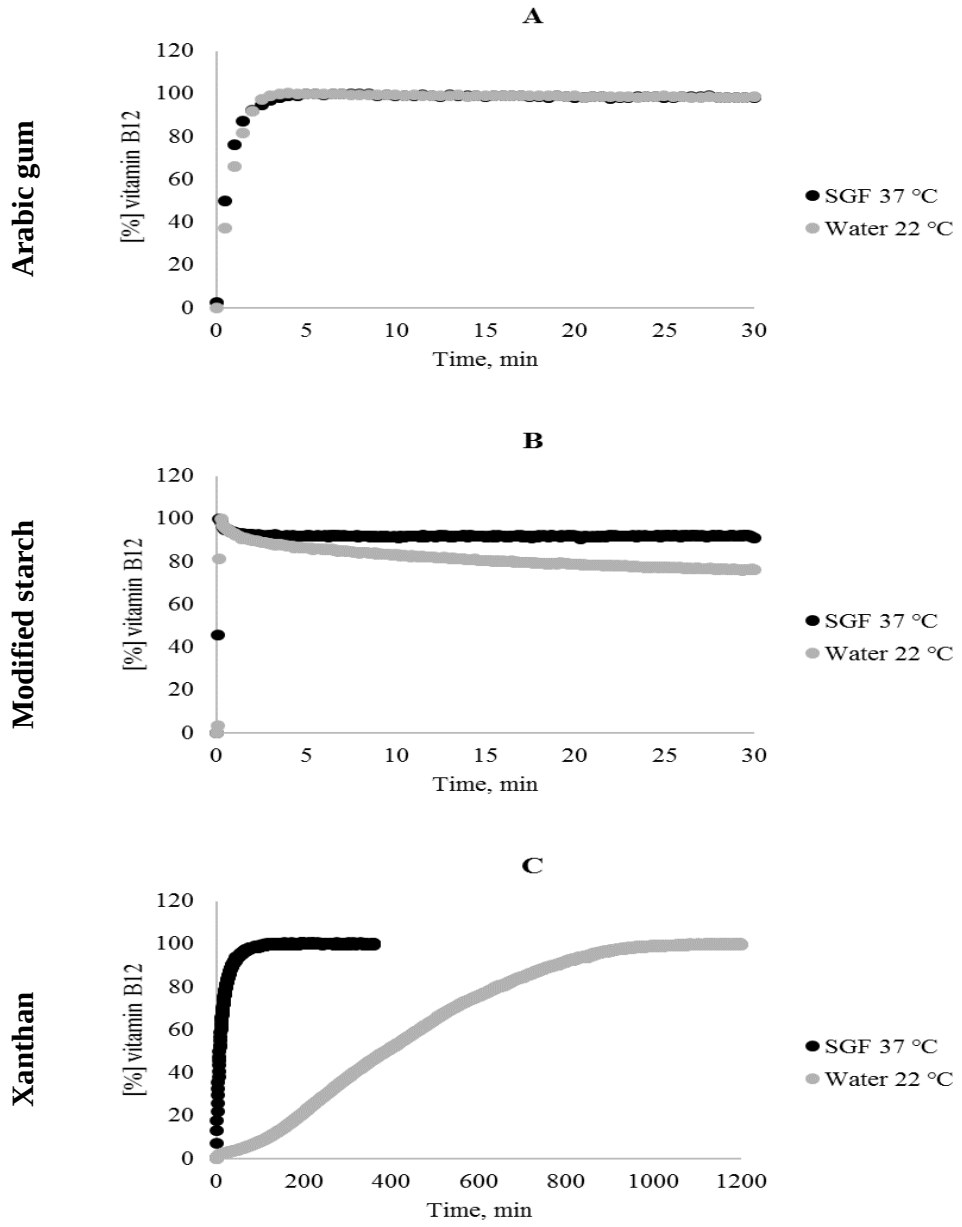

Figure 5. 5. Vitamin B12 release profile performed in: SGF at 37 ºC and deionized water at 22 ºC from microcapsules of (A) – arabic gum, (B) – modified starch and (C) – xanthan

Comparing Figures 5.4 and 5.5, with Figures 5.2 and 5.3, it is not possible to relate the morphology with the release profile. The properties of encapsulating agents will reflect on the capacity of entrapping the core material protecting it and avoiding mass losses over time. The encapsulating agent will also, affect, the morphology and size of the particles.

Another information that can be obtained from the release profiles is the encapsulation efficiency that is the percentage of drug that is successfully entrapped into the particle. About the encapsulation efficiency there are different methods and strategies to determine it: in this case and considering the specifications of our experimental design, the encapsulation efficiency can be calculated considering the release profiles, and corresponds to the amount of compound that is encapsulated in the time zero. For these experiments these values correspond to encapsulation ratios around 100% for the microparticles prepared with all the encapsulating agents, except for the case of the microparticles prepared with maltodextrin and modified starch and released in SGF, where this value is lower, around 60%.

Targeted delivery systems for bioactive compounds, like vitamin B12, can be a good solution for creating new food and pharmaceutical products. One of the big advantages of the microencapsulation is the protection. For example: a commercial instantaneous gelatin in powder can be fortified with vitamins; in this case a fast release is desired for the gelatin be homogeneous for the consumption. But during the time that it was in the package, stored, was protected from oxidation, light and interaction with other components. In other cases, as for example in pharmaceutical products, a slow release can be desired, in order to promote a more efficient absorption in the intestinal tract.

Table 5. 2. Mass loss of vitamin B12 after 4 months of storage in percentage for each type of biopolymers used as an encapsulating agent

Encapsulating agent	**Mass loss of vitamin B12 (%) after 4 months of storage for samples released in SGF**
Arabic gum	6.2
Sodium alginate	7.7
Carrageenan	12.0
Maltodextrin	11.7
Modified starch	7.6
Pectin	22.4
Xanthan	12.7

Therefore, it is important to choose effective wall materials capable to prevent or minimize the losses until they are utilized. This research work showed good results in what concerns the capsule stability for samples stored for 4 months. In Table 5.2, the mass loss of vitamin B12 is

presented in percentage for each wall material. The stability of the microparticles over the time was evaluated with SGF. The losses of vitamin ranged from 6.2 to 22.4 %, the smallest value corresponding to arabic gum and the highest to pectin.

5.5. Conclusion

The encapsulation of vitamin B12 was performed using 7 different biopolymers as encapsulating agents, using a spray-drying technique. The product yield for the spray-drying process is between 27 and 50 % depending on the encapsulating agent.

Good microparticles were produced, with different morphologies and sizes. All particles show a spherical shape, the particle surface being smooth for sodium alginate, carrageen, maltodextrin and pectin microparticles, and rough for the other 3 biopolymers, arabic gum, modified starch and xanthan. The average diameter of vitamin B12 microparticles according to volume distribution ranges from 3.17 to 6.67 μm.

Different release profiles were found, depending on the encapsulating agent and the release conditions, pH and temperature.

In terms of stability over time it can be concluded that the shelf life of microcapsules can be extended at least until 4 months. The encapsulating agents used show good protective effect.

This study proved that vitamin B12 can be microencapsulated by spray-drying technique using several biopolymers as encapsulating agents. The microparticles prepared with different encapsulating agents present different behaviours, and this fact can lead to different future applications, according to what it is expected from the final product (size and morphology of the microcapsules, needed time for the complete release of vitamin from the protective layer, type of solvent used to evaluate the release). Further, it is shown that these products are a promising solution to be used for the formulation of oral delivery systems with good stability properties.

Chapter 6

Microencapsulation of vitamin B1

In the third study, the process and the results of the microencapsulation of vitamin B1 are revealed.
This work is found in the chapter number six in the format of the published paper:

"Production of vitamin B1 microparticles by a spray drying process using different biopolymers as wall materials", *Ioana C. Carlan, Berta N. Estevinho, Fernando Rocha, The Canadian Journal of Chemical Engineering, 8, 1682-1695, 2020. DOI:10.1002/cjce.23735.*

6.1. Abstract

The aim of this work was to microencapsulate vitamin B1 by spray-drying using different encapsulating agents (arabic gum, carrageenan, chitosan, maltodextrin, modified chitosan, modified starch, pectin, sodium alginate, and xanthan) and to characterize the microcapsules and study their release. Microcapsules with a 0.25% (w/w) content of vitamin B1 were produced. The product yield results ranged from 17% - 52% and the encapsulation efficiency from 66% - 100%. Three categories of morphology, regular spherical shape, irregular spherical shape with rough surface, and irregular shape, were identified. Their sizes, determined by laser granulometry, ranged from 0.11 µm-1.32 µm, in terms of number distribution, and from 3.76 µm - 34.43 µm, in volume distribution. Controlled release studies were performed by spectrophotometric analysis, in deionized water (20 ºC) and simulated gastric fluid (37 ºC). Different release behaviours were observed from just 10 seconds (modified starch) up to more than 24 hours (xanthan). Kinetic models like zero-order, first-order, Higuchi, Korsmeyer – Peppas, and Weibull were applied. The Weibull model showed the best fitting with the experimental data. All release tests were repeated after four months and showed good stability over time. A mass loss of vitamin B1 lower than 20% was detected. This study demonstrates the possibility of encapsulating vitamin B1 using different encapsulating agents by a spray-drying technique. Depending on the intended applications, for fast release, adequate results were obtained for maltodextrin, arabic gum, modified chitosan, and sodium alginate, and for slow release, adequate results were only obtained for chitosan and pectin.

Keywords: Biopolymers, controlled release, microencapsulation, spray-drying, vitamin B1.

6.2. Introduction

Vitamin B1 was the first compound classified as a vitamin, in 1911 by the Polish biochemist Kazimierz Funk [35,62,187]. Known also as thiamine, vitamin B1 is a co-enzyme precursor responsible for the metabolism of carbohydrates. In addition, it grants a good function of the cardiovascular system, prevents neurological disorders, strengthens the immune system, withstands stress, and improves mood and memory. Due to its important role in the human body, vitamin B1 is considered an essential micronutrient and is required daily because it cannot be stored in the human body as a deposit [57,187,220,221]. The recommended dietary allowance (RDA) for women is 1.1 mg/day and 1.2 mg /day for men [37]. With a balanced and varied food diet, the necessary intake of vitamin B1 should not be a concern, and the most important natural sources of B1 are yeast, grains, and meat. It can also be found in lower amounts in some fruits, vegetables, and dairy products [61,187].

In case of vitamin B1 deficiency, it is necessary to obtain medical assistance and follow a treatment plan until the deficit is corrected.

Well-known widespread cases of vitamin B1 deficiency occurred in Asian countries where people used to consume polished white rice [4,35,187,221]. The deficiency led to a disease is named Beriberi and is associated with severe symptoms in the neurologic and cardiovascular systems. Nowadays, this deficiency remains common in some undeveloped countries where food products are not fortified with essential micronutrients. It can affect alcohol addicts, pregnant or lactating women, as well as a small percentage of people with diabetes, Alzheimer's disease, depression, or dementia [37,220,221].

Depending on the type of symptoms, a vitamin B1 deficiency can occur in three forms, either alone or a combination: Wernicke's encephalopathy (ophtalmoplegia, ataxia, confusion), Kosakoff's syndrome (pathological condition: amnesic disorder), and/or Beriberi (nutritional polyneuropathy). Patients affected by a vitamin B1 deficiency can experience some of the following symptoms: low appetite, decreased general growth, weakness, cheilosis, heart problems, gastrointestinal disorders, fatty liver, anemia, and some neurological effects [61,187,221,222].

Also, the poor stability of vitamin B1 to external factors and the Vitamin B1 losses during the classical methods of cooking, should be a concern. External factors like pH, humidity, temperature, oxygen, and metal ions (in solutions with Fe^{3+} and Cu^{2+}) easily affect the stability of vitamin B1 [62,187]. Boiling or blanching lead to the thermal breakdown of vitamin B1;

therefore, in order to avoid this situation, it is highly recommended to use steaming for cooking [64,65].

For the prevention or treatment of a deficiency, pharmaceutical products, nutraceuticals, and food supplements with vitamin B1 microparticles are a possible alternative. Recently there has been an increased demand for functional foods [25,89,102,130]. Being categorized as a functional food product means to be able to "provide a health benefit beyond the traditional nutrients it contains," according to The Institute of Medicine's Food and Nutrition Board (IOM/FNB, 1994) [223]. Enriching products with vitamins using methods like fortification must first meet the needs of the consumers, but should also be done in a simple and economical way so that it is feasible for the industry.

Choosing microencapsulation as a delivery tool for this vitamin is a good option because it can overcome stability problems [25–28,87]. Some approaches to microencapsulate vitamin B1 have already been published with good results for methods like spray-drying [147,224,225], emulsion-solvent evaporation with continuous oil-phase [145], water-in-oil-in water double emulsions [146], emulsion coacervation [225], interfacial polymerization [144], freeze-drying [226], and even nanoencapsulation [227,228]. Although some authors have succeeded in microencapsulating vitamin B1, the number of published studies on this topic is still low and more research should be done to explore different methods, experimental approaches, and conditions.

Spray-drying is defined as a one-step atomization process, capable of transforming liquid feed into dried microparticles, in a fast, simple, and economical way [25–28,98,194]. Among other microencapsulation methods, spray-drying has been selected by several authors as an adequate option for producing microparticles suitable for the food industry because it can facilitate the targeted transport of sensitive compounds, like vitamins. Some examples, besides vitamin B1, are vitamin B6 [147], vitamin B12 [32,229,230], vitamin C [32,87,207,231,232], and vitamin A [112,215].

The success of spray-drying processes depends also on the encapsulating agent itself; therefore, it is important to select adequate materials like biopolymers. Biocompatibility, bioavailability, biodegradability, no toxicity, and good stability are the characteristics that make biopolymers desirable as delivery vehicles for pharmaceuticals, food, and even cosmetics [26,27,29,89,98,100,102,112,130].

The aim of this work was to develop vitamin B1-loaded microparticles using a spray-drying process. As encapsulating agents, nine different biopolymers were selected in order to compare the results of vitamin B1 – biopolymer formulations. The purpose of using more encapsulating

agents was to follow their behaviour in the encapsulation process and to determine the similarities and differences between them. The chosen biopolymers were Arabic gum, carrageenan, chitosan, maltodextrin, modified chitosan, modified starch, pectin, sodium alginate, and xanthan.

The microencapsulation process was evaluated by two parameters: product yield and encapsulation efficiency. Also, the microparticles were characterized regarding their size and morphology after in-vitro tests were performed to study the release of vitamin B1 from the microparticles. Using the data obtained from the release profiles, specific kinetic models were tested to determine the release mechanism. Further, after four months of storage, vitamin B1 microparticles samples were used to determine the stability overtime.

The interest of the application of vitamin B1 microparticles in the food and pharmaceutical industries has been growing in the last few years. Regarding the food industry, the microencapsulated product represents 1% - 5% of the final product, and the expected maximum cost for a microencapsulation process was estimated to be ~€ 0.1/kg [27].

6.3. Materials and methods

Materials

All reagents used were of analytical grade purity. Thiamine hydrochloride was used as an encapsulated material and was provided by Sigma – Aldrich, Germany (T4625, CAS No. 67-03-8). The nine biopolymers used as encapsulating agents were purchased from different suppliers: Arabic gum (Fluka, Germany – 30888, Lot #BCBK8649V), carrageenan (Sigma-Aldrich, USA – 1001761179 C1013 -1006, Lot # SLBH9868V), chitosan (Sigma Aldrich, Germany – Cat. No. 448877), maltodextrin (Sigma-Aldrich, USA – 1001841656 419672, Lot # MKBN6629V), modified chitosan (China Easter Group (Dong Chen) Co. – Batch no. SH20091010), modified starch (Alfa Aesar GmbH&Co KG, Germany – 36673, Lot D04X013), pectin (Sigma-Aldrich, Switzerland – 101582340 76282, Lot #BCBN5335V), sodium alginate (Sigma-Aldrich, USA – 1001503523 180947, Lot # MKBH8463V), and xanthan (Sigma-Aldrich, USA –1001900732 G1253, Lot # MKBQ9467V).

For the preparation of chitosan solution (1% (w/V)), it was necessary to add a solution of acetic acid (1% (V/V)) with a viscosity of 200 mPa·s (25 °C). Simulated gastric fluid was prepared using 37% hydrochloric acid from Sigma-Aldrich (product code: 25,814-8, CAS-No.: 7647-01-0) and sodium chloride from AppliChem Panreac ITW Companies (product code: 131,659.1211, Lot 0000542745, CAS-No: 7647-14-5).

For the preparation of all of the solutions, deionized water collected from a water purification device with a 0.45 µm filter, type MilliporeTM (Massachusetts, USA), was used.

Experimental solutions

All solutions required for the spray-dryer process were prepared following the experimental procedure described in previous works [32,229,230]. First, solutions with a 200 mL volume and a 1% (w/V) content of encapsulating agent were let for 2 hours under continuous stirring at a speed of 1200 rpm. After, 4 mL solutions of vitamin B1 with a content of 0.125% (w/V) were homogenized with a shaker for 10 minutes. Then, before the feed solution was ready to be pumped to the spray-dryer, each encapsulating agent solution was mixed with a vitamin B1 solution for 30 minutes at a speed of 500 rpm. Similar solutions were prepared for empty microcapsules, without the vitamin B1 solutions.

Simulated gastric fluid (SGF) was prepared according the information provided by the European Pharmacopeia 7.0 (2010). 2.0 g of sodium chloride and 7.0 mL of hydrochloric acid

37% were dissolved in 1 L of stock solution; pH corrections were required to reach the recommended value of 1.2.

The experimental solutions were prepared in the same conditions: at room temperature (around 21 ºC) and with deionized water.

Spray-drying process

The equipment used to produce the microparticles was a Mini Spray Dryer B-290 from BÜCHI (Flavil Switzerland) with a standard 0.5 mm nozzle, and for each experiment, the following conditions were selected: solution flow rate: 4 mL/min (15%), air flow rate: 32 m^3/h (80%), air pressure: 6 bar, and inlet temperature: 120°C. The value of the outlet temperature varied for all experiments: between 50 °C – 67 °C for the formation of microcapsules with vitamin, and between 60 °C – 68 °C for the formation of empty microparticles, without vitamin. This working configuration was chosen based on the optimized experimental conditions of previous work and other similar studies [27,32,87,122,229,230].

For the evaluation of the spray-drying process performance two parameters were determined: the product yield, Equation (6.1.), and the encapsulation efficiency, Equation (6.2.):

$$\text{Product yield (\%)} = \frac{\text{amount of recovered powder}}{\text{initial amount of solid materials}} \cdot 100 \quad (6.1.)$$

The product yield is defined as the ratio between the recovered amount of powder and the initial amount of solid raw materials used for the preparation of the feed solution.

The encapsulation efficiency (EE), was determined as the ratio between the amount of vitamin encapsulated and the total amount released. Free vitamin (not encapsulated: unentrapped or at the surface of the microparticles) is determined by measuring the amount of vitamin right after dispersion of the microparticles in the solvent:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{total vitamin released} - \text{free vitamin}}{\text{total vitamin released}} \cdot 100 \quad (6.2.)$$

The powder collected at the end of the experiments was kept protected with aluminum foil from direct light sources, and at temperature of 4°C in the fridge, until its use in further tests and analyses.

Characterization of microparticles

- **Scanning electron microscopy**

Scanning electron microscopy (SEM) was used to observe the physical appearance of the microcapsules. The analysis was performed with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M (Eindhoven, The Netherlands). The samples preparation for SEM involved two operations: first, each sample was fixed on a brass stub using double-sided adhesive tape, and second, the samples were coated with a thin layer of gold in vacuum, in a Jeol JFC 100.

- **Laser Granulometry Analysis**

The microparticles were characterized in terms of number and volume average size using the laser granulometry technique in a Coulter-LS 230 (Miami, USA). Each sample was previously ultrasound-irradiated in an ethanol dispersant solution to prevent the possible aggregation of microparticles. All readings were the result of three tests and each run was ~30 seconds.

Controlled release studies

To prove the success of the microencapsulation process, release studies of the vitamin B1 from the microparticles, under controlled conditions, were done. This evaluation was done by an analytical method using a spectrophotometer from Sarspec SPEC RES+ UV/VIS (Portugal) equipped with an external device used for selecting the desired temperature and intensity of stirring inside the cuvette. The temperature and the magnetic stirring were operated via optical fiber connectors.

All encapsulating agents were analyzed in two different dissolution mediums: deionized water (dW) and SGF. The readings were done in the UV domain at specific absorbance values of vitamin B1: 261 ± 4 nm in deionized water (pH = 5.6) at 20 °C and 246 ± 1 nm in SGF (pH = 1.2) at 37 °C. The acquisition of data was set up to register in a continuous mode every 30 seconds for the following encapsulating agents: Arabic gum, carrageenan, chitosan, maltodextrin, modified chitosan, pectin, sodium alginate, and xanthan; and for modified starch, every 5 seconds.

- **Validation of analytical method**

For the validation of the analytical method, several parameters were calculated: specificity, inter-day precision, linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, and intra-day precision. 10 standard solutions were prepared with deionized water (20 °C) and another 10 with SGF (37 °C) within a range of 0.0005 g/L-0.01 g/L.

With deionized water, three specific peaks were identified for vitamin B1, corresponding to the following wavelengths: 195 ± 2 nm, 235 ± 2 nm, and 261 ± 4 nm. To eliminate the risk of potential interferences of the encapsulated material with the encapsulating agents, the spectrum of each biopolymer was verified. Their detected wavelengths were as follows: Arabic gum: 225 ± 1 nm, carrageenan: 228 ± 3 nm, chitosan: 229 ± 1 nm, maltodextrin: 227 ± 3 nm, modified chitosan: 220 ± 1 nm, modified starch: 197 ± 1 nm, pectin: 230 ± 1 nm, sodium alginate: 199 nm, and xanthan: 232 nm. Therefore, to avoid any interference problem, the chosen wavelength was 261 ± 4 nm. The calibration curve ($y = 58.739x - 0.0085$) proved to be linear in the selected range interval, with a good coefficient of correlation, $R^2 = 0.999$. The LOD was 0.146 mg/L and the LOQ was 0.482 mg/L. The accuracy determined by the percentage of recovery, expressed by the ratio between the obtained and the expected concentration of vitamin B1, was calculated for the last three concentrations and the values were 94.3%, 96.1%, and 98.2%. The intra-day precision, expressed for the last three concentrations had the follows values: 0.764, 0.800, and 0.855.

All the previous procedures were repeated to determine the behavior in the SGF. With the SGF solutions only one specific peak was identified, corresponding to the wavelength 246 ± 1 nm. Inter-days tests (n = 3) were validated as well. The calibration curve ($y = 74.769x + 0.0174$) proved to be linear in the selected range interval, with a good coefficient of correlation, $R^2 = 0.999$. The LOD was 0.151 mg/L and the LOQ was 0.505 mg/L. The accuracy, for the last three concentrations had the following values: 102.5%, 101%, and 98.1 %, and the intra-day precision, for the last three concentrations had the following values: 0.218, 0.253, and 0.208.

- **Release studies of fresh and 4 months samples**

For the detection of vitamin B1, the presented spectrophotometric method was chosen with optimized conditions similar to previous experiments with vitamin B12 and vitamin C [32,181,229,230,233].

The amount of vitamin B1 microparticles used for each experiment (4 mg/sample) was calculated through the mass balance of the reagents (assuming that during the spray-drying process, the ratio of the vitamin/encapsulating agent is constant). In all tests, with deionized water or SGF, the temperature was controlled, the intensity of magnetic stirring adjusted, and the microparticles added on the top of the analyzing liquid in the cuvette. All encapsulating agents behaved differently in terms of the time necessary for a complete release of the vitamin

B1. To further evaluate the stability of the vitamin B1 microparticles, samples were analyzed again after four months of storage. Release tests of the old samples were repeated according to the method described for fresh samples.

- **Kinetic models**

Different kinetic models, like zero order, first order, Higuchi, Korsmeyer-Peppas, and Weibull, were applied for microparticles of vitamin B1. The specific equations (Equations 6.3 -6.7.) of each kinetic model are presented in Table 6.1.

Table 6. 1. Kinetic models applied to study the release of vitamin B1

Models		
Zero order	$Q_t = Q_0 + K_0 t$	(6.3.)
First order	$Q_t = Q_0 e^{-K_t t}$	(6.4.)
Higuchi	$Q_t = K_H \sqrt{t}$	(6.5.)
Korsmeyer – Peppas	$Q_t/Q_\infty = K_K t^n$	(6.6.)
Weibull	$M_t = M_\infty \left[1 - e^{-\left(\frac{t - t_0}{\tau_d}\right)^\beta}\right]$	(6.7.)
Parameters		
Q_0 = amount of active compound released at time t = 0 (%) $Q_t(M_t)$ = amount of active compound released at time t (%) Q_t/Q_∞ = fraction of active compound released at time t (%) M_∞ = fraction of active compound released at infinite time t (%) t = time (min) K_0 = Zero order constant release K_1 = First order constant release K_H = Higuchi constant release K_K = Korsmeyer – Peppas constant release n = release mechanism exponent (diffusional): t_0 = lag time measured as a result of dissolution process parameter τ_d = the time (min) when 63.2% of active compound was released β = shape parameter of the curve		

The numerical value of the n exponent of the Korsmeyer-Peppas equation will determine the mechanism for the release of the active compound as follows: $n < 0.43$ (Fickian diffusion: case I transport); $0.43 < n < 0.85$ (anomalous transport: diffusion mechanisms with swelling release, case II transport); $n = 0.85$ (case II transport) and $n > 0.85$ (super case II transport).

The shape parameter, β, from the Weibull model describes the aspect of the release curve. Therefore, for β = 1, the curve will have an exponential profile, for β > 1 it will be sigmoidal with a turning point, and for β < 1 the release will show a steeper increase than in the case of β = 1 [27,134,137,139,209,210].

The evaluation of these mathematical models was done in order to gain a better understanding of how the active material is being released from the encapsulating agent.

Statistical analysis

All tests were done in triplicate, reproducing the same procedure and experimental conditions. The final results are presented as a mean of the triplicates. For every test, the coefficient of variation (CV) was determined as the percentual value between the standard deviation and the mean value of the triplicates. The reproducibility of this work has been proven as the CV presented values lower than 10%.

6.4. Results and discussion

Microencapsulation of vitamin B1

The microencapsulation of vitamin B1 by the spray-drying technique using different biopolymers proved to be possible; however, it was also challenging since each encapsulating agent showed a different behaviour. The product yield was calculated for both types of microcapsules, with and without active material, to gain a better understanding of the influence of the vitamin on the microencapsulation process. The obtained results are presented in Figure. 6.1.

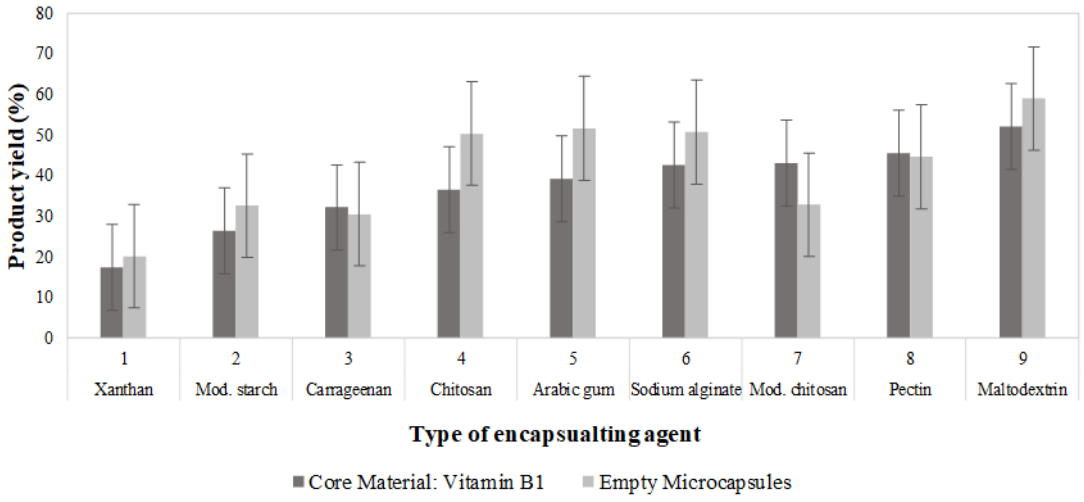

Figure 6. 1. The product yield (%) presented in the ascending order for the microparticles with vitamin B1 active core material, followed by the results for empty microparticles (results include STDEV bars).

As it can be observed in Figure 6.1, the values of the product yield for vitamin B1 microparticles varied within a range of only 17% for xanthan to 52% for maltodextrin. The first five encapsulating agents (xanthan, modified starch, carrageenan, chitosan, and Arabic gum) presented values for the product yield of < 40%; however, the last four agents (sodium alginate, modified chitosan, pectin, and maltodextrin) showed higher values, between 40% - 52%. Regarding the results of the microparticles without vitamin content, a general trend of larger than or equal to the amount of powder collected in the end of the process was observed,

compared with those with the vitamin. Only for modified chitosan microparticles it was observed a difference of around 10%, the vitamin B1 microparticles recording a higher product yield than the empty microparticles. The product yield varied between 20% - 59%.

The low values of the product yield can be explained by the small amount of solid materials used and as well by the losses accumulated during the drying stages.

Similar studies done with vitamin B1 or other water-soluble vitamins do not present large differences. In the study by Chatterjee et al [147], vitamins B1 and B6 were encapsulated with ferulic acid-grafted chitosan; product yields of 63.6% - 65.1% were obtained, for a higher inlet temperature of 140 °C. Desai et al [207,232] used cross-linked chitosan (TPP-chitosan) as wall material for vitamin C in two studies. In both, better values of product yield were observed; in the first study, at an inlet temperature of 170 °C, the results were between 58.5% - 60.6% [207], while in the second study the results were between 54.5% - 67.5% [232].

In other work [32,229,230], the authors studied the encapsulation of vitamin B12 and vitamin C using sodium alginate, chitosan, and modified chitosan as encapsulating agents. The results for the vitamin B12 and vitamin C are as follows, respectively: sodium alginate: 41.8% and 43.6%, chitosan: 55.6% and 44.5%, and modified chitosan: 42.4% and 45.4%. When analyzing these results in comparison with the results for the vitamin B1 of the present work, no significant differences were found for the sodium alginate and modified chitosan. In terms of the chitosan, the results of vitamin B1 are smaller than the ones obtained previously for vitamin B12 and vitamin C. In another study where the experimental conditions were optimized using modified chitosan and vitamin B12 [229], the product yield reported was ~ 55%. In a different work where the authors microencapsulated vitamin B12 with seven encapsulating agents [230], the product yield was reported as between 27% - 50%, depending on the encapsulating agent used. One can observe higher values of product yield for Arabic gum, carrageenan, modified starch, and xanthan microparticles, and almost the same values for maltodextrin, pectin, and sodium alginate microparticles.

In Figure 6.2, excellent results obtained for vitamins' B1 encapsulation efficiency can be observed. Maltodextrin is the only encapsulating agent with a lower value, 66%. Carrageenan, Arabic gum, and modified chitosan present values of 94% - 96%. The chitosan, pectin, modified starch, sodium alginate, and xanthan reached full entrapment.

Chatterjee et al [147] obtained EE values of 91 ± 2.31% for vitamin B1 and 83 ± 3.17% for vitamin B6. In another study of the microencapsulation of vitamin B1, Jing and Zhao [224] reported a value of EE of 85% when using cross-linked gelatin as a wall material.

Desai et al [231,232] used cross-linked chitosan (TPP-chitosan) as wall material for the encapsulation of vitamin C in two studies. In both studies, better product yield but lower EE were observed. T first study worked with an inlet temperature of 170 °C and the results were as follows: product yield of 58.46% - 60.56%, EE of 52.74% - 67.25% [232]; as for the second study, a product yield of 54.5% - 67.5% and an EE, of 45.72% - 68.7% were reported [231].

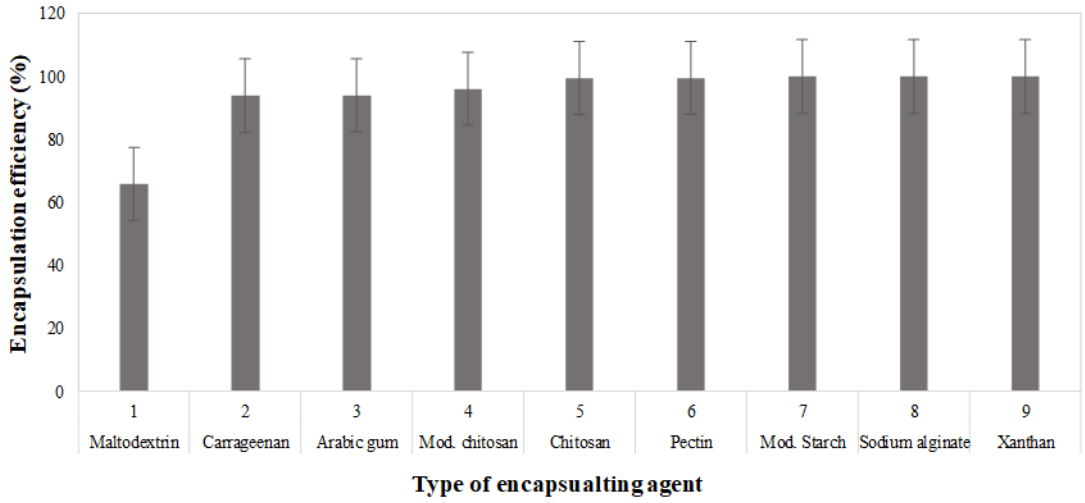

Figure 6. 2. The encapsulating efficiency (%) presented in the ascending order of the microparticles with vitamin B1 active core material (results include STDEV bars).

SEM and laser granulometry for vitamin B1 microparticles

Through SEM images (Figures 6.3. - 6.5.), the morphology of the microparticles with and without vitamin B1 for each biopolymer used as encapsulated agent was observed. Since differences in the morphology of the microcapsules were observed, the nine biopolymers were divided into three groups, named A, B, and C. Group A includes carrageenan, modified chitosan, pectin, and sodium alginate. All of them show a regular spherical shape and smooth surface, as can be observed in Figure 6.3. Group B includes Arabic gum, maltodextrin, and chitosan. In this case, Figure 6.4, the microparticles present a deformed spherical shape with a rough surface.

The last and the smallest group, composed of modified starch and xanthan microparticles, is group C. As can be clearly observed, group C cannot be easily defined neither in terms of shape nor surface, as these microparticles present an irregular shape.

The analysis of the SEM images shows the phenomenon of particle aggregation, particularly in the case of modified starch. On the other hand, significant differences were not found concerning the morphology of the particles with or without vitamin B1.

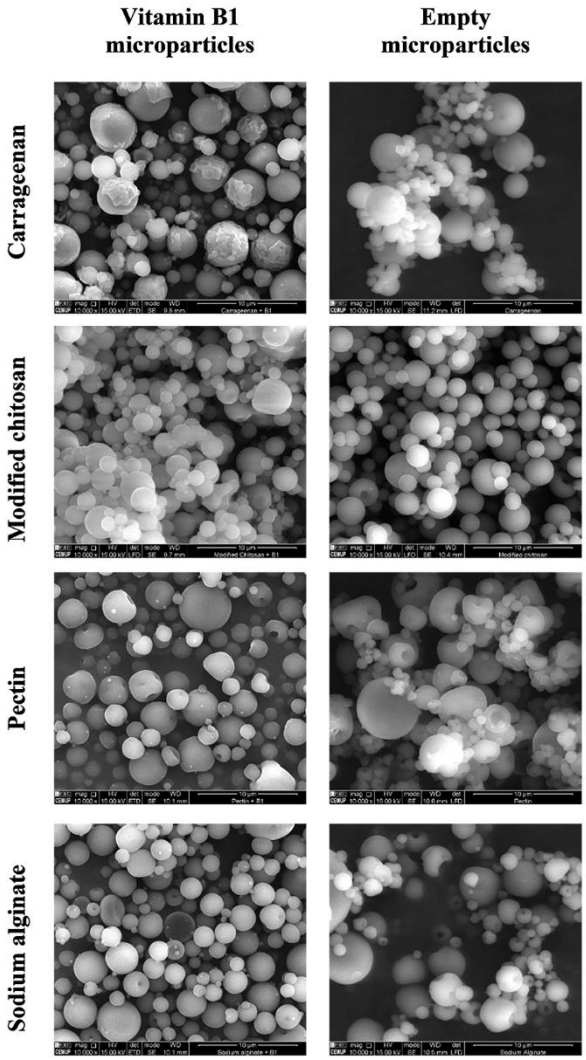

Figure 6. 3. SEM images of group A of microparticles (spherical shape and smooth surface) with and without vitamin B1. Magnification of 10.000x, beam intensity (HV) 15.00kV, distance between the samples and the lens (WD) around 10 mm.

It can be concluded that the choice of encapsulating agent is crucial to obtain particles with the desired properties. The desired properties refer to the application of the final product. The morphology, size, size distribution, and shape are parameters that are important in the characterization of the microparticles and they will have a crucial role in the acceptance of the final product.

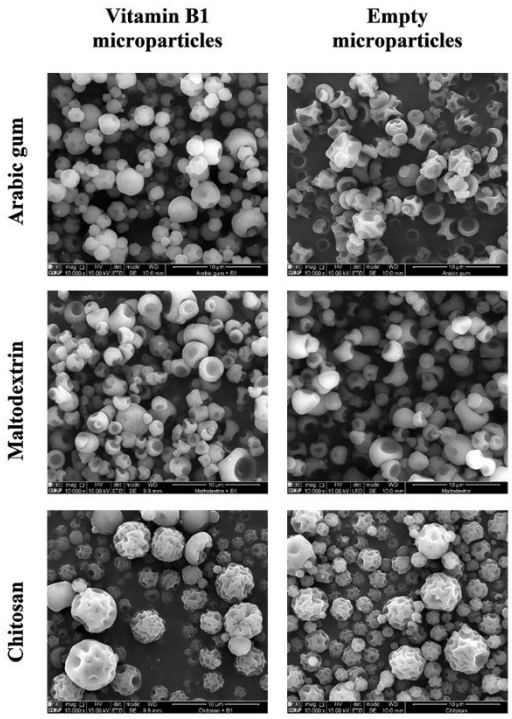

Figure 6. 4. SEM images of group B of microparticles (deformed spherical shape and rough surface) with and without vitamin B1. Magnification of 10.000x, beam intensity (HV) 15.00kV, distance between the samples and the lens (WD) around 10 mm.

These encapsulating agents were also studied by other authors who confirmed these types of microparticles morphology. Some examples are acid ferulic grafted chitosan, a type of modified chitosan, with vitamins B1 and B6 [147]; Arabic gum, carrageenan, chitosan, maltodextrin, modified chitosan, modified starch, pectin, sodium alginate, and xanthan with vitamin B12 [32,229,230]; chitosan, modified chitosan, and sodium alginate with vitamin C [32,229], and Arabic gum with vitamin A [215].

The size characterization of the produced particles, with and without vitamin B1, was done using the laser granulometry technique.

Two types of measurements were made: number and volume average, presented as the mean value and the standard deviation in µm (Table 6.2).

The values obtained for the differential number for microparticles loaded with vitamin B1 ranged from 0.11 µm - 1.32 µm. The first five types of microparticles from Table 6.2 show small sizes, the modified chitosan in position 6 shows an intermediate size, and the remaining agents from positions 7 - 9 present the largest sizes.

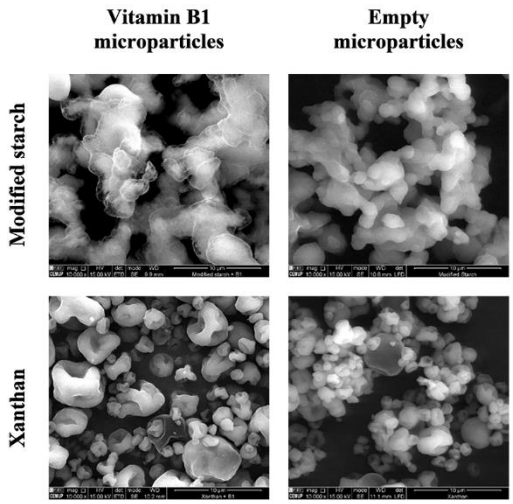

Figure 6. 5. SEM images of group C of microparticles (irregular shape) with and without vitamin B1. Magnification of 10.000x, beam intensity (HV) 15.00kV, distance between the samples and the lens (WD) around 10 mm.

In terms of volume distribution, the vitamin B1 microparticles confirm what can be seen in the SEM images, which is that some microparticles tend to aggregate, and their sizes range from 3.76 µm - 34.34 µm. The last and the largest value, relative to the modified starch microcapsules with vitamin B1 content and considering the corresponding SEM images, suggests an unsuccessful spray-drying process. As shown in Figure 6.5., the microparticles do not seem to have independent shapes, and look more like large aggregations.

For both types of measurements, number and volume, the sizes for the vitamin B1 microcapsules and the empty ones are, in general, significantly different. Only in a few situations were nearly the same values observed. For the number analysis, the values for maltodextrin and modified chitosan were similar, and for volume analysis, carrageenan and

xanthan were similar. This suggests the effect of vitamin B1 on the chemical structure of the components of the microparticles.

In a previous study [229], the authors obtained the microparticles of vitamin B12 and modified chitosan with sizes of 0.1 µm - 0.9 µm, and in the study by Chatterjee et al [147], much larger vitamin B1 and B6 microparticles with acid-grafted ferulic chitosan with sizes of 4.5 µm and 4.8 µm were produced (the values expressed by the mean number distribution). In another previous study, the authors microencapsulated vitamin B12 with different biopolymers [230], concluding that the sizes of the particles depend on the used biopolymer, which also occurs in the present work.

Table 6. 2. Particle mean diameter for differential number and volume distribution done by a laser granulometry analysis for vitamin B1-loaded microparticles and for empty microparticles.

	Mean diameter (± STDEV) µm			
	differential number		**differential volume**	
	Vitamin B1 microparticles	**Empty microparticles**	**Vitamin B1 microparticles**	**Empty microparticles**
1. Arabic gum	0.11 (± 0.00)	0.57 (± 0.02)	10.13 (± 0.13)	4.22 (± 0.00)
2. Carrageenan	1.19 (± 0.03)	0.54 (± 0.03)	7.07 (± 0.13)	7.44 (± 0.12)
3. Chitosan	0.13 (± 0.01)	0.76 (± 0.02)	9.08 (± 0.44)	4.25 (± 0.05)
4. Maltodextrin	0.14 (± 0.01)	0.11 (± 0.01)	5.03 (± 0.03)	7.24 (± 0.06)
5. Mod. chitosan	0.60 (± 0.01)	0.60 (± 0.05)	14.58 (± 0.62)	9.31 (± 0.21)
6. Mod. starch	0.12 (± 0.00)	3.62 (± 0.02)	34.34 (± 1.20)	42.00 (± 2.95)
7. Pectin	1.32 (± 0.04)	0.54 (± 0.05)	3.76 (± 0.05)	4.66 (± 0.01)
8. Sodium alginate	1.00 (± 0.00)	0.60 (± 0.01)	5.55 (± 0.01)	10.18 (± 0.03)
9. Xanthan	0.11 (± 0.00)	0.72 (± 0.02)	7.96 (± 0.27)	7.56 (± 0.06)

Controlled release profile studies

Due to its advantages, microencapsulation is widely used for the design and validation of new food or pharmaceutical products. For this reason, it is important to study the release profiles of the active material. Current release systems were targeted for possible oral delivery applications; therefore, tests were done in dW at room temperature (around 21 ºC) and in SGF

at 37 °C, in order to simulate the conditions of the human body and its temperature. While it may be possible to use water as a medium for products like effervescent micronutrient supplement tablets, syrups, or juices prepared by dissolving a powder product into a proper amount of water, these are not the only feasible products for the incorporation of vitamin B1 microparticles. Any type of food or pharmaceutical product can be enriched with essential micronutrients if the active material can be released (dissolved) in SGF. As gastric fluid is found in the stomach, it is important to know if it is possible for the vitamin to reach the small intestine where it is absorbed and further sent through the human body by the blood vessels. For products like food supplements for any age, i.e., cereal bars, breakfast food, products for those who practice sports, preliminary tests in SGF are mandatory. The purpose of these tests is to determine how a certain encapsulating agent will influence the release of the encapsulated material.

The evaluation of the release of vitamin B1 from the microparticles produced with different biopolymers is presented in Figure 6.6. This figure presents the experimental release profiles done in dW and SGF and obtained for different biopolymers, adjusted by the Weibull model (a kinetic model discussed later in this section). According to their common characteristics, four groups of encapsulating agents were formed.

Group 1 (Figure 6.6) shows the encapsulating agents with fast release, ie, up to 5 minutes in both dW and SGF, and includes carrageenan, maltodextrin and Arabic gum microparticles loaded with vitamin B1. In group 2 (Figure 6.6), the release profiles of modified chitosan and sodium alginate microcapsules are presented, and these two agents require up to 15 minutes for dW and 30 minutes for SGF. For the case of microparticles from group 3 (Figure 6.6), the release time significantly increased as follows: chitosan in dW 80 minutes and in SGF 180 minutes; pectin in dW 100 minutes and in SGF 12 hours.

The last group, group 4 (Figure 6.6), includes the encapsulating agents discussed previously as not being able to fit in the normal standards of microparticles: modified starch and xanthan. For modified starch, the release is complete after a few seconds, and for xanthan, if the release in SGF seems to take only 40 minutes, when it comes to dW, the release is slow, i.e., after 24 hours the process was not finished.

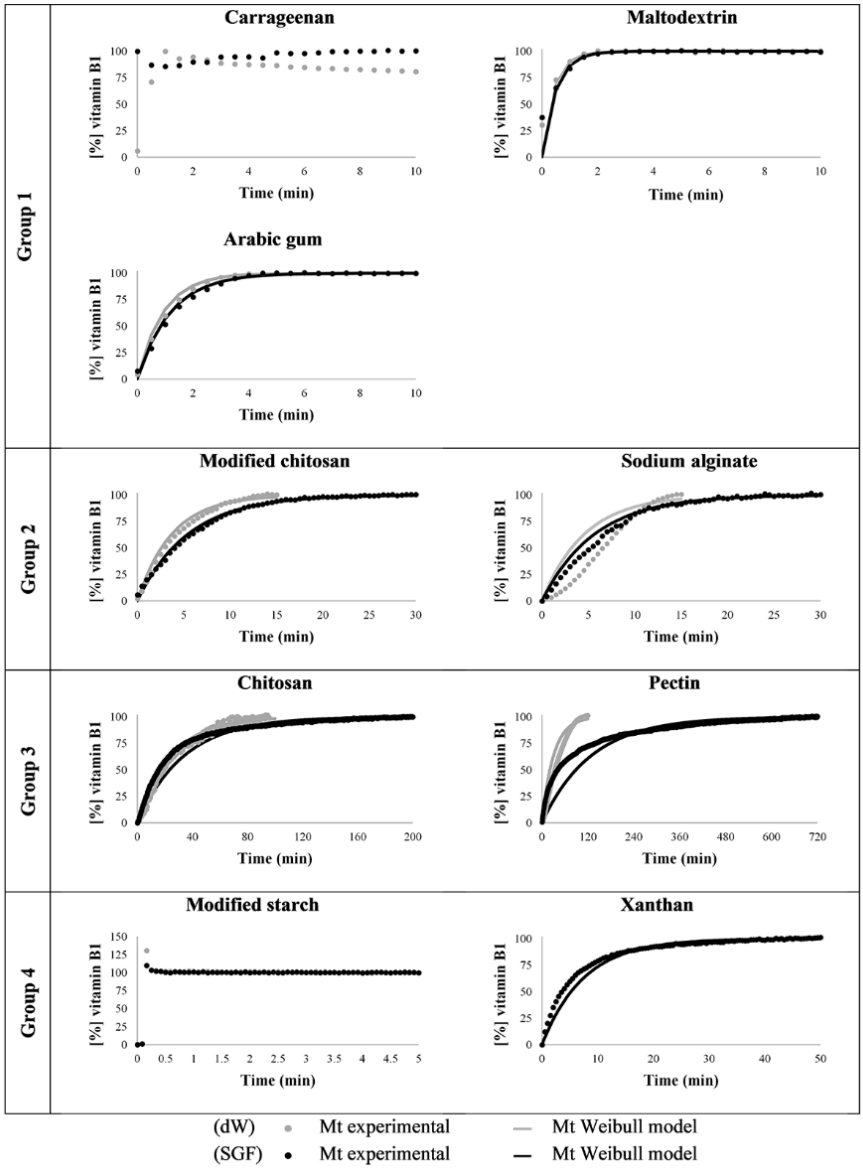

Figure 6. 6. Release profiles for vitamin B1 from microparticles prepared with different encapsulating agents.

Table 6.3 presents the time necessary for the complete release of vitamin B1. Depending on the reason of incorporating vitamin B1 in products, the release time is an essential factor in selecting an encapsulating agent. For both food and pharmaceutical products, according to the desired effect, both slow or fast release could be suitable.

Table 6. 3. Time necessary for the complete release of vitamin B1

	Complete release reached after	
	dW	**SGF**
Modified starch	10 s	10 s
Carrageenan	1 min	0 min
Maltodextrin	2 min	3 min
Arabic gum	5 min	4.5 min
Modified chitosan	12.5 min	24 min
Sodium alginate	15 min	30 min
Chitosan	80 min	130 min
Pectin	100 min	12 h
Xanthan	>24 h	40 min

The release of vitamin B1 from the microparticles in both dissolution mediums showed similar trends for most of the microparticles. Small differences can be observed between the time required for the vitamin the be released from dW and SGF, and usually the release in dW was faster. In the case of chitosan, the time to total release in SGF was higher than in water; however, in the first 20 minutes, the release in SGF was faster. Pectin was six times slower to release in SGF, and xanthan was slow in water.

In Table 6.4, the mass loss of vitamin B1 in percentage, after four months of storage and determined after release tests were repeated using the same experimental conditions, is presented. During this time, the samples were preserved in a controlled ambient condition, at a low temperature (4 °C), protected them from light or humidity.

As can be observed, all of the microcapsules presented a mass loss of less than 20%, proving the adequate stability of the microcapsules. Maltodextrin and carrageenan were the agents that registered the highest values of mass loss. Sodium alginate, pectin, modified chitosan, chitosan, and Arabic gum presented losses less than 10%.

Comparing the release profiles of vitamin B1 microparticles with our previous work on vitamin B12 [230], a similar behavior of the vitamins and encapsulating agents can be observed. The release time proved to be almost the same.

Different kinetic models were applied to determine which fits better the data from the release profiles of vitamin B1. The parameters and the correlation coefficients of these models are presented in Table 6.5, with only one exception, the first order model, because it showed weak

fitting to the experimental data. This can be explained by the fact that this model is applicable to microparticles with liquid cores, which is not our case.

Table 6. 4. Stability evaluation of microcapsules for four months of storage

	Mass loss of vitamin B1 (%)
1. Maltodextrin	17.0
2. Pectin	7.5
3. Sodium alginate	7.5
4. Modified chitosan	3.2
5. Arabic gum	6.2
6. Chitosan	4.0
7. Carrageenan	18.1
8. Modified starch	(*)
9. Xanthan	(*)

NOTE: (*) No data

In general, good fitting was observed, considering the values of the R^2: zero order 0.882 - 0.996, Higuchi 0.909 - 0.994, Korsmeyer – Peppas 0.966 - 0.997, and Weibull 0.971 - 0.998.

Weibull is the model that best fitted the experimental data (Figure 6.6). This type of model is characteristic of microparticles with a matrix type, as is the specific case of microparticles produced by a spray-drying process. The results of this model matched the release profiles determined in both dissolution mediums well.

However, the other models also showed adequate results: zero order model (described by a constant and slow release), Higuchi (specific for water soluble and low soluble core materials), and Korsmeyer - Peppas (a model describing the diffusion mechanism through one of its parameters, n). In the case of this last model, Korsmeyer – Peppas, and considering the information mentioned earlier about this parameter, the results showed the following: anomalous transport (case II transport) for modified chitosan, pectin, and xanthan in SGF; and super case II-transport for chitosan and sodium alginate in both mediums and modified chitosan and pectin only in dW. For these reasons it can be concluded that the release of vitamin B1 from biopolymer microparticles is the result of a combination of mechanisms.

Table 6. 5. Parameters and correlation coefficients of the kinetic models

		Zero order[(1)]			Higuchi		Korsmeyer-Peppas[(2)]			Weibull[(3)]			
		Q_0	K_0	R^2	Kh	R^2	Kk	n	R^2	β	Ƭd exp	Ƭd	R^2
1. arabic gum	**dW**	12.690	39.617	0.949	57.236	0.993	57.957	0.591	0.994	1.129	1.268	1.056	0.990
	SGF	8.398	41.092	0.996	50.961	0.974	49.353	0.724	0.989	1.121	1.383	1.335	0.993
2. carrageenan	**dW**	*											
	SGF	*											
3. chitosan	**dW**	2.746	1.122	0.989	13.979	0.973	2.519	1.010	0.973	1.128	24.850	27.626	0.983
	SGF	1.180	3.558	0.991	21.940	0.982	3.698	0.969	0.979	0.821	23.387	27.856	0.978
4. maltodextrin	**dW**	40.058	43.798	0.882	56.335	0.993	88.659	0.270	0.987	0.789	0.433	0.332	0.982
	SGF	42.054	37.810	0.961	44.611	0.989	81.933	0.298	0.978	1.013	0.482	0.506	0.989
5. mod. chitosan	**dW**	2.369	16.171	0.995	32.986	0.982	18.065	0.956	0.997	1.120	4.357	4.182	0.997
	SGF	9.587	9.587	0.990	27.148	0.987	20.261	0.628	0.992	0.976	5.946	5.339	0.987
6. mod. starch	**dW**	*											
	SGF	*											
7. pectin	**dW**	0.340	1.495	0.994	12.796	0.990	0.708	1.236	0.988	1.376	44.776	41.747	0.994
	SGF	4.352	1.818	0.975	8.329	0.994	5.131	0.619	0.966	0.636	76.319	81.561	0.991
8. sodium alginate	**dW**	6.593	8.604	0.993	33.626	0.909	4.099	1.294	0.982	1.658	7.879	7.588	0.971
	SGF	5.312	8.114	0.985	29.425	0.980	10.262	0.990	0.991	1.178	6.758	6.698	0.998
9. xanthan	**dW**	*											
	SGF	7.753	11.484	0.958	28.391	0.992	20.497	0.700	0.995	0.781	5.774	5.883	0.995

6.5. Conclusion

Considering the benefits and the functions of vitamin B1, it is necessary to manufacture more food and nutraceutical and pharmaceutical products enriched with this vitamin. These products are essential for human health because they can maintain or correct the level of vitamin B1 and prevent deficiencies of vitamin B1.

Using spray-drying as a tool to protect and preserve sensitive compounds like vitamins comes with several advantages. It is simple and fast to produce polymeric delivery systems and enhance the stability of the encapsulated material against possible external factors.

The present research has led to the microencapsulation of vitamin B1 using a spray-drying method. Several biopolymers were selected as encapsulating agents with the purpose of understanding the role of encapsulating material on the final product. the microparticle.

This study demonstrates the possibility of encapsulating vitamin B1 using different encapsulating agents by a spray-drying technique. Depending on the intended applications, for fast release, adequate results were obtained for maltodextrin, Arabic gum, modified chitosan, and sodium alginate, and for slow release, adequate results were obtained for chitosan and pectin.

Different release behaviours have been observed, i.e., from just 10 seconds (modified starch) to more than 24 hours (xanthan).

Interest of the application of vitamin B1 microparticles in the food and pharmaceutical industries has been growing in the last few years. Regarding the food industry, the microencapsulated product represents 1% - 5% of the final product, while the maximum expected cost for a microencapsulation process is estimated at ~€ 0.1/kg.

Given the overall results, spray-drying seems to be a feasible method to produce stable vitamin B1 microparticles, and future work should continue with studies about the possibility of incorporating these microparticles into real products.

Chapter 7

Microencapsulation of vitamins B2 and B3

Chapter number seven gathers the results of microencapsulation of vitamins B2 and B3. This is the fourth and last study of this research.
This study is in the format of submitted paper:

"Innovation and improvement in food fortification: Microencapsulation of vitamin B2 and B3 by a spray-drying method and evaluation of the simulated release profiles", *Ioana C. Carlan, Berta N. Estevinho, Fernando Rocha, Journal of Dispersion Science and Technology, accepted for publication.*

7.1. Abstract

Food fortification helps to maintain, improve or correct nutrients requirements. The current paper explored the possibility of using spray-drying method to produce microparticles with vitamin content suitable for incorporation in fortified food products. Vitamin B2 and vitamin B3 were microencapsulated with six different biopolymers (chitosan, modified chitosan, gum arabic, maltodextrin, sodium alginate, pectin), aiming with this research to highlight the similarities and differences. For these two categories of microparticles were determined: product yield, encapsulation efficiency, size and external morphology. Afterwards, in vitro studies were performed under two different in *vitro* simulated conditions to evaluate: the time required for the vitamin to be released, the best kinetic models (Zero order, Higuchi, Korsmeyer-Peppas and Weibull) to describe the experimental release profiles and the main release mechanisms involved. Stability of the microparticles over 4 months of storage was also evaluated. The overall results lead to a successful microencapsulation process as for both vitamins the encapsulation efficiency reached values higher than 99% and the product yield ranged from 45 to 55% in the case of vitamin B2, and to 58% for vitamin B3. Trough SEM characterization microparticles: with regular or irregular spherical shape and with smooth or rough surface were distinguished. The mean size of the microparticles loaded with vitamin B2 ranged in the interval 0.10 – 0.16 µm and the ones with vitamin B3 between 0.11 and 0.84 µm, considering a number distribution. With faster (minutes) or slower (hours) release, both types of microparticles showed a very good fitting especially to Weibull kinetic model and proved good stability over time, since a vitamin loss lower than 10% was registered.

Keywords: Biopolymers, controlled release, food fortification, kinetic models, microencapsulation, spray-drying, vitamin B2, vitamin B3.

7.2. Introduction

Concerns regarding personal health, wellbeing and environmental protection led consumers to change the way they think about food [234]. Following the latest food diets it was noticed a growing tendency for the consumers to choose organic food, products with natural ingredients, processed as little as possible or not at all. Therefore, food industry is facing the challenge to create new products and to adapt old manufacturing techniques to these new demands [6].

A solution for both consumers and industry is food fortification, a process of nutrients supplementation with the purpose of improving the properties of food products. Even though this process is not a new practice for this industry branch, there are still many food products that could benefit from fortification [235]. Food fortification can be done voluntary or mandatory according to the legislation of every country or region. In the case of voluntary fortification, food producers add nutrients to improve the composition of some products with the purpose to increase sales and to promote healthy foods. Instead, mandatory fortification it is applied to staple foods, regulated by specific laws and considered a public health measure against nutritional deficiencies [86].

There is a big variety of nutrients used for fortification and the most important are the vitamins and the minerals. Both categories are defined as essential micronutrients, because are required daily in relatively small quantities.

The current study will focus on two water soluble vitamins from B complex, namely B2 and B3. Water-soluble class of vitamins gathers vitamins C and the B complex group. All these vitamins have in common the following characteristics: the capacity to dissolve in water, cannot be stored by the human body and it is mandatory to ensure a minimum daily dose [28].

The first steps for the discovery of vitamin B2 were done in 1872, when a yellow pigment with fluorescent properties, named lactochrome, was found. Until the early 1930s it was speculated that vitamin B2 was the factor responsible for the cure of pellagra, a dietary deficiency of that time. Yet the actual form of vitamin B2 was identified, isolated and synthesized between 1932 and 1939. While the discovery of vitamins continued, in 1937 it was established that the compound responsible for pellagra was actually vitamin B3 (nicotinic acid) [77,236].

Vitamin B2, well-known under names like riboflavin or lactoflavin, is a precursor for FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide), two co-enzymes with an

essential role in the cellular metabolism. Riboflavin has antioxidant and anti-inflammatory properties, stimulates healthy fetal development, reduces blood pressure, strengthens immune and digestive systems [28].

Vitamin B3 is the generic name for niacin, nicotinamide, nicotinic acid and vitamin PP. NAD (nicotinamide adenine dinucleotide) and NADP (nicotinamide adenine dinucleotide phosphate) are specific co-enzymes required for cellular oxidation-reaction processes. Other essential functions of vitamin B3 are: boast brain activity, improves cholesterol levels, reduces the risk of heart diseases and prevents birth defects [77,78].

Both vitamins B2 and B3 are involved in the metabolism of carbohydrates, fats and proteins so the two vitamins produce energy from foods, act as co-enzymes, promote healthy skin, nails, hair and vision. The recommended dietary allowance (RDA) for vitamin B2 is 1.1 mg/day for women and 1.3 for men, but for vitamin B3 is required a higher amount, of 14 mg/day for women and 16 mg/day for men [187]. The RDA should be reached easily from normal food, if the diet is made up of varied natural products. The most important natural sources of vitamin B2 are meat (organ products, lean meat, poultry), dairy products (milk, cheese, yogurt), eggs, vegetables (with dark green colour – asparagus, broccoli and green leafy – spinach) and whole grain cereals [72]. In the case of vitamin B3, the richest natural sources are yeast extract, fish (yellowfin tuna, anchovies, salmon), meat (organ meat - liver, lean meat, poultry), grains and cereals, vegetables (green peas, mushrooms, sweet potatoes, asparagus) nuts and seeds (peanuts and sunflower seeds) [77].

People can suffer from nutritional disorder provoked by the lack of vitamin B2 or vitamin B3. In this situation it is mandatory to get medical assistance for fast correction of the vitamin status. The treatment will rely on specific medication and a proper diet, in which fortified products are recommended.

At high risk are people from developing countries, vulnerable ones who experience poor diets. Stress situations and alcoholism also can provoke deficiencies of these vitamins. As well pregnant women or during lactation and their infants are susceptible to develop vitamins deficiency. The following groups may also be affected,: elderly people who might suffer from malabsorption, those who chose selective food diets like vegetarians and patients with cancer, heart or kidney problems [76].

Vitamin B2 deficiency is called ariboflavinosis and the most frequent symptoms include muscular weakness, appetite and growth decrease, anemia and fatigue, anxiety with signs of depression, dermatologic disorders (cheilosis, stomatitis, glossitis), gastrointestinal inflammation, ophthalmological problems (blurred vision, photophobia) [72,237].

The signs of mild Vitamin B3 deficiency are dermatologic disorders, fatigue with loss of appetite, high cholesterol, digestive problems and brain impairment. The severe vitamin B3 deficiency leads to pellagra, a disease more common for poor population from developing countries, characterized by the "4D" diarrhea, dermatitis and dementia that in a final stage will determine the death of the patient [77].

To avoid as much as possible, the cases of vitamins B2 and B3 deficiency several fortification measures were taken, and the first product to be fortified with these two compounds was flour, around 100 years ago. From flour, a basic aliment, the list of fortified food products with vitamins B2 and B3 extended to bread, bakery products, cereals, pasta, dairy products, milk, infants' formulas, energy drinks and beverages [19].

Considering all functions and benefits it is easy to understand the importance of vitamins B2 and B3 for human health. However, a suitable delivery system for these compounds must be found because they are prone to lose stability when exposed to external factors, during manufacturing processes and even storage time. One solution to overcome stability problems is to use microencapsulation, a process that has been reported to have very good results for the manufacturing of food products [125,238].

There are some microencapsulation methods described in the literature for the encapsulation of vitamins, namely vitamin B2 and B3. For example, the following methods have been used for the production of vitamin B2 microparticles: emulsification (alginate beads) [151], oil-in-water emulsion solvent evaporation (O/W) [155,239], double emulsion (W1/O/W2) [240], whey protein hydrogels [154], freeze-drying [150] and extrusion dripping [241]. For the vitamin B3 some examples are: co-crystalization [166], micro emulsification and complexation [242], water-in-oil-in-oil emulsion [163], fluidised bed spray coating [243] or sol-gel-based encapsulation [244].

One of the most important methods of microencapsulation described in the literature is the spray drying technique. It is a technique with high impact in the industry and with an extraordinary applicability in the food industry allowing the production of fortified food products and supplements with an acceptable commercial price. The spray-drying method is a

one-step process in which the feed solution is converted into a dried form by atomizing the solution into a hot air stream [27]. Spray-drying is preferred for food industry due to its features: easy handling, flexible, rapid and economical [26,194].

In this work, vitamins B2 and B3 were microencapsulated by spray-drying using as encapsulating agents some biopolymers. Natural biopolymers were selected based on their optimal characteristics for food industry: biocompatibility, biodegradability, eco-friendly, nontoxicity, good stability in water and low viscosity for high concentrations [245]. The application of the spray drying technique continues limited in the microencapsulation of vitamins namely with the application of biopolymers (high biocompatibility). For the vitamin B2, at least 6 papers were published based on the microencapsulation with whey protein isolate (WPI) [158], whey protein concentrate [246], galactomannan biopolymer and F127 [157], lycopene−gum arabic [247], arabic gum [152] and buffalo skim milk [248].

For vitamin B3 at least 3 papers were made using as encapsulating agents, whey protein concentrate [249], whey protein isolate [165] and hydroxypropyl methylcellulose (HPMC E5) [164].

So, the number of publications reported on this topic is quite small, and for this reason, new approaches must be explored.

The aim of this paper was to compare microparticles with vitamin B2 and vitamin B3 content and to highlight the similarities and differences between them. As coating materials for the microparticles the following six biopolymers from the polysaccharides class were selected: chitosan, modified chitosan, gum arabic, maltodextrin, sodium alginate, and pectin, according to preliminary studies with vitamin B1 and B12. All of them are part of the list called Generally Recognized As Safe (GRAS) and have the approval from Food and Drugs Administration (FDA) to be used as food or as pharmaceutical additives.

The microparticles were prepared at a relatively low working temperature – 120 °C, in order to avoid destroying the vitamins during the drying process. After, both types of microparticles formulations (vitamin B2 – biopolymer and vitamin B3 – biopolymer) were evaluated for product yield and encapsulation efficiency. Further, the size and morphology of the prepared microparticles were determined. In vitro release studies were made, in deionized water (dH2O) and simulated gastric fluid (SGF). Different kinetic models were studied to understand how the vitamin is being released from the microparticles and the main mechanism involved. In the

last part of the work the stability of the microparticles was evaluated by repeating the release tests after 4 months of storage.

7.3. Materials and methods

Previous studies of the authors [32,229,230] were used as model for the selection of the experimental conditions and procedures required for the microencapsulation of vitamin B2 and vitamin B3 and for the further analysis done with the obtained microparticles.

Materials

Vitamin B2 was obtained from Sigma Aldrich (China) as riboflavin from Eremothecium ashbyii, ≥98% (R4500-256, Lot #WXBC3912V, CAS: 83-88-5) and vitamin B3 from Sigma Aldrich (USA) as nicotinamide, ≥98% (TLC) powder (N3376-100G, Lot #SLBT5505, CAS: 98-92-0).

The encapsulating agents were provided from the following suppliers: chitosan – Sigma Aldrich, Germany (Cat. No. 448877), gum arabic – Fluka, Germany (30888, Lot #BCBK8649V), maltodextrin – Sigma Aldrich, USA (1001841656 419672, Lot # MKBN6629V), modified chitosan – China Easter Group, Dong Chen Co. (Batch no. SH20091010), pectin – Sigma Aldrich, Switzerland (101582340 76282, Lot #BCBN5335V) and sodium alginate – Sigma Aldrich, USA (1001503523 180947, Lot # MKBH8463V).

The chitosan solution of 1% (w/V) was prepared with an aqueous acetic acid solution of 1% (V/V) with the viscosity of 200 mPa·s (25 °C).

The materials used to prepare SGF were sodium chloride from AppliChem Panreac ITW Companies (product code: 131,659.1211, Lot 0000542745, CAS-No: 7647-14-5) and hydrochloric acid 37% from Sigma-Aldrich (product code: 25,814-8, CAS-No.: 7647-01-0).

Deionized water was collected from a water purification device with a 0.45 μm filter, type MilliporeTM (Massachusetts, USA). The materials selected had analytical grade purity.

Preparation of the experimental solutions

Different solutions were prepared for every encapsulating agent with a concentration of 1% (w/V) and a volume of 200 ml. For 2 hours these solutions remained under continuous stirring at a speed of 1200 rpm, to ensure complete homogenization.

The vitamin B2 and vitamin B3 solutions were produced separately. In the case of vitamin B2, a special place protected from light exposure was used to prepare the solutions.

Solutions with a concentration of 0.25% (w/V) vitamin B2 and a 4 ml volume were homogenized for 10 min with a shaker. Same procedure was repeated for vitamin B3, but the solutions had a 0.5% (w/V) concentration. After the vitamin's solutions were mixed with the encapsulating agent solutions and set aside for at least 30 min to mix at a speed of 500 rpm.

Solutions without core material, only with encapsulating agents, were prepared.

To obtain the stock solution of SGF it was necessary to dissolve 2.0 g of sodium chloride into 1 L of dH_2O and after added 7.0 mL of hydrochloric acid 37%. The final solution should have an acid pH of 1.2 and to reach this value additional corrections of pH might be done. This procedure followed the specifications of the European Pharmacopeia 7.0 (2010).

For all solutions, deionized water was used, at room temperature.

Preparation of the microparticles

The microparticles loaded with vitamins B2 and B3 were produced with a Mini Spray Dryer B-290 equipment from BÜCHI (Flavil Switzerland) with a standard 0.5 mm nozzle. The configuration used for all experiments was: solution flow rate – 4 mL/min (15%), air flow rate – 32 m3/h (80%), air pressure – 6 bar and inlet temperature – 120 °C. The only parameter that varied during the spray-drying processed was the outlet temperature. The following temperatures were registered: 63 – 72 °C for the solutions with vitamin B2 content, 55 – 63 °C for the solutions with vitamin B3 content and 60 – 68 °C for the solutions without vitamin content.

To avoid degradation, the samples collected from the main equipment were covered with aluminum foil and stored in the fridge at 4 °C until further analysis.

Characterization of the microparticles

The morphology of the microparticles was examined with a Fei Quanta 400 FEG ESEM/EDAX Pegasus X4M equipment (Eindhoven, The Netherlands) by scanning electron microscopy (SEM). Before every analysis, the samples were placed on a brass stub using double-sided adhesive tape, and then were covered with a thin layer of gold in a Jeol JFC 100 equipment.

The size of the microparticles was determined with a Coulter-LS 230 (Miami, USA) using laser granulometry method. For every sample triplicates of 30 seconds runs each were performed to

consider the average as valid value. Prior the tests, the samples were ultrasound-irradiated in ethanol dispersant solution to avoid the aggregation of microparticles.

In vitro studies for the release of vitamins B2 and B3

In vitro studies were performed for two related reasons: first to demonstrate the presence of vitamins inside the microparticles and determine the encapsulation efficiency and second, to evaluate the release process. The release profiles were used also to determine the time necessary for complete release. Some kinetic models that can describe the release and stability of the microparticles overtime were studied.

It was chosen to analyze the samples using a spectrophotometric method with an equipment from Sarspec SPEC RES+ UV/VIS (Portugal). The spectrophotometer was customized with an external device for temperature and magnetic stirring system, both operated by optical fiber connectors.

Before proceeding to in vitro studies the analytical method [183–185] was validated, to prove that spectrophotometry is suitable for the evaluation of the release of vitamins B2 and B3. Therefore, specific parameters were determined. In Table 7.1., the results for: linearity range (equation of calibration curve, coefficient of correlation R^2), limit of detection (LOD), limit of quantification (LOQ), accuracy and precision are presented.

To avoid possible interference of vitamin B2 or vitamin B3 with the encapsulating agents the values of their wavelengths were verified. And as it can be observed in Table 7.2., the wavelengths are different, so the specificity rule is also respected.

The configuration selected for the acquisition of data during the release studies was readings every 10 seconds in continuous mode (UV domain) at 266 ± 2 nm for vitamin B2 and at 261 ± 1 nm for vitamin B3. Other authors confirmed similar values of the reading wavelength, or with small differences, for both vitamin B2 [71] and vitamin B3 [167].

The microparticles with vitamin B2 and the microparticles with vitamin B3 were analysed separately. Samples of 3 mg from each formulation of microparticles with vitamin B2 were released on the top of the dissolution mediums. For the release tests with vitamin B3 a different amount of microparticles, 5 mg per test, was used.

The amount of microparticles was determined from the mass balance of the reagents, considering that the ratio between the core material and the encapsulating agent remained

constant during the spray-drying process. The cuvette, in which the samples were placed, was set to maintain a constant value of stirring and a specific temperature for each medium: so, for dH_2O 22 °C and for SGF 37 °C.

Table 7. 1. Parameters calculated to validate the analytical method.

		Equation	R^2	LOD (mg/L)	LOQ (mg/L)	Accuracy (**) (%)	Precision (**)
Vitamin B2	**dH_2O (*)** (20 °C)	y = 65.698x + 0.0372	0.996	0.317	1.055	102.2 101.9 95.9	0.880 0.228 0.679
	SGF (*) (37 °C)	y = 65.698x + 0.0372	0.997	0.301	1.003	104.3 99.0 97.1	0.356 0.312 0.389
Vitamin B3	**dH_2O (*)** (20 °C)	y = 21.815x + 0.0045	0.999	0.310	1.032	101.6 99.9 99.0	1.694 0.280 0.236
	SGF (*) (37 °C)	y = 33.073x + 0.0236	0.999	0.555	1.849	101.3 100.6 97.6	0.311 0.318 0.430

(*) **Linearity range**: 0.0005 – 0.012 mg/ml (vitamin B2), 0.00125 – 0.03 mg/ml (vitamin B3)
(**) and (***) calculated for the last 3 concentrations

The release time was different for every formulation. Besides the comparison of the behavior of different biopolymers used as encapsulating agents, it was made also a comparison between fresh and old samples. So, the tests were repeated in the same conditions after 4 months during which the samples were stored and preserved.

Table 7. 2. Specificity – detected wavelengths of the encapsulating agents and core.

Wavelength of encapsulating agents:	
Gum arabic	225 ± 1 nm
Chitosan	229 ± 1 nm
Maltodextrin	227 ± 3 nm
Modified chitosan	220 ± 1 nm
Pectin	230 ± 1 nm
Sodium alginate	199 nm
Wavelength of core materials:	
Vitamin B2	266 ± 2 nm
Vitamin B3	261 ± 1 nm

After the release profiles were established, the experimental data was adjusted to several kinetic models, and after evaluating the fitting degree, it was determined which model describes best the process of release for each of these two vitamins. The selection of kinetic models included zero order, Higuchi, Korsmeryer-Peppas and Weibull models. The specific equations and parameters [27,134] are presented in Table 7.3.

Table 7. 3. Kinetic models applied for the release of vitamins B2 and B3 from the microparticles.

Equation	Parameters
Zero order model $Q_t = Q_0 + K_0 t$ **(7.1)**	Q_0, Q_t= amount of active compound released at time zero, t (%) Q_t/Q_∞= fraction of active compound released at time t (%) t = time (min) K_0, K_H, K_K = constant of release for Zero order, Higuchi Korsmeyer – Peppas model τ_d = the time (min) when 63.2% of active compound was released n = release exponent (diffusional) n < 0.43 (Fickian diffusion: case I transport with rate as a function of time $t^{-0.57}$) 0.43 < n < 0.85 (anomalous transport: diffusion mechanisms with smelling release, case II transport, rate as a function of time, t^{n-1}) n = 0.85 (case II transport, rate as a function of time – zero order release) n > 0.85 (super case II transport and rate as a function of time, t^{n-1}) β = shape parameter of the curve β = 1 (the curve will have an exponential profile) β > 1 (will be sigmoidal with a turning point) β < 1 (the release will show a steeper increase then in the case of β = 1)
Higuchi model $Q_t = K_H\sqrt{t}$ **(7.2)**	
Korsmeyer – Peppas model $Q_t/Q_\infty = K_K t^n$ **(7.3)**	
Weibull model $M_t = M_\infty\left[1 - e^{-\left(\frac{t-t_0}{\tau_d}\right)^\beta}\right]$ **(7.4)** $\ln\left(\ln\left(1/(1-M)\right)\right) = Z + \beta\ln(t)$ **(7.5)** $\tau_d = e^{(Z/-\beta)}$ **(7.6)**	

Statistical analysis of the experimental data

The statistical analysis was evaluated by the coefficient of variation (CV) and for all tests the values were lower than 10%. Each test was performed three times and the final result was calculated as the mean of the triplicates.

7.4. Results and discussion

The functionality of these two type of microparticles, with vitamin B2 and vitamin B3 content, was determined by the evaluation of several parameters like: product yield, encapsulation efficiency, size, morphology, release profiles, stability over time and kinetic models [238]. The results of this work are presented and discussed in the following subsections.

Spray-drying process

The challenge of working with water-soluble vitamins comes from poor stability and predisposition to fast degradation. This work proposed the use of microencapsulation as an alternative method to produce stable transport vehicles for vitamins B2 and B3.

Vitamin B2 is a yellow crystalline compound relatively stable to heat or variations of pH. However, the stability is easily affected by the exposure to light. So, handling must be done in the dark or under subdued red light because vitamin B2 is light-sensitive compound and will suffer fast and irreversible photochemical degradation [72].

Vitamin B3 is a white compound with needle-shaped crystals aspect, stable in dry form and solutions. Considered the most stable water-soluble vitamins, its activity is not affected by external factors like heat, light, acid, alkali or oxidation [81].

But both vitamins are predisposed to degradation during processing or storage, therefore measures must be taken to avoid nutritional losses.

The microencapsulation of vitamins B2 and B3 was performed with a Mini Spray – Dryer and it was possible to produce different types of microparticles: formulations of vitamin – biopolymer (vitamin B2 or vitamin B3 – chitosan, modified chitosan, gum arabic, maltodextrin, sodium alginate or pectin), plus formulations of empty microparticles.

The performance of this process was determined by the product yield and the encapsulation efficiency. The specific equations of these process factors are presented below in the Equations 7.7. and 7.8.

$$\text{Product yield (\%)} = \frac{(\text{amount of recovered powder})}{(\text{initial amount of solid materials})} \cdot 100 \quad (7.7)$$

$$\text{Encapsulation efficiency (\%)} = \frac{(\text{total core released} - \text{"free" core})}{(\text{total core released})} \cdot 100 \quad (7.8)$$

The product yield (Equation 7.7.) measures the percentual ratio between the output (amount of obtained powder) and the input of materials (amount of solid materials from the feed composition). Results of the product yield of microparticles with vitamin B2, vitamin B3 and empty microparticles are presented in Figure 7.1.

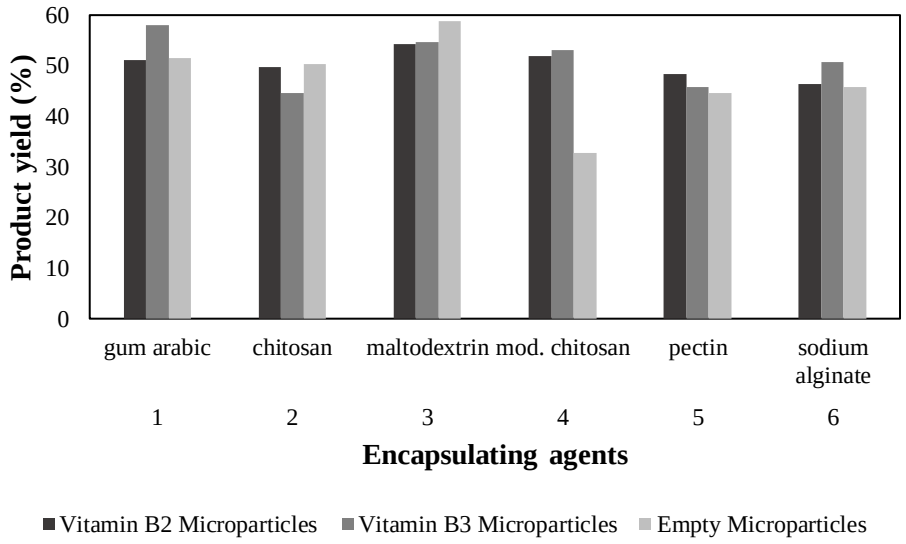

Figure 7. 1. Product yield (%) of microparticles loaded with vitamin B2, with vitamin B3, and of empty microparticles.

The results obtained for microparticles with and without core material were compared because it is important to understand how the core will influence the microencapsulation process. The values of the product yield ranged in the following intervals: for vitamin B2 microparticles 47 – 54%, for vitamin B3 microparticles 47 – 54% and for empty microparticles 33 – 59% (Figure 7.1). Some differences were noticed between the values of the product yield, from 4.18% until 7.20%, these values expressed as coefficients of variation. For modified chitosan an exception was noticed as the coefficient of variation was 24.82%. In the case of this encapsulating agent. the product yield of empty microparticles had a very low value comparing with vitamin loaded microparticles.

Since the equipment is laboratory scale it is well known that the performance of the spray-drying process will be diminished. This can be justified by the low amounts of solid materials required for the fed solution and by the losses that occur during the drying stages. Small batches

lead to moderate results of product yield, fact indicated by values typically not higher than 70% [250].

The authors tested vitamin C and vitamin B12 with similar experimental conditions and comparable results were observed. In a first study [32] three encapsulating agents (sodium alginate, chitosan and modified chitosan) with vitamin C (43.6, 44.5 and 45.4%) and vitamin B12 (41.8, 55.6 and 42.4) were studied. Vitamin B12 was analyzed in two different studies, one in which experimental conditions are optimized with modified chitosan [229] and other in which different biopolymers were used to produce loaded microparticles [230]. From the last study six out of nine encapsulating agents are the same as the encapsulating agents from this study. The results are: gum arabic – 48%, chitosan – 55.5%, maltodextrin – 49.9%, modified chitosan – 55.8%, pectin – 43% and sodium alginate 43.6%. So, for all formulations lower values of product yield, except for chitosan and modified chitosan, were determined.

Other authors have studied the microencapsulation by spray-drying method of water-soluble vitamins like vitamin C and vitamin B1.Desai et al. chose cross-linked chitosan (TPP-chitosan) for protecting vitamin C and obtained in a first study a product yield between 60.15 and 62.83% [207] (T_{inlet} = 170 °C) and in a second work 54.5 – 67.5% [231] (T_{inlet} = 175 °C). Another type of chitosan, namely acid-grafted chitosan, was used in a study of Chatterjee et al. [147] for the microencapsulation of vitamins B1 and B6 (T_{inlet} = 140 °C). The product yield of this study had even higher values, 63.6 – 65.1%.

The product yield of vitamin B3 microencapsulation process was reported by Paidi et al., (2015). Paidi et al. reported a product yield of around 26% for a temperature similar with the current study (T_{inlet} = 120 °C), but a feed flow rate lower than 15% and aspiration volume of 70%. After changing the conditions to T_{inlet} = 110 °C and increasing the flow rate to 15% and the aspiration to 80% a higher product yield of 42.03 ± 0.65% was obtained, proving with this the effect of increasing the aspiration rate.

In terms of the encapsulation efficiency (EE) (Equation7.2.), not all core material will get inside the microparticles, therefore some material, known as free core, will remain “unentrapped” or at the surface of the microparticles. Figure 7.2. presents the encapsulation efficiency of each type of microparticles and the results lead to a very good encapsulation of these vitamins. For vitamin B2 the minimum value was of 99.3% (gum arabic) and the maximum of 99.8% (modified chitosan); as for vitamin B3 the minimum was 98. 9% (modified chitosan) and the

maximum 100.0% (sodium alginate). Because these values are very close to 100%, it can be concluded that full entrapment of vitamins B2 and B3 has been reached.

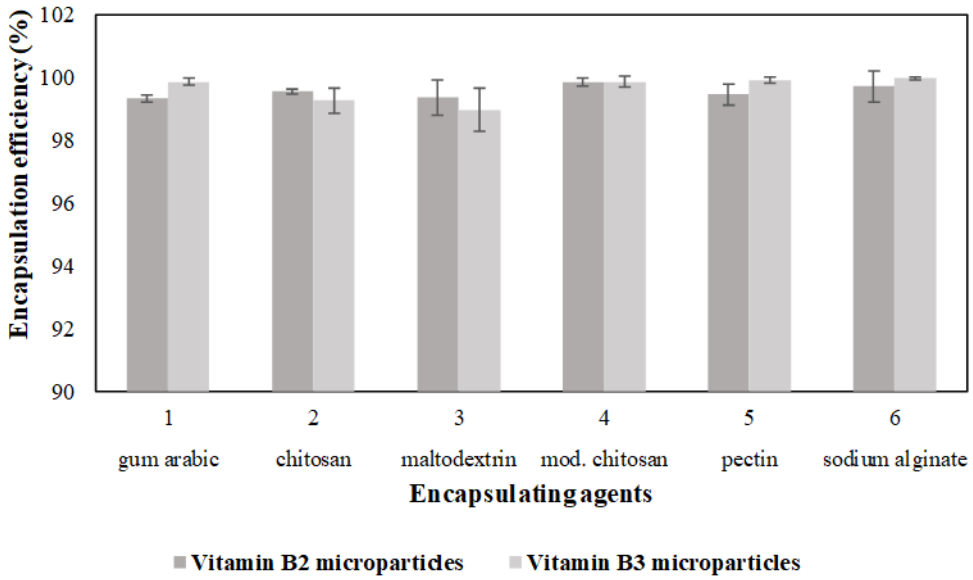

Figure 7. 2. Encapsulation efficiency (%) for microparticles loaded with vitamins B2 and B3, the bars represent standard deviations.

Similar studies in which spray-drying technique was used to microencapsulate water-soluble vitamins obtained good results, but with lower values. Farias et al. [157] selected galactomannan biopolymer as encapsulating agent for the microencapsulation of vitamin B2 and obtained values of 87.14 – 88.53% for EE. For this study, the same type of equipment as in the current work was used and three types of formulation (with two different surfactants and without) at 130 °C for T_{inlet} and 100 °C for T_{outlet} were prepared. In another study [147] good EE results for vitamin B1 of 91 ± 2.31% and for vitamin B6 of 83 ± 3.17%, were obtained.

In the case of vitamin C encapsulated with cross-linked chitosan Desai et al. obtained moderate values of EE: 45.05 – 58.30% in the first study [207] and 45.72 – 68.7% in the second study [231].

SEM and laser granulometry analysis

The morphology of the microparticles was studied using SEM. In Figure 7.3. images of the different particles prepared.

With or without core material, the microparticles prove to look alike. Differences appear between encapsulating agents and 3 types of morphologies were identified: A, B and C. Type A has regular spherical shape with smooth surface and is specific for modified chitosan. Types B and C have both irregular shape but round appearance and rough surface. On the surface of types B and C some shallows can be observed. Type B with big shallows is characteristic for sodium alginate, maltodextrin, pectin and gum Arabic. Type C with small shallows was identified only for chitosan.

Almost identical morphology of microparticles was observed in some previous studies with vitamin B12 as core and chitosan, modified chitosan, gum arabic, maltodextrin, pectin and sodium alginate as encapsulating agents [32,229,230], with vitamin C and chitosan, modified chitosan, cross-linked chitosan and sodium alginate [32,207,231], as well with both vitamins B1 and B6 with acid ferulic grafted chitosan [147,251].

Regular shape for particles with vitamin B2 content was reached with calcium pectinate-alginate beads prepared by freeze-drying [150], with alginate/chitosan nanoparticles produced by ionotropic pre-gelation [252], and with ovomucin nanoparticles [253].

Vitamin B2 loaded microparticles with irregular structure and surface, were observed also in the study of Farias et al. [157]. In this case, the authors prepared different formulations with a biopolymer named galactomannan and with the surfactant F127 using spray-drying method.

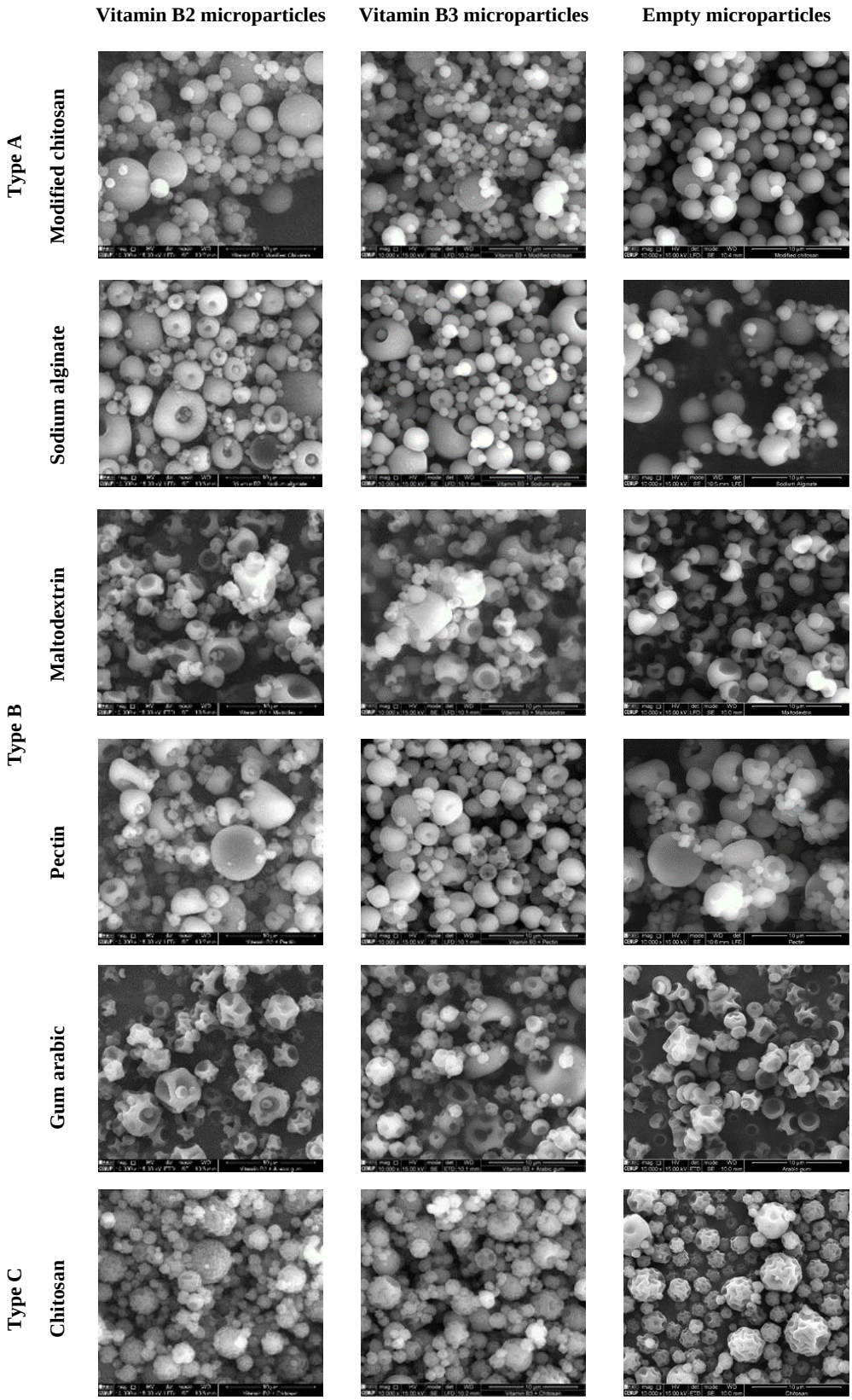

Figure 7. 3. SEM images of vitamin B2, vitamin B3 and empty microparticles (magnification of 10.000x, beam intensity (HV) of 15.00kV and distance between the samples and the lens (WD) around 10 mm).

Measurements with laser granulometry were made to determine the size of microparticles with vitamin content and for empty ones as well. The results for number distribution and for volume distribution are presented in Tables 7.4 and 7.5, respectively.

Table 7. 4. Mean diameter of microparticles determined from differential number distribution for vitamins B2 and B3 loaded microparticles and for empty microparticles.

	Mean diameter (± STDEV) µm - differential number-		
	Vitamin B2 microparticles	**Vitamin B3 microparticles**	**Empty microparticles**
1. Gum arabic	0.12 ± 0.00	0.56 ± 0.02	0.57 ± 0.02
2. Chitosan	0.16 ± 0.01	0.69 ± 0.015	0.70 ± 0.02
3. Maltodextrin	0.10 ± 0.00	0.11 ± 0.00	0.11 ± 0.00
4. Modified chitosan	0.11 ± 0.00	0.18 ± 0.03	0.60 ± 0.05
5. Pectin	0.10 ± 0.00	0.11 ± 0.00	1.77 ± 0.05
6. Sodium alginate	0.11 ± 0.00	0.84 ± 0.07	0.60 ± 0.02

The mean diameter for vitamin B2 loaded microparticles determined from the number distribution showed values in the interval 0.10 – 0.16 µm, and for vitamin B3 higher values of 0.11 – 0.84 µm. According to the volume distribution, the values for vitamin B2 microparticles are in the range 4.12 – 6.84 µm, and for vitamin B3 values of 4.03 – 9.78 µm. These measurements confirm the small size of the microparticles and the tendency to form quite compact groups (aggregates), fact confirmed also by the SEM images.

As shown in Tables 7.4. and 7.5. the presence of core material will lead, in general, to the production of smaller or approximately equal size microparticles for number and volume measurements, in most of the formulations.

Similar behavior was observed in the work of Azevedo et al. [252] in which nanoparticles of alginate/chitosan with (104.0 ± 67.2 nm) and without (119.5 ± 49.9 nm) vitamin B2 are compared.

Table 7. 5. Mean diameter of microparticles determined from differential volume distribution for vitamins B2 and B3 loaded microparticles and for empty microparticles.

	Mean diameter (± STDEV) µm - differential volume-		
	Vitamin B2 microparticles	**Vitamin B3 microparticles**	**Empty microparticles**
1. Gum arabic	4.12 ± 0.01	4.09 ± 0.00	4.22 ± 0.00
2. Chitosan	4.78 ± 0.03	5.14 ± 0.21	4.25 ± 0.05
3. Maltodextrin	6.84 ± 0.09	7.59 ± 0.12	7.24 ± 0.06
4. Modified chitosan	6.70 ± 0.08	9.78 ± 0.38	9.31 ± 0.15
5. Pectin	5.05 ± 0.20	4.03 ± 0.03	4.66 ± 0.01
6. Sodium alginate	4.79 ± 0.02	4.77 ± 0.14	10.18 ± 0.03

In other studies, higher values were determined in terms of number and volume for the size of the vitamin B12 microparticles prepared by the authors [230]. Measurements for number distribution were found for vitamin B1 (4.5 µm) and B6 (4.8 µm) by Chatterjee et al. [147] with acid-grafted chitosan microparticles and for vitamin B2 (160 nm) by Akbari et al. [253] with ovomucin nanoparticles.

The selection of an encapsulating agent is very important because will determine specific morphology and size. Therefore, the materials used as encapsulating agents must be linked to the final application of the microparticles, according to the desired shape and size to be reached. In addition, the effect of the core material and possible interactions between the core and the encapsulating must be taken into consideration. As it can be observed in the case of gum arabic, chitosan and sodium alginate, the microparticles with vitamin B3 as a core have much bigger size in terms of number distribution as the same type of microparticles with vitamin B2 as a core.

Controlled release studies

If properly implemented, vitamin-fortification can sustain the daily recommended intake or to correct a possible deficit. Towards the importance of preliminary tests done before developing food products, the release mechanism of vitamins B2 and B3 were investigated under controlled conditions.

Two dissolution mediums were selected to be tested: dH_2O at 22 °C – to simulate room temperature, and SGF at 37 °C – to simulate human body temperature. Only one wavelength was tested for each vitamin, because both compounds are relatively stable to pH changes.

Prepared microparticles are likely to be suitable for food products to be delivered through the oral route. dH_2O system was selected for possible applications like juices, syrups, instant soups, effervescent tablets or any type of product prepared from a powder dissolved in water. On the other hand, tests in SGF are relevant for those food products or nutraceuticals that will release the vitamin B2 content only after consumption in the stomach.

In Figure 7.4. the release profiles of vitamins B2 (1 – 6. A.) and B3 (1 – 6. B) from the biopolymer microparticles, are presented. The release profiles were determined after the analysis in dH_2O and in SGF and the results from Figure 7.4. present two types of profiles: one built with experimental data and one built according to Weibull kinetic model.

An important information obtained from these release profiles is the time necessary for the core materials to be completely released from the microparticle. As can be seen in Table 6, differences between encapsulating agents and between dissolution mediums are registered.

Vitamin B2 can be released from the produced microparticles in following time intervals: less than 30 min (gum arabic – in both mediums, and modified chitosan, maltodextrin, sodium alginate – only in dH_2O), around one hour (modified chitosan and maltodextrin in SGF), around one hour and 30 min (pectin in dH_2O and sodium alginate with chitosan in SGF), around 5 hours (chitosan in dH_2O) and about 21 hours (pectin in SGF).

Regarding vitamin B3 particles the release was reached in the interval of times as follows: less than 30 min (gum Arabic, modified chitosan, maltodextrin and sodium alginate – in both mediums), around one hour and 30 min (pectin in dH_2O) and around 5 hours (chitosan in both mediums). The release of vitamin B3 from pectin microparticles in SGF medium was not accomplished due to possible degradation.

It is not possible to define a pattern for the effect of the encapsulation agent and the release medium on the release profile and release time. In general, Arabic gum gives the faster releases, and chitosan and pectin the slower ones.

So before selecting an encapsulating agent it is important to know what is expected from the product: fast or slow release of the core material. In most of the cases of food application, a fast release is expected, so the core material offers the nutritional value as fast as possible.

However slow release has the advantage of releasing the core gradually, so the effect will be prolonged in time.

Stability over time is another aspect that must be considered, because is important to know if after being incorporated in real products, the microparticles will not lose their properties and will not be deteriorated. Table 7.7. presents how much of the initial amount of both vitamins is lost after 4 months of storage. Very good results can be observed for almost all types of formulations since it was registered a loss smaller than 10%. The only agent with a higher loss is chitosan with a loss of 18.5%. Even if chitosan is reported to be a good encapsulating agent used for many applications, modified chitosan, should be used because will guaranty better results as it can be seen in this work.

Zero order, Higuchi, Korsmeyer-Peppas and Weibull kinetic models were applied to the experimental data to study the release mechanism of vitamins B2 and B3. The results are presented in Table 7.8. and Table 7.9.; for each model specific parameters and the correlation coefficients R^2 were determined.

Zero order model is representative for constant slow release and in this work R^2 ranged in the interval 0.965 – 0.998 for vitamin B2 and between 0.963 – 0.997 for vitamin B3.

The following kinetic models describe more complex mathematical models of drug release from matrix system.

The results of Higuchi in terms of R^2 are 0.957 – 0.994 for vitamin B2 and 0.954 – 0.996 for vitamin B3.

Korsmeyer-Peppas model is specific for drug release from polymeric systems by diffusion mechanism, and from the values of the parameter n the exact type or release can be evaluated. For vitamin B2, n parameter registered values higher than 0.85, so the release mechanism is described by super case II transport for the microparticles prepared with gum arabic, chitosan and modified chitosan. The other encapsulating agents (maltodextrin, pectin and sodium alginate) share anomalous and super case II transport because for release in SGF n has values lower than 0.85 and in dH2O higher than 0.85. Anomalous transport is known as a diffusion mechanism with swelling release case II transport. The R2 for Korsmeyer-Peppas model showed values in the interval 0.907 – 0.998.

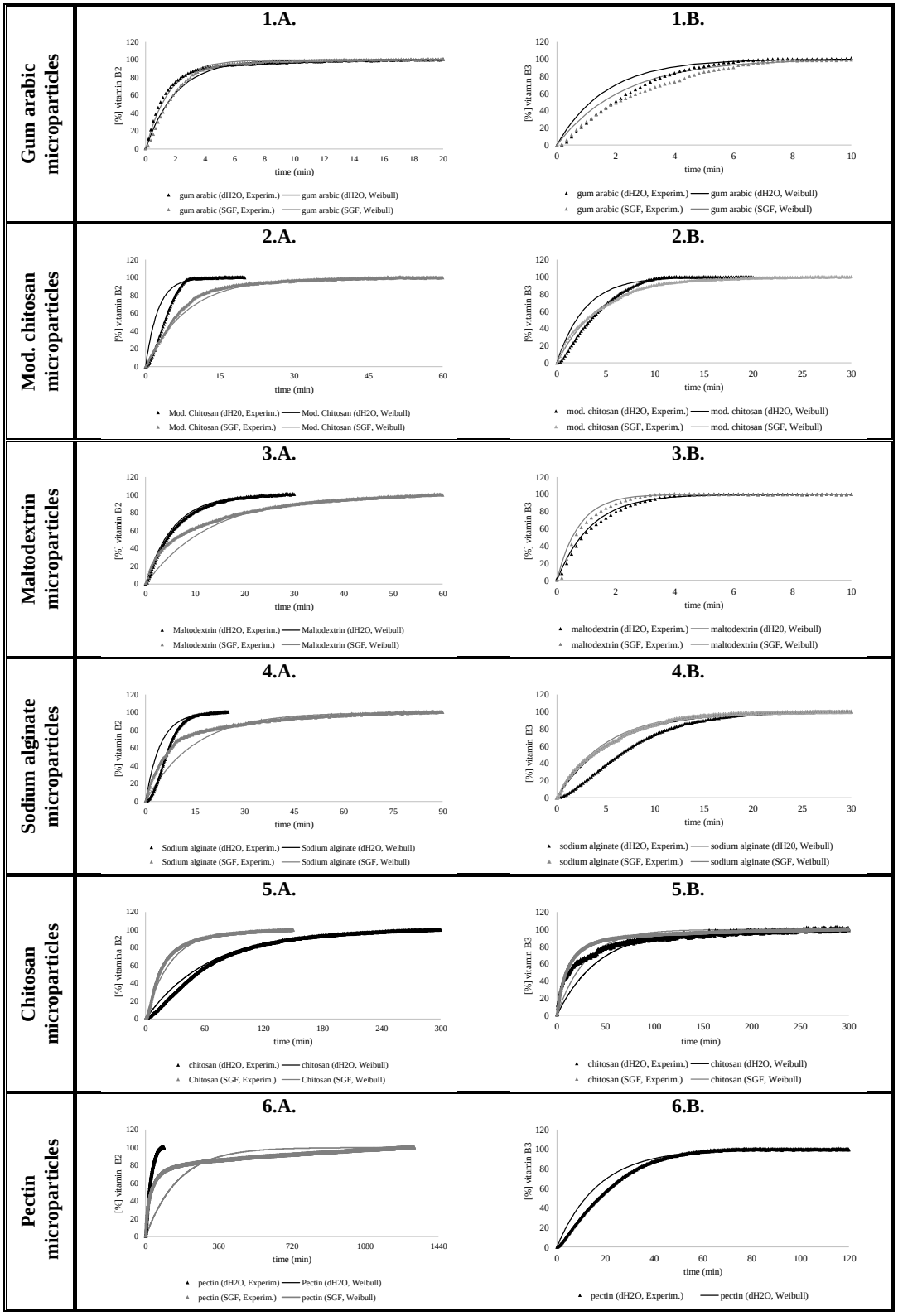

Figure 7. 4. Release profiles for vitamins B2 and B3 from different biopolymers microparticles.

Table 7. 6. Release time of vitamin B2 and vitamin B3 for the different formulations.

	Complete release reached after: (hh:mm)			
	dH_2O		SGF	
	Vitamin B2	**Vitamin B3**	**Vitamin B2**	**Vitamin B3**
Gum arabic	00:16	00:07	00:10	00:11
Mod. chitosan	00:11	00:11	00:48	00:23
Maltodextrin	00:23	00:05	00:55	00:03
Sodium alginate	00:19	00:24	01:13	00:26
Chitosan	04:35	04:35	01:20	00:11
Pectin	01:15	01:33	20:29	-

Besides one exception, all formulations with vitamin B3 have a release type super case-II transport. The exception are chitosan microparticles with vitamin B3 which are characterized by anomalous transport. R^2 for vitamin B3, in the case of Korsmeyer-Peppas model is in the interval 0.868 – 0.994. All four models showed a good fitting, but Weibull model was considered more appropriate for this work. The choice is supported by the fact that this model was created to describe the release from matrix microparticles, a type of microparticles specific for spray-drying method. For Weibull model R^2 ranged between 0.947 and 0.996 for vitamin B2 and between 0.937 and0.999 for vitamin B3.

Table 7. 7. Mass loss of vitamins B2 and B3 after 4 months of storage.

	Mass loss of vitamin (%) after 4 months of storage	
	Vitamin B2	**Vitamin B3**
1. Gum Arabic	1.08	7.27
2. Modified chitosan	6.09	8.33
3. Maltodextrin	2.95	3.62
4. Sodium alginate	0.39	2.83
5. Chitosan	18.50	5.27
6. Pectin	6.20	5.31

In a previous study of the authors[230], was studied the release of vitamin B12 from similar type of microparticles was studied. Regarding the time necessary for the release, lower values were observed, meaning it is faster to release vitamin B12; in terms of stability, after 4 months a

higher mass loss for the encapsulating agents: gum arabic, maltodextrin, pectin and sodium alginate, was registered. Also, it was opted for Weibull model as best model to fit the experimental data.

Table 7. 8. Kinetic models applied for the evaluation of release profiles: specific parameters and correlation coefficients for vitamin B2.

		Zero order (1)			Higuchi		Korsmeyer-Peppas (2)			Weibull (3)			
		Q_0	K_0 ($mg \cdot min^{-1}$)	R^2	Kh ($mg \cdot min^{-0.5}$)	R^2	Kk ($mg \cdot min^{-n}$)	n	R^2	β	$T_{d\ exp}$ (min)	T_d (min)	R^2
1. Gum arabic	**dW**	2.09	55.25	0.995	57.01	0.984	53.61	0.85	0.995	0.73	1.33	1.49	0.970
	SGF	1.19	36.18	0.996	53.72	0.977	34.27	1.20	0.989	1.28	1.77	1.99	0.997
2. Chitosan	**dW**	0.34	0.97	0.995	9.90	0.987	1.24	1.05	0.983	1.15	67.83	73.20	0.993
	SGF	3.42	4.16	0.993	18.34	0.979	2.66	1.21	0.982	1.01	19.00	25.20	0.965
3. Maltodextrin	**dW**	0.03	12.12	0.991	30.87	0.986	9.60	1.20	0.976	1.16	6.00	6.41	0.991
	SGF	0.43	17.14	0.978	22.52	0.987	13.48	0.78	0.907	0.78	10.00	10.16	0.981
4. Mod. chitosan	**dW**	5.74	14.32	0.996	43.94	0.960	2.61	1.55	0.998	1.79	4.67	4.43	0.995
	SGF	1.42	9.30	0.998	29.18	0.986	9.43	1.01	0.981	0.97	7.17	8.13	0.982
5. Pectin	**dW**	1.74	3.05	0.998	16.10	0.990	1.94	1.14	0.988	1.23	24.50	26.07	0.996
	SGF	4.42	2.88	0.965	10.31	0.991	5.68	0.72	0.965	0.42	48.67	66.18	0.947
6. Sodium alginate	**dW**	8.18	9.35	0.990	34.25	0.957	2.97	1.54	0.987	1.72	7.33	7.63	0.993
	SGF	4.34	9.03	0.981	24.85	0.994	12.84	0.80	0.987	0.73	8.50	10.47	0.982

Experimental data applied as following: (1) – the linear part of the curve, (2) – till Q_t/Q_∞ <0.6 and (3) – till complete release is reached.

Table 7. 9. Kinetic models applied for the evaluation of release profiles: specific parameters and correlation coefficients for vitamin B3.

		Zero order (1)			Higuchi		Korsmeyer-Peppas (2)			Weibull (3)			
		Q_0	K_0 ($mg \cdot min^{-1}$)	R^2	Kh ($mg \cdot min^{-0.5}$)	R^2	Kk ($mg \cdot min^{-n}$)	n	R^2	β	$T_{d\ exp}$ (min)	T_d (min)	R^2
1. Gum arabic	**dW**	0.58	25.03	0.991	50.05	0.977	20.03	1.67	0.934	1.57	-1.51	2.61	0.975
	SGF	1.43	23.34	0.983	42.60	0.988	21.69	1.30	0.936	1.30	-1.39	2.90	0.985
2. Chitosan	**dW**	18.88	2.21	0.887	9.38	0.954	8.77	0.71	0.868	0.62	-2.01	26.31	0.961
	SGF	5.58	5.04	0.976	19.15	0.992	8.28	0.82	0.988	0.58	-1.63	16.98	0.965
3. Maltodextrin	**dW**	1.14	56.17	0.995	59.66	0.977	59.82	1.07	0.993	1.12	-0.29	1.30	0.997
	SGF	1.29	74.56	0.963	70.12	0.954	175.00	2.19	0.933	1.42	-0.08	1.06	0.937
4. Mod. chitosan	**dW**	3.15	15.57	0.995	39.36	0.982	9.09	1.47	0.982	1.55	-2.30	4.40	0.994
	SGF	0.63	23.54	0.983	32.54	0.996	20.76	0.88	0.942	0.97	-1.40	4.24	0.994
5. Pectin	**dW**	0.32	2.95	0.996	17.21	0.990	1.84	1.19	0.991	1.31	-4.08	22.27	0.998
	SGF	-											
6. Sodium alginate	**dW**	3.63	8.19	0.997	29.91	0.977	3.82	1.42	0.994	1.51	-3.20	8.34	0.999
	SGF	2.16	15.53	0.977	31.24	0.994	13.82	1.10	0.881	1.08	-1.79	5.27	0.978

Experimental data applied as following: (1) – the linear part of the curve, (2) – till Q_t/Q_∞ <0.6 and (3) – till complete release is reached.

The purpose of these tests was to check how a certain encapsulating agent will influence the release of the core material. The release of vitamins B2 and B3 was studied from different types of microparticles because a specific application can be chosen according to the time required for complete release, the kinetic model that describes the release and the capacity to maintain the stability over time.

7.5. Conclusion

Microencapsulation is a suitable method to preserve vitamins for several reasons: enhanced stability of sensitive core materials, the possibility of releasing the core under controlled conditions and to the development of new products since the interaction with other ingredients is minimized.

Due to the big number of biochemical functions that could not be achieved without the activity of vitamin B2 and vitamin B3, it is important to manufacture more food products fortified with this vitamin.

The main goal of the current study was to produce vitamin B2 and vitamin B3 loaded microparticles with a spray-drying method. Overall results prove the influence of the encapsulating agent and of the core material on the final properties of the microparticles. Differences between formulations were observed in terms of product yield, encapsulation efficiency, size, morphology, release profiles, kinetic models and stability over time, but every type of formulation vitamin – biopolymer can be valorised for promising applications.
Further studies should be made to explore the possibility of incorporating these microparticles into real products.

Chapter 8

Comparison study

The comparison study of this research is in Chapter 8 where are analysed and compared the four experimental studies performed for this work. In this chapter, the similarities and differences between the different obtained microparticles and the effect of different experimental conditions are discussed

Vitamins B1, B2, B3 and B12 are the four vitamins from the B-complex that were studied and tested during this PhD program.

All of them have essential role for human health and are considered compounds with high interest for different applications in medical, pharmaceutical and food industries.

For this research, it was decided to valorise the microencapsulation process and to present a spray-drying approach with the aim to ensure stability for vitamin B1, B2, B3 and B12 by developing biopolymeric delivery systems.

The microencapsulation of each vitamin was performed with the same type of lab-scale equipment and the same configuration. A Mini Spray-Dryer BÜCHI B-290 with a standard 0.5 mm nozzle was used as equipment and the experimental conditions configuration selected was: solution flow rate – 4 mL/min (15%), air flow rate – 32 m3/h (80%), air pressure – 6 bar and inlet temperature – 120 °C.

The following biopolymers were used as encapsulating agents: gum arabic, chitosan, modified chitosan, carrageenan, maltodextrin, modified starch, sodium alginate, pectin and xanthan. These materials have been selected due to their properties: biocompatibility, bioavailability, biodegradability, no toxicity and excellent stability.

The performance of the microencapsulation process was determined through two specific parameters, namely the product yield and the encapsulation efficiency.

The work included four main experimental studies prepared independently of each other, using the same spray-dryer equipment, however the experimental setups were changed according the aim of each study.

In the first study, entitled "*Optimization of work conditions"*, the optimization of the experimental conditions was made. For this purpose, vitamin B12 was the model core material, and the encapsulating agent was the biopolymer modified chitosan. This formulation vitamin B12 - modified chitosan was used to analyse the effect of changing different experimental parameters.

After a set of optimized work conditions was decided, other three studies were developed, in which more encapsulating agents were used, in order to identify similarities and differences. For the second study "*Microencapsulation of vitamin B12",* seven biopolymeric materials were used as encapsulating agents; for the third study – "*Microencapsulation of vitamin B1",* were

prepared nine biopolymers and for the last study, the fourth one, "*Microencapsulation of vitamins B2 an B3"*, six biopolymers for each vitamin.

In the first study, found in Chapter 4, the microencapsulation of vitamin B12 with modified chitosan (1% w/V) was presented. Different formulations of microparticles were produced with the following amount of core material: 1, 2, 3, 4 and 5 % (w/w). The main aim of this work was to study if the amount of vitamin used as core material has any effect on the final microparticles and as well if modified chitosan has the capacity to protect vitamin B12.

Small differences were observed among all the formulations, as the product yield ranged in the interval 56 – 58%. Because of this, it was decided for the next studies to prepare formulations with only one concentration of the vitamin.

In the remaining studies, the product yield varied between 27 and 50% in the second study (Chapter 5) for the microparticles with 2% of vitamin B12, between 17 and 52% in the third study (Chapter 6) for the microparticles with 0.25% of vitamin B1, between 45 and 55% in the last study (Chapter 7) for the microparticles with 0.50% of vitamin B2, and between 45 and 58% for the microparticles with 1% of vitamin B3. Comparing the microparticles prepared with the same encapsulating agent but different vitamin, it was concluded that only the product yield of maltodextrin, pectin, and sodium alginate microparticles showed similar values.

The encapsulation efficiency was calculated for all the studies, except the first one. Very good results were obtained, near 100%, excepting the cases of maltodextrin and modified starch (around 60%) in the second study and maltodextrin (around 66%) in the third study.

The external morphology of the microparticles was determined by scanning electron microscopy (SEM) and their size was evaluated using laser granulometry method. The microparticles produced with the same encapsulating agent proved to look alike in all the studies. 3 categories of morphologies were identified: regular spherical shape with smooth surface (modified chitosan), spherical shape with rough surface or with shallows aspect (carrageenan, chitosan, gum arabic, maltodextrin, sodium alginate, pectin) and with irregular shape (modified starch and xanthan).

The size of each type of microparticles seemed to be different and the results of differential number distribution are vitamin B12 microparticles: 0.96 – 2.74 μm (second study), vitamin B1 microparticles: 0.11 – 1.32 μm (third study), vitamin B2 microparticles: 0.10 – 0.16 μm (fourth study) and vitamin B3 microparticles: 0.11 – 0.84 μm (fourth study).

The number of encapsulating agents used to microencapsulate vitamins B1 and B12 was reduced for the studies with vitamins B2 and B3, because the obtained results for carrageenan, modified starch and xanthan led to the conclusion that these encapsulating agents are not suitable for this work.

Release studies of the vitamins from the microparticles were performed to evaluate the entrapment of the vitamins after the microencapsulation process. During the release tests of the first, several conditions were studied and optimized: stirring mode (static solution and stirred solution), release medium (deionized water and simulated gastric fluid – SGF) and the temperature (room temperature – 22 °C and human body temperature – 37 °C). The experiments showed the best results for release of vitamin in SGF at 37 °C under continuous stirring.

Different behaviours were observed comparing the release of the vitamins in deionized water or in SGF. As well it was proved that the release of the vitamin is a function of the encapsulating agent. And for all 4 studies it was concluded that there are vitamins which tend to be released from the microparticles very fast, only some minutes are necessary, or very slow, requiring several hours.

The stability overtime of the microparticles was evaluated with samples that were kept for 4 months protected from external factors like light, humidity and temperature. For all studies a mass loss of less than 20% was obtained, proving good stability of the microparticles produced by spray-drying method.

The fitting degree of the following kinetic models: zero order (studies no. 3 - 4), first order (study no.3), Higuchi (studies no. 3 - 4), Korsmeyer-Peppas (studies no. 3 - 4) and Weibull (studies no. 1, 3 - 4), was evaluated. All the models showed a good value for R^2, the correlation coefficient. The only exception is first order, a model who proved weak fitting to the experimental data. Best results were observed for Weibull model and this fact can be explained by the applicability of this model: the release from matrix microparticles, very common for spray-drying method.

Differences between formulations in terms of product yield, encapsulation efficiency, size, morphology, release profiles, kinetic models and stability over time, were observed, but overall results are considered very good and useful for future research. This work should be continued with studies for the incorporation of the microparticles into real products.

Chapter 9

Conclusions

The aim of Chapter 9 is to present a critical synthesis of the main findings achieved from the entire research. The main conclusions of this work are correlated to the most significant results.

Vitamins are essential micronutrients for human health and nutrition; however, they are characterized as sensitive compounds to environmental parameters, which makes them susceptible to degradation processes during storage and cooking.

As well, vitamins are among the ingredients with a high demand for incorporation into food and pharmaceutical products, due to their dual function of prevention and treatment. Therefore, the consumer intensive request also contributed to finding a method of working with vitamins, which should be easy to implement, not involve very high costs and lead to high nutritional products.

Using the microencapsulation technique to ensure a proper delivery system for vitamins, it is possible to overcome some of these limitations and to improve their final industrial applications.

For this doctoral program were selected four water-soluble micronutrients from the B complex to be used as core materials in microencapsulation studies trough spray-drying method. The selected vitamins were B1, B2, B3 and B12 and behind the motivation of working with these compounds stands their essential role for overall health. As delivery systems were chosen different biopolymers since they are GRAS compounds characterized by biocompatibility, biodegradability, non-toxicity, and high availability.

Spray-drying method has been already used for a wide variety of compounds as it offers enhanced stability, especially for sensitive materials, the interaction with other ingredients is minimized, leads to the possibility of releasing the core under controlled conditions and altogether the production process is simple, reproducible and implies a relatively low cost. Consequently, spray-drying is considered to be the most appropriate microencapsulation method to develop new products with vitamin content.

The main goal of the current dissertation was to produce different types of microparticles loaded with vitamins B1, B2, B3 and B12 using the spray-drying technology. The big influence of the encapsulating agent and of the core material on the final properties of the microparticles, was highlighted through the overall results.

Differences between formulations were observed in terms of product yield, encapsulation efficiency, size, morphology, release profiles, kinetic models, and stability over time, although every type of formulation vitamin – biopolymer can be valorised for promising applications.

The first study, in which was microencapsulated vitamin B12 with modified chitosan, was used a model for the further research, since the focus of the work was to find the best experimental conditions. The results obtained showed which parameters affect the microencapsulation process and the release of the vitamin from the microparticles. It was concluded that all formulations reached almost the same product yield value of 56 -58%, they had the same spherical aspect, however, their size increased with the concentration of the vitamin. Regarding release studies, was observed that the best results were for SGF medium under continuous stirring at 37 ºC and the release profiles fitted Weibull model. Also, the stability tests proved good results because were registered very low vitamin losses for three and six months storage.

For the second study was used vitamin B12 but the number of encapsulating agents was increased to seven biopolymers. Depending on what encapsulating agent was used the product yield reached values from 27 till 50%, and the encapsulation efficiency was near 100% for all agents with only one exception. All the microparticles presented a spherical shape, but their surface was either smooth or rough, and their size differed too. The release profiles showed that vitamin B12 can be used for different applications as the release time ranged from few seconds to some hours. As well the stability experiments performed after four months concluded that losses are very small.

In the next studies, were used the remaining vitamins, namely vitamin B1, vitamin B2 and vitamin B3. The release tests were performed in the same way as in the second study and the stability evaluated after four months. For all vitamins were observed different shapes and sizes, however in most of the cases for the same encapsulating agent the microparticles with different vitamins had a similar aspect.

Vitamin B1 was evaluated in the third study and for this work was used the highest number of biopolymers, namely nine encapsulating agents. In terms of product yield the process registered values from 17 to 52%, and for the encapsulation efficiency eight of the formulations had high values of 94 – 96% and only one of 66%. The release profiles showed different delivery behaviours: fast release made in few minutes and slow release that required up to 24 hours. Different kinetic models were tested, although the one that fitted the best was Weibull. After four months of storage the microparticles showed good stability.

For the last study, was opted to perform experiments with two vitamins, B2 and B3, and six encapsulating agents for each. The product yield for vitamins B2 and B3 was considered high

as it reached values from 47 to 54%, and spray-drying method is known to register moderate values. However, the encapsulation efficiency was very high as for both vitamins the values were from 98 up to 100%. The release tests showed that every encapsulating agent presents a different time of releasing, from few minutes until some hours. Like in the previous studies, the Weibull model fitted the best the results and after four months the microparticles were considered to have good stability.

Until recently, the topic of encapsulation of B vitamins by a spray-drying attracted the interest of few scientists, therefore new results are valuable sources for this engineering field. And to obtain microparticles with quality, it is necessary to develop and experiment as many trials as possible.

For the future, more research is expected for the optimization of spray drying process with B vitamins. As well, it is important to explore the possibility of using more than one B vitamin as core material and to study combinations of biopolymeric wall materials, which can also help increase the final product application and implicitly the bioavailability of B vitamins.

Chapter 10

Final remarks

The last chapter is divided into two parts: limitations and future perspectives. The first part briefly presented the aspects and actions that limited the work and good development of the research. In the second part, some recommendations for future work are made.

10.1. Limitations

This research's findings provide important insights about the microencapsulation of vitamins B1, B2, B3 and B12 for potential incorporation of the microparticles into food, nutraceutical, or pharmaceutical products. However, the experimental part of the work is subject to a few limitations that must be highlighted to understand the obtained results better.

The identified limitations are related to time, material, and equipment issues.

The spectrophotometer had some small problems related to the lifetime of the lamps and some fibers or software malfunctioning. Consequently, some experiments had to be repeated.

Another task that was not easy to accomplish was working with light-sensitive compounds, namely vitamins B2 and B12. To avoid the degradation of these vitamins, the manipulation of the material had to be done fast and using a significantly reduced intensity of direct light.

All samples prepared for release tests had very small weight (3 – 5 mg); therefore, caution must be applied to succeed an exact weighing.

Summing up the work results, this study has great potential to be continued or to serve as a model for similar research.

10.2. Future perspectives

It is expected that the industry sectors producing foods, nutraceuticals, and pharmaceuticals continue growing in the next years from the financial point of view and as a research field. And microencapsulation is an important technology used for this purpose, confirmed by its market value evaluated at USD 7.88 billion in 2018 globally.

Due to constant high demand, research and development departments are always seeking to discover new products and the challenge now is to develop products with improved properties, increased availability and affordable prices. Moreover, the products that contain microparticles fit very well in this category.

Given the overall results of current work, spray-drying seems to be a promising method to produce stable microparticles with B vitamins and further studies should be made to explore the possibility of incorporating these microparticles into real products.

The microencapsulation of the rest of the vitamins belonging to B-Complex should be performed. It can be speculated the possibility of using a combination of vitamins and encapsulating agents.

Multivitamin microparticles would increase the nutritional value of the product in which are incorporated and would be helpful for the cure of more medical conditions. Among the most important advantages of multivitamin products are increased energy levels, improved mood, reduced stress and anxiety, boost immunity and overall reduced risk of diseases.

Mixing different biopolymers to obtain the composition of the encapsulating agent will offer benefits like increased stability, different release time, and maybe a lower price.

The same methodology presented for this work should be respected for new types of microparticles. New experiments can be considered using new encapsulating agents and also new dissolution mediums such as simulate intestinal fluid (SIF).

The addition of these microparticles to the composition of food, nutraceutical and pharmaceutical products should be made in order to understand if the whole process is suitable for future industrial production.

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