

Microencapsulation of Thyme Oil
by Coacervation:
Production, Characterization and Release Evaluation



Microencapsulation of Thyme Oil by Coacervation: Production, Characterization and Release Evaluation

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Isabel Maria Duque Martins

Supervision: Professor Alírio Egídio Rodrigues

Co-supervision: Professor Maria Filomena Filipe Barreiro



Laboratory of Separation and Reaction Engineering

Associate Laboratory -LSRE/LCM

Department of Chemical Engineering

Faculty of Engineering

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Isabel Maria Duque Martins, 2009-2012

Laboratory of Separation and Reaction Engineering (LSRE)

Department of Chemical Engineering – University of Porto

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Abstract

In this work polylactide (PLA) microcapsules have been produced by coacervation having in view the encapsulation of *Thymus vulgaris* L. (thyme oil), an antioxidant and antimicrobial active agent. Biodegradable microcapsules of PLA have received extensive attention as drug delivery systems since they can be hydrolysed in the body, and its degradation products easily resorbed or eliminated. The core material, thyme oil, is extracted from an aromatic and medicinal plant of increasing economic importance in North America, Europe and North Africa.

The novelty of the developed process consists on dissolving PLA in dimethylformamide (DMF) which is a good solvent for PLA but in addition has high solubility in water. Upon contact with water the PLA dissolved in the DMF solution precipitate covering the oily droplets. As so, an easy and executable method of coacervation was put in practice allowing the encapsulation of an oily active principle by simply preparing an o/w emulsion.

Several nonionic surfactants with different hydrophilic-lipophilic balance (HLB) values were evaluated focusing the encapsulation efficiency of polar and apolar compounds of oil. Thus, Tween[®] 20, Tween[®] 80, Tergitol[™] 15-S-9 and a combination of Tergitol[™] 15-S-9 with Span[®] 85 have been used covering the range between 11 and 16.5. For all the studied cases, microcapsules have shown a spherical shape and the obtained particle size distribution in volume was bimodal, with a mean size comprised between 30 and 40 μm . The amount of encapsulated thyme oil reaches a maximum of 65% when using Tergitol[™] 15-S-9, a polyglycol ether surfactant with a HLB value of 13.3. The study confirmed the encapsulation efficiency dependence on the surfactant HLB, putting also in evidence a preferential encapsulation of apolar compounds of thyme oil in detriment of polar ones.

The release behaviour of the thyme oil itself and of its individual components, through the PLA microcapsules wall, was evaluated by using the microcapsules

solution during the first day period after production and using GC-FID to discriminate and quantify individual components. The developed diffusion model was applied to single-layer microcapsule systems resulting that the release of the polar compounds of thyme oil was faster than the apolar ones. The diffusion coefficient in first hour of release was $1.39 \times 10^{-15} \text{ m}^2/\text{s}$ for thymol and $5.21 \times 10^{-17} \text{ m}^2/\text{s}$ for *p*-cymene. However, the diffusion was slower if considering a 5 days period thus obtaining diffusion coefficients of $3.81 \times 10^{-17} \text{ m}^2/\text{s}$ for thymol and $1.43 \times 10^{-18} \text{ m}^2/\text{s}$ for *p*-cymene.

Complementary studies considering the production and characterization of vanillin, thymol and *p*-cymene, used as model core materials, have been also performed. The obtained microcapsules presented similar morphology as the thyme oil ones, i.e., spherical shape, but with a somewhat smaller mean particle size (21 μm for vanillin, 25 μm for thymol and 37 μm for *p*-cymene). The vanillin release has been monitored along with time, but no amount was detected in the outside solution of microcapsules pointed out that the vanillin stayed entrapped in the produced microcapsules. However, the results show that the release of thymol and *p*-cymene is faster in the first hour keeping almost constant in next days. The diffusion coefficient in first hour of release was $1.99 \times 10^{-16} \text{ m}^2/\text{s}$ for thymol and $4.34 \times 10^{-16} \text{ m}^2/\text{s}$ for *p*-cymene. However, the diffusion is slower for a period of 5 days with the diffusion coefficients of $3.34 \times 10^{-19} \text{ m}^2/\text{s}$ for thymol and $3.45 \times 10^{-18} \text{ m}^2/\text{s}$ for *p*-cymene. The release rate for thymol was slower when used as model core material, since it was observed that only 40% of the encapsulated oil was released during the first day.

Resumo

A execução do presente trabalho experimental tem como objetivo a encapsulação do óleo essencial de *Thymus vulgaris* L. (óleo de tomilho), um agente antioxidante e antimicrobiano. As microcápsulas foram produzidos por coacervação usando poli(ácido láctico) (PLA) como material de revestimento. As microcápsulas biodegradáveis de PLA apresentam um elevado interesse como sistemas de libertação de medicamentos, uma vez que este tipo de polímero pode ser hidrolisado no organismo, sendo os seus produtos de degradação facilmente reabsorvidos ou eliminados. Por outro lado, o material encapsulado, óleo de tomilho, é extraído a partir de uma planta aromática e medicinal de crescente importância econômica na América do Norte, Europa e África do Norte.

A novidade do processo desenvolvido consiste em dissolver o PLA em dimetilformamida (DMF). A dimetilformamida é um bom solvente para o PLA e ainda tem uma elevada solubilidade em água. Em contato com a água o PLA dissolvido na solução de DMF precipita revestindo as gotículas de óleo. Apresenta-se assim, um método de coacervação simples e de fácil execução que permite a encapsulação de um princípio ativo oleoso partindo apenas de uma emulsão óleo/água.

Diversos agentes tensioativos não iónicos com diferentes valores de balanço hidrofílico-lipofílico (HLB) foram estudados avaliando os valores de eficiência de encapsulação dos compostos polares e apolares do óleo de tomilho. Foram estudados os seguintes agentes tensioativos: Tween[®] 20, Tween[®] 80, Tergitol[™] 15-S-9 e uma combinação de Tergitol[™] 15-S-9 com Span[®] 85, cobrindo uma gama de valores de HLB entre 11 e 16,5. Para todos os casos estudados as microcápsulas obtidas apresentaram forma esférica e uma distribuição de tamanho em volume bimodal, com um tamanho médio compreendido entre 30 e 40 µm. A quantidade de óleo de tomilho encapsulado apresentou um valor máximo de 65% quando se utilizou Tergitol[™] 15-S-9, um éter poliglicólico com um valor de HLB de 13,3. Este

estudo confirmou a dependência dos valores de eficiência de encapsulação do agente tensoativo usado, colocando também em evidência uma encapsulação preferencial dos compostos apolares de óleo de tomilho em detrimento dos polares.

A difusão do óleo de tomilho e dos seus componentes individuais, através da parede das microcápsulas de PLA foi avaliada usando como meio reacional a solução de microcápsulas. O estudo foi feito durante o primeiro dia após a produção das microcápsulas e utilizou-se a análise cromatográfica GC-FID para discriminar e quantificar todos os componentes. O modelo de difusão desenvolvido foi aplicado a sistemas de microcápsulas simples (microcápsulas de parede única), obtendo-se uma difusão mais rápida dos compostos polares do óleo de tomilho quando comparada com os compostos apolares. Os coeficientes de difusão na primeira hora de libertação foram de $1.39 \times 10^{-15} \text{ m}^2 / \text{s}$ para o timol e $5.21 \times 10^{-17} \text{ m}^2 / \text{s}$ para o *p*-cimeno. No entanto, a difusão foi mais lenta se se considerar um período de 5 dias obtendo-se assim os coeficientes de difusão de $3.81 \times 10^{-17} \text{ m}^2 / \text{s}$ para o timol e $1.43 \times 10^{-18} \text{ m}^2 / \text{s}$ para o *p*-cimeno.

Estudos complementares considerando a produção e caracterização de microcápsulas usando vanilina, timol e *p*-cimeno como compostos modelo, foram também realizados. As microcápsulas obtidas apresentaram morfologia semelhante à já obtida usando o óleo de tomilho como material do núcleo, ou seja, forma esférica mas com um tamanho médio de partícula um pouco menor (21 μm para vanilina, 25 μm para timol e 37 μm para *p*-cimeno). A difusão da vanilina foi monitorizada ao longo do tempo, contudo não foi detectada na solução exterior às microcápsulas, salientando assim que provavelmente toda a vanilina ficou retida no interior das microcápsulas produzidas. No entanto, os resultados mostram que a libertação de timol e *p*-cimeno foi mais rápida na primeira hora mantendo-se praticamente constante nos dias seguintes. Os coeficientes de difusão na primeira hora de libertação foram de $1.99 \times 10^{-16} \text{ m}^2/\text{s}$ para o timol e $4.34 \times 10^{-16} \text{ m}^2/\text{s}$ para o *p*-cimeno. No entanto, a difusão é mais lenta

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CHAPTER 1:

Introduction

“Learning is a treasure that will follow its owner everywhere. “

[Chinese Proverb]

1.1 Relevance and motivation

Nowadays, scientific advance is being used in the development of innovative products. The industry of food, cosmetics, personal care and beauty has become a multi-billion dollar international market (Costa *et al.*, 2006; Wesselingh *et al.*, 2007; Zev, 2005). In fact, the value growth in the beauty and personal care industry has been significant in emerging markets, such as Brazil, China, India, Indonesia and Argentina, see Figure 1.1 (Euromonitor, 2011). To have success in such competitive and demanding sector, the products must differentiate which can be achieved by means of using emergent technologies, such as microencapsulation. Many primary products do not achieve a market until they are added to more commonly used products to create materials with high added value. If good use is made of primary products its value can increase and contribute, for example, to enhance innovation in cosmetics (Michael, 2009). In this context it is imperative to expand microencapsulation technologies to other fields contributing for the creation of other innovative products of high added value in response to human needs.

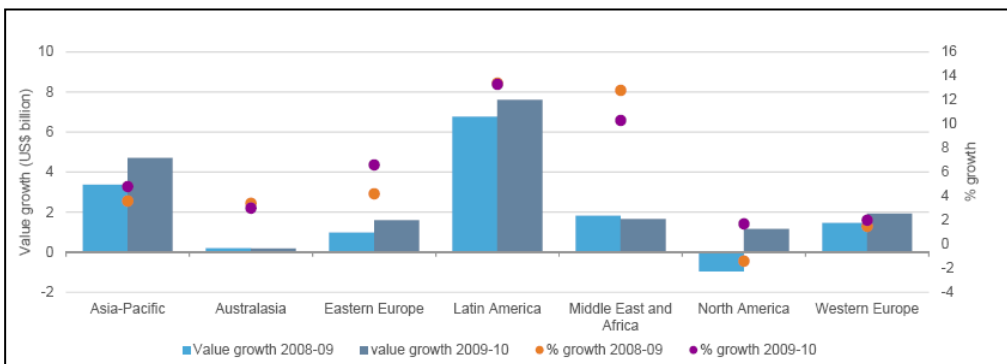


Figure 1.1. Comparison by region of value sales and growth rates in the beauty and personal care industry; time period: 2008-2010. Source: Euromonitor International (Euromonitor, 2011)

Microencapsulation provides an important tool for cosmetic and/or pharmaceutical industry, enabling the choice of various delivery mechanisms based on several core materials (Fairhurst *et al.*, 2008). The introduction of microencapsulated products in cosmetics can provide a system that delivers an active agent to a specific place, such as the skin or hair, when it is needed. The microcapsules containing the active agent are added to a cream or gel, and burst open when it is rubbed in, delivering its contents straight to the target place. In skin care there are many applications of delivery systems; microcapsules are used in sunscreens, anti-wrinkle products, skin whitening/bleaching, antioxidant delivery, flavor and fragrance delivery, sensory markers, such as warming, cooling or tingling and coloring. In hair care, some applications include nutrient delivery, antistatic agents, relaxing chemicals for ethnic hair, coloring/dyeing, conditioning agents, humectants and deodorants (Elder *et al.*, 2005; Li *et al.*, 2005). The advantage of microencapsulation use in these fields is that it enables consumers to benefit from a substance that might otherwise cause degradation of the base cream or gel. Many active agents presented in cosmetics are unstable compounds such as, for example, some essential oils. The encapsulation of these oils in a core-shell material or matrix has been investigated for various reasons, such as, protection from oxidative decomposition, evaporation or merely to support them. A practical example is the encapsulation of essential oils to mask unattractive colours and unpleasant smells when added to a cream, which makes the product unsuitable for use. Protect the oil in capsules, enables its addition to the cream without any problem. Using a microencapsulated system not only ensures that the active agent can be blended successfully into the cream, but also increases its shelf life. As the personal care business continues to represent one of the biggest growing areas in chemical industry, the development of efficient formulations promotes the advance of emergent technologies, such as microencapsulation.

At present, the number of microencapsulation techniques amounts to several hundred and that number is expected to grow as new materials for microencapsulation emerge and new active principles requiring a specific microencapsulation process are identified. Taking into account the limitations of some microencapsulation processes and the physicochemical characteristics of the active agent to be encapsulated, several techniques were developed. Most of the methods currently used at industrial level could be grouped into the categories presented in the Table 1.1.

Table1.1. Microencapsulation processes, core materials nature and microcapsules size range (Lamprecht *et al.*, 2000).

<i>Microencapsulation technique</i>	<i>Core material</i>	<i>Particle size (μm)</i>
Coacervation (phase separation)	Solid/Liquid	2-1200
Interfacial polymerization	Solid/Liquid	2-2000
Spray drying	Solid/Liquid	6-600
Solvent evaporation	Solid/Liquid	5-500
Centrifugal extrusion	Solid/Liquid	1-5000
Air Suspension	Solid	35-5000
Extrusion	Solid/Liquid	1 - 5000
Fluid bed coating	Solid/Liquid	20 - 1500
In situ polymerization	Solid/Liquid	2-2000
Spinning disc	Liquid	5 – 1500

The choice of the appropriate technique depends on the core material properties, the involved manufacturing constraints and the product end-use requirements.

Among the listed techniques coacervation is widely used to encapsulate essential oils. Typical examples of essential oils encapsulation are given in Table 1.2.

Table 1.2. Survey of essential oils encapsulated by coacervation and their major applications. Adapted from (Magdassi *et al.*, 1996).

Core Material	Method	Application	Reference
Mint, orange or eucalyptus oils	Complex coacervation	Cosmetics and food	(Arneodo <i>et al.</i> , 1986; Blake <i>et al.</i> , 2003; Jun-Xia <i>et al.</i> , 2011)
Orange oil	Heat denaturation	Food and pharmaceuticals	(Janda <i>et al.</i> , 1995)
Rosemary oil	Simple coacervation	Food and pharmaceuticals	(Fredj <i>et al.</i> , 1984; Ribeiro <i>et al.</i> , 1997)
Rose perfume oil	Complex coacervation	Cosmetics	(Golz-Berner <i>et al.</i> , 2003; Maekawa <i>et al.</i> , 1975)
Lemon oil	Complex coacervation	Cosmetics	(Arneodo <i>et al.</i> , 1988; Weinbreck <i>et al.</i> , 2004)
Citronella oil	Simple coacervation	Insect repellents	(Scher Herbert, 1977; Solomon <i>et al.</i> , 2011)
Peppermint oil	Complex coacervation	Pharmaceuticals	(Dong <i>et al.</i> , 2011; Ribeiro <i>et al.</i> , 1997)
Cinnamon oil	Simple coacervation	Food	(Soper, 1997; Xing <i>et al.</i> , 2011)

Recent published patents in the area of microencapsulation suggests that both industrial and academic sectors are running to explore and develop new applications in this area, including a broad range of cosmetics or personal care products (Figure 1.2). The cosmetic or personal care business is worth pursuing in view of the wide-ranging potential they hold.

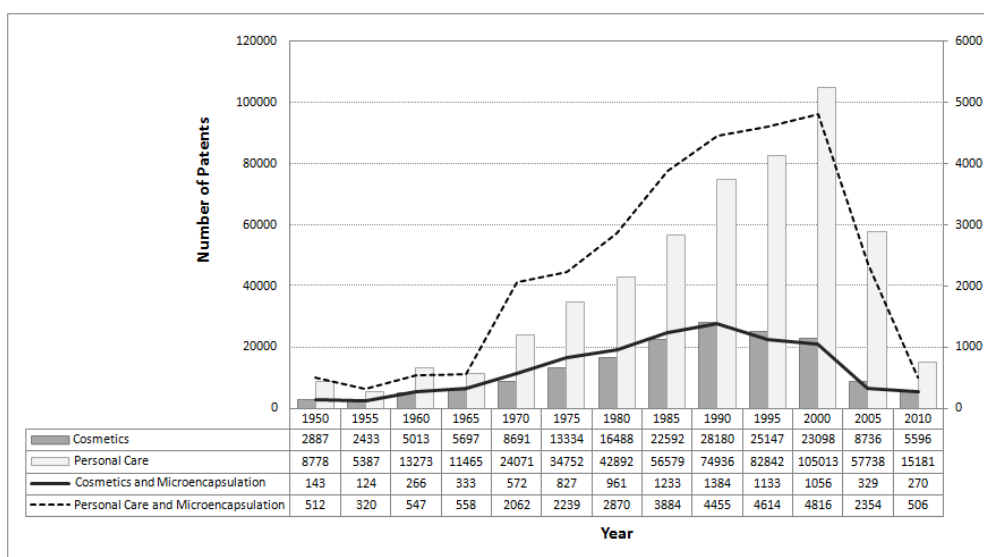


Figure 1.2. Number of patents published in the period from 1950 to 2010 (obtained on free patents online database, November 2011; Keywords: cosmetics, personal care and microencapsulation)

1.2 Objectives and outline

The goal of this thesis is to develop a methodology to obtain microcapsules containing active principles, such as essential oils. More specifically, the objective of this work is to develop a coacervation process to produce polylactide (PLA) microcapsules containing thyme essential oil, having in view cosmetic applications. Generally, PLA is used to encapsulate water soluble active principles such as drugs, pesticides and dye-stuffs by coacervation, or oily active principals by means of microspheres production or by using double emulsion techniques (o/w/o). However, the objective of this work is to encapsulate thyme essential oil, a water insoluble active principle, in a shell-like capsule by using an oil-in-water (o/w) emulsion.

Chemical and structural characterization will be performed in order to understand microcapsule's properties and behavior. Size control, wall thickness and encapsulation efficiency will be accessed by using several techniques, namely laser dispersion to obtain size distributions in number and volume; optical microscopy (OPM), scanning electron microscopy (SEM) and cryogenic scanning electron microscopy (CryoSEM) to study morphology and evaluate the wall thickness; gas chromatography with flame ionization detection (GC/FID) to quantify the encapsulation efficiency and gas chromatography with mass detection (GC/MS) to determine the composition. Release of the encapsulated essential oil will be studied by developing a theoretical model for its diffusion across the PLA capsule and by performing experimental assays.

This thesis is divided in eight chapters according to figure 1.3. The first chapter, "Introduction", presents the relevance and motivation and describes the main objectives and outline of the work. In second chapter the state of the art concerning microencapsulation of essential oils using PLA, a biodegradable polymer, and its application in cosmetics field is presented. The third chapter, called "Materials and Experimental Methods" describes the materials and experimental techniques used for the synthesis, characterization and evaluation of microcapsules performance. In chapter four, "Microencapsulation of thyme oil by coacervation", PLA microcapsules production method development is presented in detail. Chapter five entitled "PLA-based thyme oil microcapsules production: evaluation of surfactants" presents a study of different surfactants and their effect on microencapsulation efficiency, namely in what respects polar and apolar compounds of thyme oil. In chapter six, "Release of thyme oil from polylactide microcapsules", the experimentally obtained results for thyme oil release are compared with the calculated ones based on a general diffusion model used to predict mass transport phenomena across PLA microcapsules. The seventh chapter called, "Release studies of vanillin, thymol and cymene from polylactide

microcapsules” presents the release results using vanillin, thymol and cymene (thyme oil components) as model core materials. Finally, in chapter eight called “Conclusions and Future work” the main contributions of the thesis are highlighted and suggestions for future work listed.

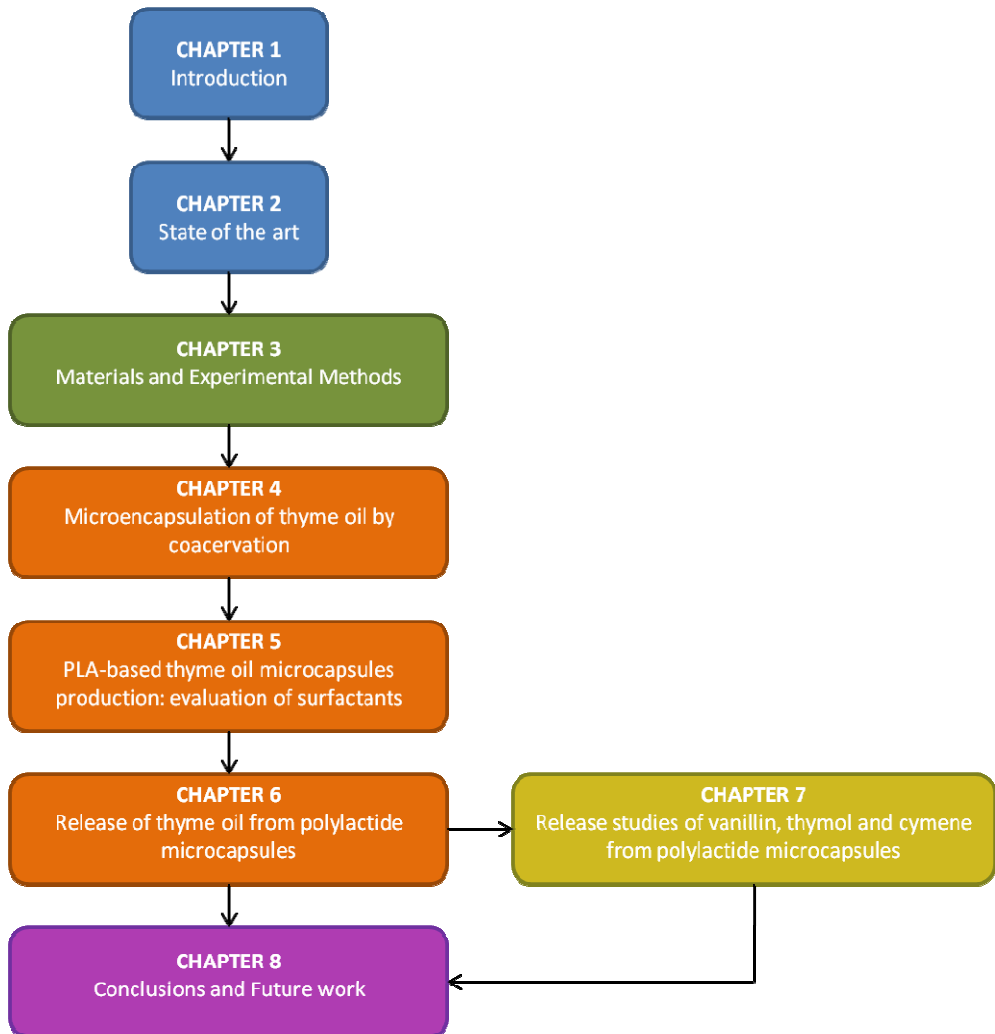


Figure 1.3. Thesis organization.

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CHAPTER 2:

State of The Art

“Microencapsulation is both an art and a science.”

[Lipo Technologies Inc.]

In this chapter the state of the art concerning microencapsulation of essential oils is presented. First the definition of microcapsules is given and a description of the main microencapsulation techniques is presented. A survey of polymeric materials commonly used as wall materials, with a special focus on biodegradable polymers, is also given. This chapter also describes the various types of core materials used in microencapsulation, making a more detailed reference on the essential oil of thyme which is the active agent studied in this work. Release studies of essential oils are also addressed.

2.1 Microcapsules: definition

Microcapsules are small particles with a size between 1 and 1000 μm comprising an active agent surrounded by a natural or synthetic polymeric membrane. Microcapsules are composed by two parts, namely the core and the shell, as represented schematically in Figure 2.1. The core (the internal part) contains the active agent (e.g., an essential oil), while the shell (the external part) protects the core from the outer environment (Ghosh, 2006). Encapsulation can be achieved by a wide range of methods or techniques, providing isolation, entrapment, protection or controlled release of sensitive or reactive materials (e.g. flavours and fragrances) from/across the surrounding matter.

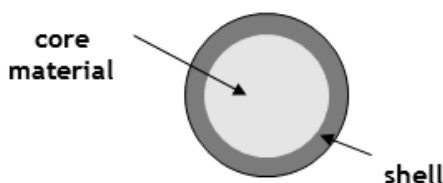


Figure 2.1. Idealized continuous core/shell microcapsule.

Microencapsulation is used to protect fragrances or other active agents from oxidation caused by heat, light, moisture, from contact with other substances over a long shelf life, to prevent evaporation of volatile compounds and to control the release rate (Ghosh, 2006 ; Lumsdon *et al.*, 2005). The encapsulated agent can be released by many actions, for example, mechanical, temperature, diffusion, pH, biodegradation and dissolution.

Encapsulation systems can be classified according to four main morphologies: reservoir, double wall, matrix and polynucleated structures (Figure 2.2). Each system could be achieved through different encapsulation processes. The main

purpose is to entrap an active agent into a protective structure in order to be adapted to a specific finished product. The choice of carriers or filming agents used to obtain the protective matrix/wall will confer unique properties in terms of controlled release, solubility or moisture resistance.

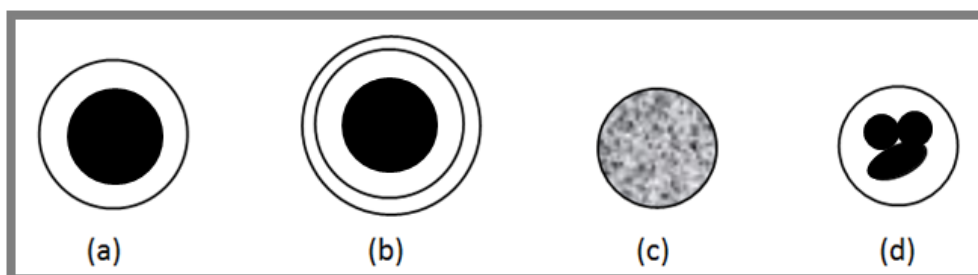


Figure 2.2. Different morphologies of microcapsules: (a) reservoir type, (b) double wall, (c) matrix, (d) Polynucleated (Silva *et al.*, 2003).

The compatibility of the core material with the membrane shell is an important criterion for enhancing the efficiency of microencapsulation. On the other hand the size of core material also plays an imperative role for diffusion, permeability or controlled release applications (Ghosh, 2006).

2.2 Microencapsulation techniques

A large number of methods have been purposed for microcapsule's production, in order to be adapted to different types of core and wall materials, as well as, to generate particles with various sizes, wall thickness and permeability, thus adjusting the release rate of the active principle.

The selection of the technique and wall material depends on the final application of the product, considering physical and chemical stability, concentration, required particle size, release mechanism and manufacturing costs. Generally the

techniques used for microencapsulation can be divided into two major categories, namely chemical and physical methods; the latter can be subdivided into physico-chemical and physico-mechanical techniques (Ghosh, 2006). Table 2.1 presents a brief description of some used techniques.

Table 2.1. Chemical, Physico-chemical and Physico-mechanical methods used for microencapsulation (adapted from Jyothi *et al.* (2009))

Microencapsulation Techniques		References
Chemical	Interfacial Polymerisation	(Hirech <i>et al.</i> , 2003; Mizuno <i>et al.</i> , 2005; Torres <i>et al.</i> , 1990)
	“In Situ” polymerisation	(Bang <i>et al.</i> , 2005; Marshall <i>et al.</i> , 1963)
Physico-Chemical	Coacervation	(Jegat <i>et al.</i> , 2000; Madan, 1978; Nori <i>et al.</i> , 2011; Soper <i>et al.</i> , 2000)
	Sol-Gel Encapsulation	(Ahn <i>et al.</i> , 2008; Nguyen-Ngoc <i>et al.</i> , 2007; Ochiai <i>et al.</i> , 1987)
	Supercritical CO ₂ assisted microencapsulation	(Chen <i>et al.</i> , 2009; Fages <i>et al.</i> , 2004)
Physico-Mechanical	Atomization	(Ascheri <i>et al.</i> , 2003; Bauckhage <i>et al.</i> , 1996)
	Spray Drying	(Bodmeier <i>et al.</i> , 1988; Yin <i>et al.</i> , 2009)
	Fluid-Bed Coating	(Anwar <i>et al.</i> , 2010; Sun <i>et al.</i> , 1997)
	Solvent Evaporation	(Hung <i>et al.</i> , 2010; Tice <i>et al.</i> , 1985)

The mentioned techniques are widely used for microencapsulation of several pharmaceuticals. Among these techniques, fluidized bed or air suspension method, coacervation and phase separation, spray drying and spray congealing, pan coating, solvent evaporation methods are usually used. Depending on the physical nature of the core substance to be encapsulated the chosen technique could differ.

Spray drying is the most frequently used technique to encapsulate flavours (Figure 2.3). It is a physico-mechanical method developed in the 1930s and is a very attractive and versatile process (Desai *et al.*, 2005). Spray drying is a simple process, similar to a one stage drying operation (Figure 2.4), capable of producing a wide range of microcapsules at good yield. It is also used to produce commercial capsules loaded with fragrance or flavour oils (Thies, 2000). The process is adaptable to a wide range feedstock and product specifications, almost any feedstock that can be used: solutions, suspensions, slurries, melts and pastes (Andrews, 2011).

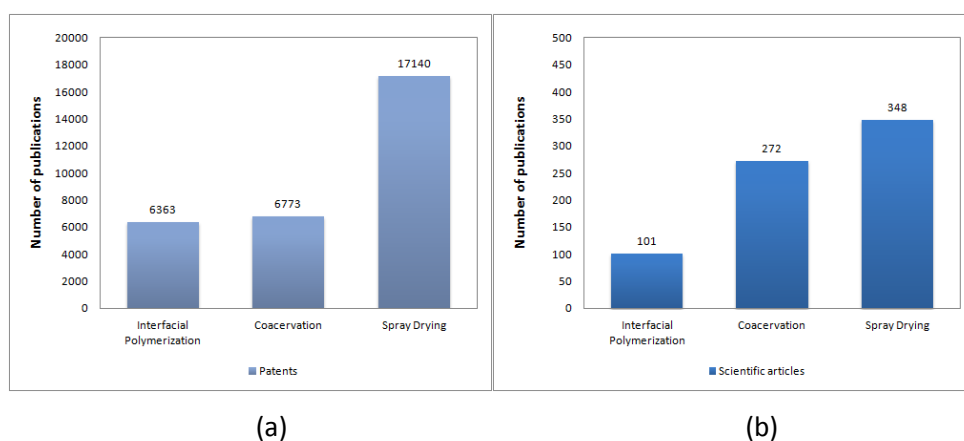


Figure 2.3. Microencapsulation methods by publication type ((obtained on: (a) free patents online database and (b) Web of Science, February 2011; Keywords: microencapsulation and interfacial polymerization or coacervation or spray drying).

Using spray drying, active principles with different solubility properties can be encapsulated with various wall materials and the partitioning of active principle between two immiscible phases is avoided (Finch *et al.*, 2000).

Nevertheless spray drying has some disadvantages: the equipment is very bulky and expensive (Andrews, 2011). Moreover, it produces a fine microcapsules powder which needs further processing such as agglomeration, and the overall thermal efficiency is low (uses large volumes of heated air passing through the chamber without contacting a particle, thus not contributing directly for the drying). On the other hand, the use of spray drying technique in microencapsulation is limited by the number of wall materials available that have good solubility in water (Gharsallaoui *et al.*, 2007; Moretti *et al.*, 2002).

Taking into account the limitations of some processes and the physicochemical characteristics of essential oils, coacervation is a more suitable technique to encapsulate this type of active agents.

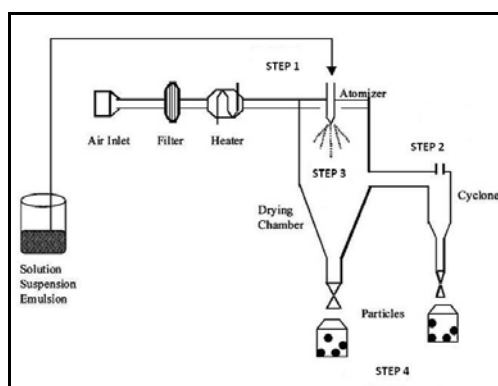


Figure 2.4. The main process steps involved in spray drying: STEP 1 – atomization; STEP 2 – spray-air contact; Step 3 – droplet drying and Step 4 – product separation. Adapted from Ré (2006).

In general, several aspects may affect the encapsulation efficiency of microcapsules (content of core material effectively encapsulated). Fig.2.5 illustrates the factors that can influence encapsulation efficiency using coacervation technique.

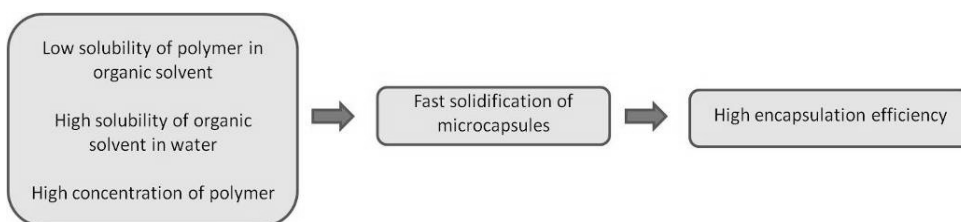


Figure 2.5. Factors influencing encapsulation efficiency. Adapted from Jyothi *et al.* (2009).

The retention of active agent in the membrane wall is ruled by factors related to the chemical nature of the core, including its molecular weight, chemical functionality, polarity and volatility, the wall material properties and the microencapsulation technique chosen. Table 2.2, shows the maximum active agent encapsulation yield for different coating techniques, as well as, the particle size range achieved.

Table 2.2. Characteristics of encapsulation process. Adapted from Madene *et al.* (Madene *et al.*, 2006)

<i>Microencapsulation Techniques</i>	<i>Particle size (μm)</i>	<i>Max. Load (%)</i>	<i>References</i>
Interfacial Polymerisation	2-2000	35-70	(Judefeind <i>et al.</i> , 2009)
Simple Coacervation	20-200	<60	(Richard <i>et al.</i> , 2000)
Complex Coacervation	5-200	70-90	(Richard <i>et al.</i> , 2000)
Spray Drying	1-50	<40	(Richard <i>et al.</i> , 2000)

2.2.1 Coacervation

The term coacervation was introduced in 1930 by Bungenberg de John and Kruyt (Jong *et al.*, 1930) describing a process in which aqueous colloidal solutions were separated into two liquid phases, one rich in colloid (coacervate) and the other poor in colloid. In their studies, Bungenberg de Jong described the conditions under which complex coacervation of gelatin/gum arabic occurred, such as pH, ionic strength, polymer concentration, polymer ratio, and temperature (Bungenberg De Jong, 1949). Coacervation is thus defined as the formation of macromolecular aggregates as a result of phase separation - partial desolvation of a homogeneous colloidal polymeric solution. According to the International Union of Pure and Applied Chemistry (IUPAC), coacervation is defined as the separation of colloidal systems into two liquid phases. Figure 2.6 shows the optical microscopy images of orange microcapsules obtained using coacervation technique.



Figure 2.6. Optical microscopy images of microcapsules of orange flavour with whey protein/gum arabic coacervates, before drying (Weinbreck, 1977).

The coacervation techniques are divided in two main groups: aqueous and organic. The coacervation in aqueous phase can only be used to encapsulate water insoluble materials (hydrophobic core materials presented in solid or liquid state). On the other hand, the coacervation in organic phase allows the encapsulation of hydrosoluble material, but requires the use of organic solvents (Kas, 2000).

Coacervation in aqueous phase can be classified into simple and complex, according to the involved phase separation mechanism (see Figure 2.7). In simple coacervation, the polymer is salted out by the action of electrolytes, such as sodium sulphate, or desolvated by the addition of a water miscible non-solvent, such as ethanol, or by increasing/decreasing temperature. These conditions promote the macromolecule-macromolecule interactions in detriment of the macromolecule-solvent interactions. On the other hand, complex coacervation is essentially driven by the attractive forces of oppositely charged polymers (Gander *et al.*, 2006). The coexistence of a coacervate phase made of concentrated polyelectrolytes and a diluted equilibrium phase depends on pH, ionic strength and polyion concentrations (Ducel *et al.*, 2004).

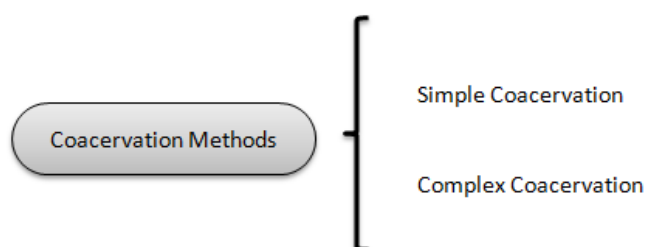


Figure 2.7. Coacervation methods used for microencapsulation.

Based on the experimental results of Bungenberg de Jong and Kruyt (Jong *et al.*, 1930), Overbeek and Voorn developed the first quantitative theory on complex coacervation (Overbeek *et al.*, 1957). In their study the complex coacervation of whey protein/gum arabic was a spontaneous phenomenon.

There are a significant number of patents related to microencapsulation by coacervation involving several kinds of core materials and applications. Table 2.3 presents a summary of the patents related to coacervation technique.

Table 2.3. Patent processes for microencapsulation by coacervation.

<i>Patent Assignee</i>	<i>Summary of Invention</i>	<i>Reference</i>
The National Cash Register Company	Encapsulation process by complex coacervation. The present invention includes gelatine as the organic hydrophilic polymeric material.	(Georg, 1972)
Bend Research, Inc.	A complex coacervation process to obtain microcapsules with mosquito repellent.	(Baker <i>et al.</i> , 1989)
The Procter & Gamble Company	Encapsulation of a cosmetic cleansing composition with dual blooming perfume system.	(Tanner, 1995)
The Johns Hopkins University School of Medicine	Controlled release of pharmaceutically active substances from coacervate microcapsules.	(Leong <i>et al.</i> , 1998)
The Procter & Gamble Company	Process for obtain a better conditioning shampoo composition. The compositions provide improved hair conditioning performance, including improved wet hair feel.	(Baravetto <i>et al.</i> , 1999)
Unilever Home & Personal Care, USA, division of Conopco	Methods for producing a fabric care composition which comprises an amine or amide-epichlorohydrin resin or derivative thereof. The invention presents a method of treatment of fabric.	(Carswell <i>et al.</i> , 2001)
Givaudan Roure Flavors Corporation	Enzymatically protein-encapsulating oil particles by complex coacervation.	(Soper <i>et al.</i> , 2001)
The Procter & Gamble Company	Process for obtain a packaged product having a liquid reservoir containing a cleaning product, and a means for their delivering.	(Lawson <i>et al.</i> , 2003)
Mainelab	Method for encapsulating active substances by coacervation of polymers in non-chlorinated organic solvent.	(Benoit <i>et al.</i> , 2004)

<i>Patent Assignee</i>	<i>Summary of Invention</i>	<i>Reference</i>
Philip Morris Products S.A.	Method for preparing microcapsules by coacervation.	(Dardelle <i>et al.</i> , 2009)
E.I. du Pont de Nemours and Company	Methods for encapsulation a water insoluble oils by coacervation.	(Friedmann <i>et al.</i> , 2009)
L'Oréal	Methods for preparing core/skin microcapsules by coacervation.	(Simmonet <i>et al.</i> , 2009)
Philip Morris Products S.A.	Solid flavor encapsulation by applying complex coacervation and gelation technology.	(Sengupta <i>et al.</i> , 2011)

Complex coacervation, also called phase separation, was developed in the 1950s by National Cash Register Company, USA. It is based on the ability of cationic and anionic water-soluble polymers to interact in water to form a liquid polymer-rich phase called complex coacervate. The charges must be sufficiently large to induce interaction, but not large enough to cause precipitation. Complex coacervation is the separation of an aqueous polymeric solution into two miscible liquid phases: a dense coacervate phase and a dilute equilibrium phase. The dense coacervate phase wraps as a uniform layer around suspended core materials. Complex coacervation is affected by pH, ionic strength, temperature, molecular weight, and concentration.

The general outline of the coacervation process consists in three steps that occur under continuous stirring (see Figure 2.8). The first step consists in the formation of an oil-in-water (o/w) emulsion (dispersion of the oil in a aqueous solution containing a surface-active hydrocolloid), the second comprises the formation of the coating (deposition the polymer coating upon the core material), and the last one is the stabilization of the coating (coating hardening, using thermal, crosslinking or desolvation techniques, to form self sustaining microcapsules) (Soest, 2007).

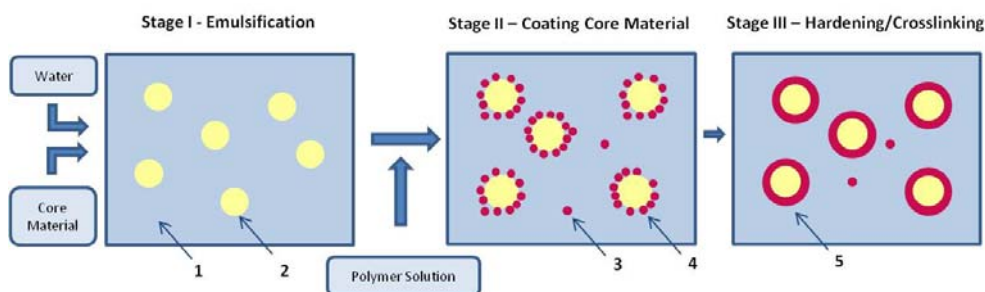


Figure 2.8. General process scheme for microcapsule preparation by coacervation. 1) water; 2) core material 3) polymer; 4) deposition the polymer coating upon core material; 5) microcapsules.

The coacervate wall formation is driven by the surface tension difference between the coacervate phase, the water and the hydrophobic material.

Coacervation offers many possibilities for the encapsulation of various types of active agents (solid or liquid core materials) (Benoit *et al.*, 2001; Ferres *et al.*, 1999; Lumsdon *et al.*, 2005; Magdassi *et al.*, 1996; Schobel, 1986; Soper, 1996; Whitaker *et al.*, 1991). Coacervation techniques can be useful in many industrial sectors such as, food, cosmetic or pharmaceutical. Figure 2.9 shows the number of publications for each application market considering as key words coacervation plus the intended application.

Food, cosmetic/fragrances/flavours and pharmaceuticals are the areas with the highest number of publications using coacervation as the technique to encapsulate active agents and, as previously mentioned in Table 2.2, microencapsulation of fragrances by coacervation is an efficient way of adding oil-based fragrances to products.

Active principles can be embedded in cosmetic products such as gels, creams, lotions, emulsions, bath gels, shampoos; in case of pharmaceutical products they can be applied topically, orally or parentally; and in food products can be consumed by human or animals, for example, in diet products (Duena *et al.*, 2006

).Usually, in industrial coacervation processes, one of the hydrocolloids used is gelatine; this protein is easier to use and less prone to aggregation after the wall formation. The process of gelification is achieved by lowering the temperature of the reaction mixture below the gelling point of the gellable hydrocolloid (Zupancic *et al.*, 1996).

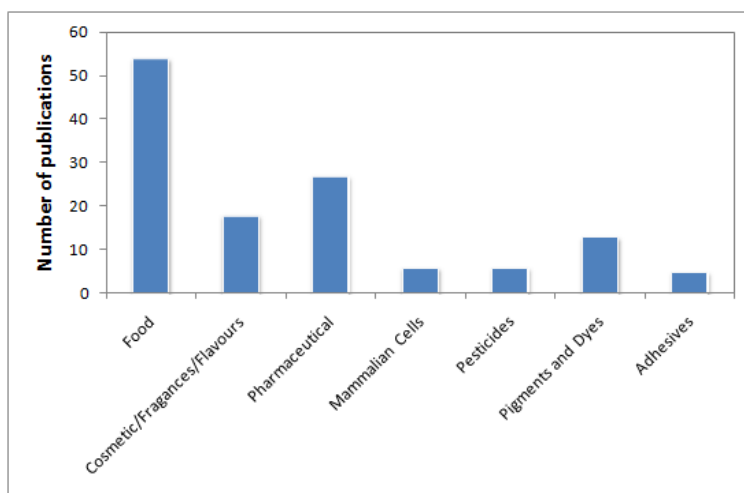


Figure 2.9. Number of publications for all years (obtained on data base 2011 web of science, February 2011; keywords: coacervation and application).

2.3 Microencapsulation application: cosmetics

New products development is an essential goal for modern society and encapsulation contribution has grown over the years (Costa *et al.*, 2006; Rodrigues *et al.*, 2009; Rodriguez Romero *et al.*, 2007; Sanchez-Silva *et al.*, 2010). The encapsulation of food ingredients, flavours and fragrances has been performed and commercialized based on different encapsulation methods (Dodge, 1988). There are numerous industrial applications, such as carbonless paper, “scratch and sniff” fragrance sampling, “intelligent” textiles, controlled release of drugs, pesticides and cosmetic active agents, i.e., there are numerous possibilities to use

microencapsulation as a technique to obtain products with high added value. Figure 2.10 shows the wide application markets of microencapsulation.

In recent years the demand for fragranced products is growing and it is expected a future expansion and an increasing diversity. Fragrances and flavours are an essential additive in consumer products such as household detergents, laundry products or cosmetics.

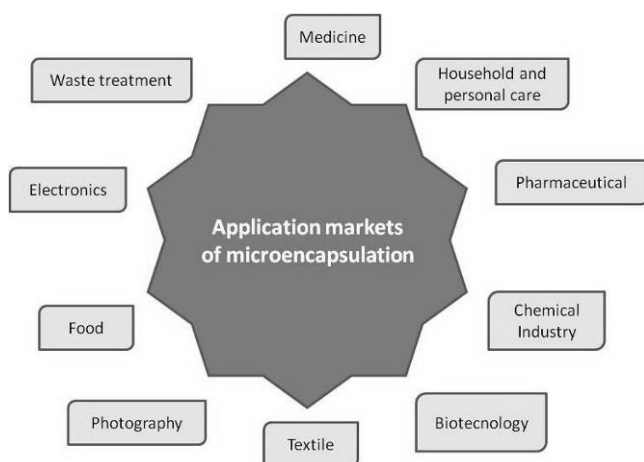


Figure 2.10. Schematic representation of the application markets for microencapsulation (Gate2tech, 2008).

Microcapsules of fragrances or perfumes can be added to the products to reach several objectives: to mask unpleasant odours of the products, to reduce losses during repeated opening of the packages; to stabilise and protect the fragrance during storage and not least, to provide the controlled release of odour (Soest, 2007).

Figure 2.11 illustrates the statistical distribution of microencapsulation over different fields of application. It is possible to observe that the sector which has the highest level of application is the chemistry industry (45%), followed by drugs (18%) and food (16%) sectors. On the contrary, the hygiene sector accounts only

with 1% (the smallest percentage), although it is important to retain that this share percentage can be higher if all the products used in health care are considered together in one category. Nevertheless, the cosmetic industry presents a significant percentage (8%) of this distribution.

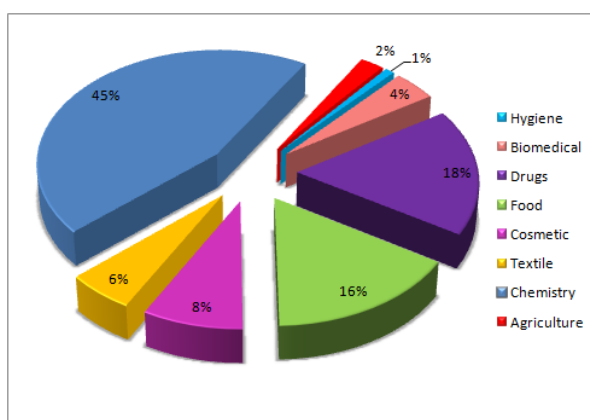


Figure 2.11. Schematic representation of the statistical distribution of microencapsulation over different fields of application (obtained on ISI web of knowledge, February 2011; timespan=all years and keywords: microcapsules and *application*) (Isi, 2011).

In recent years, encapsulation of ingredients for cosmetic and personal care products has become very popular, attractive and associated production processes technologically developed. This is a result of the added value of the generated products, but also because secure defined functions of the compounds can be preserved (Society, 2006). For example, skin care businesses can benefit from this invention since it allows obtaining products with much longer shelf life because fragrances/flavours are protected inside the capsules added to a cream.

In conclusion, cosmetic technology is growing constantly in terms of raw materials, excipients and formulations of active agents (Benita *et al.*, 1996). It is thus desirable to keep in mind that consumers are more demanding and that

microencapsulation remains a challenging art being important to increase the operative window in terms of processes and encapsulation materials (core and shell materials).

2.4 Microcapsules wall material: biodegradable polymers

Microencapsulation is the process of enclosing the active agent inside of a miniature reservoir (capsule). The capsule wall can consist of various materials, such as, wax, synthetic or natural polymers like proteins and polysaccharides. Microcapsules can have a variety of shapes: spherical, oblong or irregular; they can be monolithic or composed by aggregates and can present single or multiple walls. The wall protects the entrapping materials referred as active agent, core material, fill or internal phase. The coating is called wall, membrane, shell or capsule (Soest, 2007).

The most commonly used wall materials are polysaccharides and sugars (gums, starches, celluloses, cyclodextrines); proteins (gelatine, casein, soy proteins); lipids (waxes, paraffin, oils); inorganics (silicates, clays) and synthetic polymers (acrylic polymers, poly(vinylpyrrolidone)). Table 2.4 shows the wall materials usually used in coacervation systems.

Until 1950, polysaccharides and sugars were the principal coating material used in pharmaceutical preparations. The introduction of cellulose derived synthetic polymers such as methylcellulose and cellulose acetate phthalate (CAP), as well as, polymethacrylic synthetic esters have introduced a main advance in this area. However, a range of other materials have been the basis for microcapsules preparation. The choice between them depends on various factors such as the desired final product properties or the manufacturing process to apply (Silva *et al.*, 1998). The polymers used in microencapsulation can be classified in different forms; one possible classification is shown in Table 2.5.

Table 2.4. Wall materials used in simple and complex coacervation systems (Boh *et al.*, 2010)

Simple Coacervation	Wall Material (hydrocolloid)	Coacervation induction
	Gelatin Soy glycinin Casein Chitosan Polyvinyl alcohol	• Addition of water – miscible organic solvent (ethanol, methanol) • Addition of salt (aq. Solution of sodium-sulphate) • Heating • pH change
Complex Coacervation	Wall Material: 1st polymer (amphoteric/polycation)	Wall Material: 2nd polymer (polyanion)
	Gelatin Albumin Casein Soy glycinin Collagen Methacrylic acid polymers	Gum Arabic Carboxymethyl cellulose Carageenan Alginate Dimethylaminoethyl methacrylate

Despite several systems proposed, biodegradable polymers have emerged as potential candidates for the development of carriers for targeting compounds to specific sites in the body. These polymers are usually biocompatible, non-antigenic and highly hydrophilic in nature, thus hydrophilic compounds can easily be incorporated into of them (Nimesh *et al.*, 2006).

During the last years, numerous processes for drug encapsulation have been developed that currently use aliphatic polyesters, such us poly(lactic acid) (PLA) and copolymers of lactic and glycolic acids (e.g. PLGA) that are well known biodegradable polymers. The biodegradability of these polymers can be

manipulated by incorporating a variety of chemical groups such as ethers, anhydrides, carbonate, amides, ureas and urethanes in their main chain (Chandy *et al.*, 2002; Del Valle *et al.*, 2009; Pálínkó-Biró *et al.*, 2001; Wischke *et al.*, 2008).

Table 2.5. Representative list of polymers used in drug delivery systems (Pillai O. *et al.*, 2001).

<i>Classification</i>	<i>Polymer</i>
<u>Natural Polymers</u>	
Protein-based polymers	Collagen, albumin, gelatine
Polysaccharides	Agarose, alginate, dextran, chitosan, cyclodextrins
<u>Synthetic Polymers</u>	
<i>Biodegradable</i>	
Polyesters	Poly(lactic acid) , poly(glycolic acid), Poly(hydroxyl butyrate)
Polyamides	Poly(imino carbonates), polyamino acids
Others	Poly(cyano acrylates), polyurethanes, polyacetals
<i>Non-biodegradable</i>	
Cellulose derivatives	Carboxymethyl cellulose, ethyl cellulose, cellulose acetate
Silicones	Polydimethylsiloxane, colloidal silica
Others	Poloxamers, poloxamines

Biodegradable polymers, such as PLA and PLGA (poly(lactide-co-glycolide)), have proven, since a long time, their capacity for applications in the field of controlled delivery systems (Heya *et al.*, 1994; Lancranjan *et al.*, 1995). The degradation behavior of biodegradable polymers is a very important property in the medical field especially in tissue engineering, and drug delivery. Their properties (such as degradation rate) are strongly defined by structural characteristics like the composition of the co-polymer, molecular weight and nature of the chain end groups. Polylactide-co-glycolide copolymers can be copolymerized to get various

molecular architectures that originate a range of mechanical properties and degradation rates.

PLA is an aliphatic polyester obtained from the lactide by ring-opening polymerization usually using a stannous octoate as catalyst and heat (see Figure 2.12). Due to the methyl group presence, PLA is more hydrophobic than PLGA. Thus, PLA-based products degrade by hydrolysis much slower than PLGA-based counterparts.

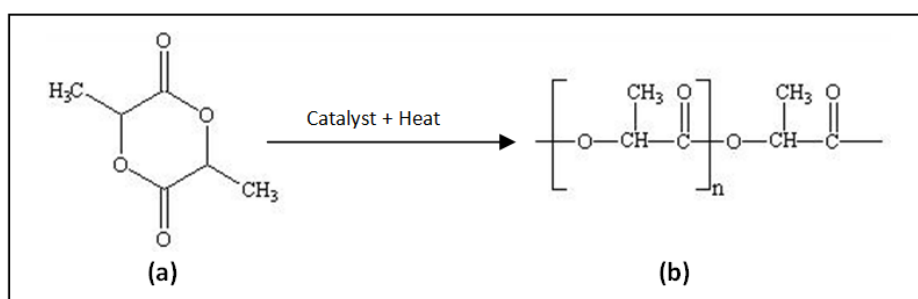


Figure 2.12. Chemical synthesis of polylactide. (a) Lactide, (b) Polylactide.

PLA microcapsules have received intensive attention as delivery systems for drug encapsulation since they don't cause adverse tissue reaction (Huang *et al.*, 1997). This type of biodegradable polymeric carriers can be hydrolyzed in the body to form products that are easily reabsorbed or eliminated (Hong *et al.*, 2000; Huang *et al.*, 1997). The adjustable physicochemical properties of PLA, such as swelling and biodegradation kinetics, or molecular interaction with potential embedded drugs, offer various possibilities in the design of controlled release systems (Blanco-Príeto *et al.*, 2004; Gander *et al.*, 1995; Tracy *et al.*, 1999; Wang, 2000). These properties of biodegradable polymers are strongly defined by structural features such as co-polymer composition, molecular weight and nature of the chain end-groups. For example, the non-esterified carboxyl end groups increase the hydrophilicity of polymer and promotes a faster and higher polymer swelling; consequently, a faster biodegradation in aqueous environment (Blanco-Príeto *et*

al., 2004). On the other hand, polylactide films are also an attractive and relevant material of increasing interest for developing food packing applications. PLA is used as anti-microbial agent for food packaging due to its retention properties towards various molecules such as bacteriocins or other proteins (Mascheroni *et al.*, 2010).

2.5 Microcapsules core: essential oil of *Thymus vulgaris* L.

The use of oils in the perfumery, cosmetics, agriculture or food industries is quite common due to its aromatic properties. In addition, some essential oils have biological activities that can be used in the preparation of pharmaceutical products and functional foods (Silva *et al.*, 2004). Properties of essential oils can change depending on its origin and composition. Some oils have medicinal properties such as antioxidant activity, acting in fighting free radicals, anti-inflammatory activity and antimicrobial activity. Table 2.6. lists a set of essential oils that were subjected to microencapsulation.

Thyme (*Thymus vulgaris*) is the common name given to the herbs of the *Thymus* species, native of the western Mediterranean region and extending to the southeast of Italy. This type of plant has a large number of species (300 to 400), most of which are aromatic shrubs or perennials. The common or garden thyme, *Thymus vulgaris*, is considered the leading brand and is used commercially as flowering and ornamental plant (Figure 2.13). This aromatic plant is having an increasing economic importance in North America, Europe and North Africa (Naghdi Badi *et al.*, 2004), and its oil, widely used in the flavour and food industries.

Thyme oil is extracted by steam distillation from the fresh or dried leaves and tall flowering plant. Ideally, thyme must be gathered when in flower and should be thoroughly dried. The oil content of the dried plant can vary from 2 to 5% in weight.

Table 2.6. Representative list of encapsulated essential oils.

<i>Essential oil</i>	<i>References</i>
Lemon	(Park <i>et al.</i> , 2001)
Thyme	(Šipailiene, 2006)
Citronella	(Hsieh <i>et al.</i> , 2006)
Vanilina	(Gumi <i>et al.</i> , 2009)
Menthol	(Mortenson <i>et al.</i> , 2008)
Eucaliptol	(Costa, 2011)
Clove	(Kim <i>et al.</i> , 2011)
Peppermint	(Adamiec, 2009)

It has a strong flavour and a pungent, spicy, penetrating, pleasant odour that are preserved by careful drying. The essential oil is located mainly in the small glands of the leaves and contains mostly thymol, linalool and the p-cymene.

The essential oils of thyme are grouped into three main types: thyme oil, which contains 42 to 60% phenols and is mainly thymol; organum oil, which contains 63 to 74% phenols and is mainly carvacrol, and thyme oil lime, which contains citral (Pérez G *et al.*, 2011). The thyme oil can be divided into two types: red thyme oil (is a crude distillate from the partially dried herb of the wild growing *Thymus Vulgaris*) and white thyme oil (is derived by re-distilling the red oil). The value of thyme oil depends a lot on its content in phenols.

Thyme oil has several components in its composition (see Figure 2.14), but its antimicrobial activity is mainly attributed to the presence of carvacrol, cinnamaldehyde, thymol, geraniol and eugenol, among others (Šipailiene *et al.*, 2006). As a pharmaceutical compound, thymol and carvacrol are used in mouthwashes, soaps and creams. Thyme oil itself, is used in the manufacture of perfumes and cosmetics. In fact, this essential oil is also used as fragrance for

soaps and detergents, where the fresh scent and antiseptic characteristics are greatly desired.



Figure 2.13. Photograph of *Thymus vulgaris* L. plant.

The essential oil of *Thymus vulgaris* L. is considered a powerful source of natural derivatives, very useful against stored product pests and has several insecticidal activities such as: fumigant and topical toxicity and repellent effects.

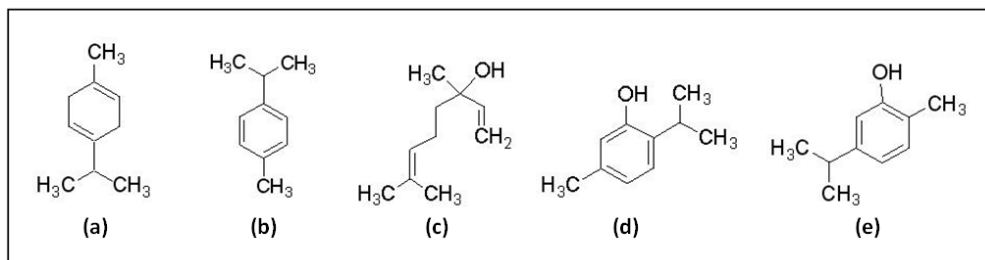


Figure 2.14. Representative scheme of various components of thyme oil. (a) γ - Terpinene, (b) *p*-Cymene, (c) Linalool, (d) Thymol, (e) Carvacrol.

Thymol, one of the constituents of thyme oil, is usually used as antimicrobial agent in food packaging. Active packaging materials have the capacity to release antimicrobial compounds into foodstuffs and can be used in order to inhibit or slow down bacterial growth during storage (Mastromatteo et al., 2009). It can also be used to control the microbiological/oxidation decay of perishable food products, to increase the shelf life of products or to maintain food quality (Hu et

al., 2011). In recent years, extensive research has been made to develop packaging strategies able to retain the active agent in the polymeric membrane and control its release.

2.6 Controlled release of oils

The protection of essential oils, perfumes, deodorants, moisturizers and other active agents in polymer carriers with the purpose of controlled release over a certain period of time has been a question of considerable research in recent years (Calkin *et al.*, 1994; Costa *et al.*, 2006; Costa *et al.*, 2008; Gumi *et al.*, 2009; Peña *et al.*, 2009; Peppas *et al.*, 1996; Peppas *et al.*, 1997; Thies, 1996). Controlled release technologies are used to deliver compounds such as drugs, pesticides, fragrances or flavours at prescribed rates, together with improved efficacy, safety and convenience (Romero-Cano *et al.*, 2002). Figure 2.15 shows the schematic representation of thyme oil release through the polymeric microcapsule wall.

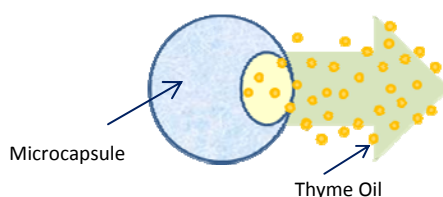


Figure 2.15. Schematic representation of thyme oil release through the polymeric microcapsule wall.

Nowadays, core-shell microcapsules are highly used in controlled release systems, especially in drug delivery, where the polymeric wall works as a permeable element with a selectivity that can determine the release behaviour of the core material (Guo *et al.*, 2005). Delivery systems for drugs and other active ingredients and size-reduction technologies, such as microencapsulation, are at the frontier of

advances in modern biotechnology. Focusing the developments in trans-dermal delivery systems microencapsulation introduces a new hope for replacing current high-risk intravenous applications and drastically reduce undesirable side effects of drugs and active ingredients (Yechiel *et al.*, 2004).

The particular properties of the polymeric network, such as, chain length, flexibility and mobility, water-uptake and swelling behaviour, plasticization extent, or potential interactions between polymer and active agent will affect the diffusion rate across the polymeric matrix, and therefore, the oil release (Wischke *et al.*, 2008).

According to Del Valle *et al.* (Del Valle *et al.*, 2009) diffusion of active agents occurs when a drug or oils passes through the polymer that forms the controlled release device. There are different classifications for primarily diffusion controlled active agent delivery systems: (a) reservoir system, where the active agent is retained in a central compartment surrounded by a polymeric membrane through which it must diffuse, thus controlling the rate of delivery, and (b) matrix systems, where no local separation between the active agent reservoir and a release rate controlling wall exists (Siepmann *et al.*, 2008). A schematic representation of these systems is shown in Figure 2.16. Nevertheless, the release of the active agent from delivery systems can be classified based on other mechanisms, such as, erosion (the product gradually dissolves in membrane wall), diffusion (the oil diffuses out of delivery system), extraction (mechanical forces during chewing or processing enlarge area of oil) and burst (a reservoir system ruptures under influence of mechanical or osmotic forces) (Ubbink *et al.*, 2001).

Several diffusion models have been proposed in the literature to describe the release of an active agent from microcapsules (Borgquist *et al.*, 2004; Cryer *et al.*, 2009; Gumi *et al.*, 2009; Kwok *et al.*, 1991; Lü *et al.*, 2000; Marucci *et al.*, 2008; Muschert *et al.*, 2009; Sanna Passino *et al.*, 2004; Tavera *et al.*, 2009).

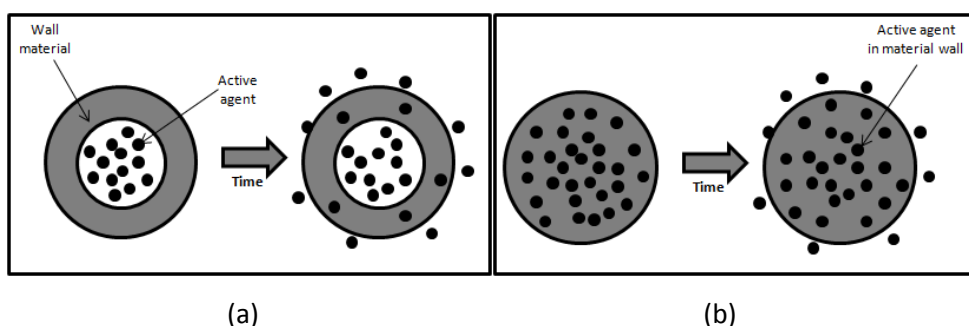


Figure 2.16. Mechanisms for active agent release: (a) reservoir system and (b) matrix system (Del Valle *et al.*, 2009).

A mathematical release model is based on equations that describe the real phenomena, such as mass transport by diffusion, dissolution of active agent, and for example, the transition of a polymer from the glassy to the rubbery state (Siepmann *et al.*, 2008). Figure 2.17 shows different types of classification for drug delivery systems. In reservoir system if the active agent concentration at the inner membrane surface continuously decreases with time and if the active agent permeability through the barrier remains constant, a first order release kinetics is obtained. However, if the initial active agent concentration exceeds the active agent solubility in reservoir device, results a constant active agent concentration (saturated solution) at the inner membrane surface, and still if the properties of the release rate controlling barrier (such as, thickness and permeability for the active agent) remain constant, obtains a zero order release kinetic.

On the other hand in the case of matrix devices, the system geometry extensively affects the resulting active agent release kinetics. In that case, for each system is necessary to develop a specific mathematical equation (Siepmann *et al.*, 2008). Table 2.7 presents a summary of the model release related to the diffusion of active agents through the polymeric membranes of microcapsules.

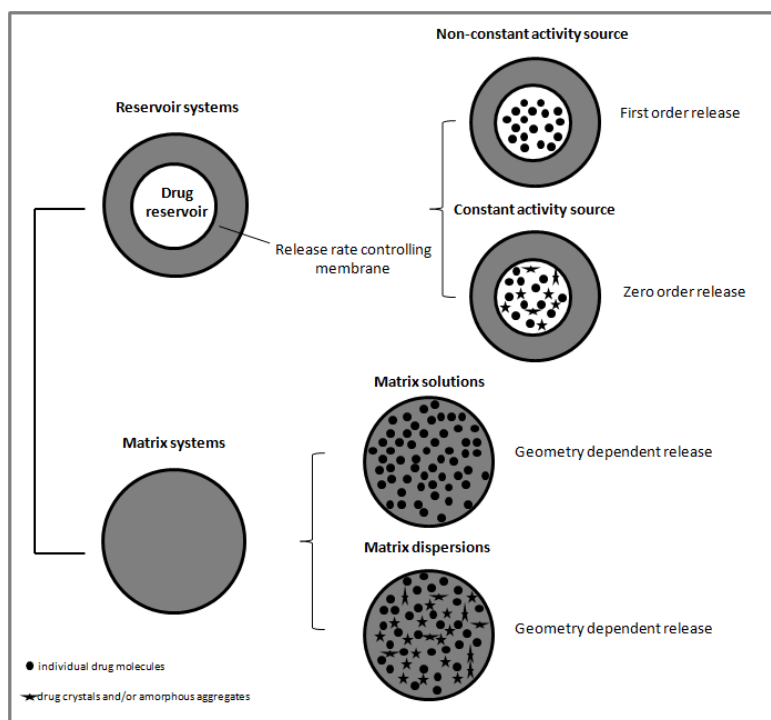


Figure 2.17. Classification scheme for diffusion controlled drug delivery systems (Siepmann *et al.*, 2008).

Figure 2.18 shows the experimental and theoretical release profiles from dye-encapsulated microcapsules in polymer shell. Analysis and comparison of the diffusion mechanism using several microcapsule's geometries and materials can provide the essential information for understanding the mass transfer behaviour in such systems.

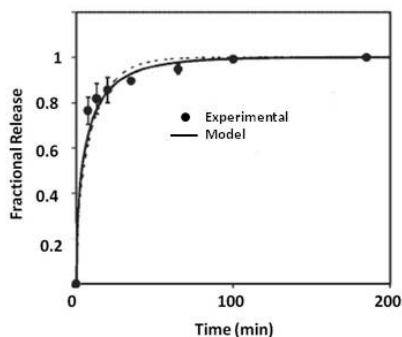


Figure 2.18. Experimental and theoretical release profiles from dye-encapsulated microcapsules in polymer shell (Tavera *et al.*, 2009).

Table 2.7. Representative list of release models of active agents through the polymeric membranes of microcapsules.

<i>Active agent</i>	<i>Release model</i>	<i>References</i>
Perfume	Zero order model for films geometry	(Peppas <i>et al.</i> , 1997)
Drug	Fick's second law model for spherical geometry	(Romero-Cano <i>et al.</i> , 2002)
Drug	Single pellet model Multiple-pellet model	(Borgquist <i>et al.</i> , 2004)
Drug	Single pellet model (solid drug coated with a semi-permeable membrane)	(Marucci <i>et al.</i> , 2008)
Dye (oils)	Single shell model	(Yow <i>et al.</i> , 2009)
Propolis	Fick's second law model for films geometry	(Mascheroni <i>et al.</i> , 2010)

The release rate of thyme oil, as described in the work of Mastromatteo *et al.* (2009) dealing with the study of active food packaging, is affected by film thickness and polymer concentration (Mastromatteo *et al.*, 2009). On the other hand, release tests performed by Passino *et al.* (2004) have shown that the diffusion of *Thymus* oil through the gelatine microcapsules is affected not only by the characteristics of the polymeric membrane but also by the type of used oil. The differences found in release might be due to the different hydrophilic characteristics of the oil. In fact, the percentage of polar compounds of oil can favour the entrapment of aqueous phase into de microcapsules during the coacervation process and consequently slows down its diffusion (Sanna Passino *et al.*, 2004).

Microencapsulation of essential oils is gaining wider acceptance in a broad range of industrial applications, creating added value products. In fact, it is predictable that, in the near future, microencapsulation techniques development will continue to grow trying to explore new possibilities for industry.

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CHAPTER 3:

Materials and Experimental Methods

“One never notices what has been done; one can only see what remains to be done.”

[Marie Curie]

In this chapter, the materials and methods used for the synthesis, characterization and performance evaluation of microcapsules are presented. The chemical materials used for the microcapsules production (core and wall materials), and the experimental/theoretical facilities for synthesis and characterization are listed and described.

3.1 Chemical compounds and reagents

The reagents used for the preparation of polylactide microcapsules are presented in Table 3.1.

Table 3.1. List of used reagents and suppliers used for microcapsule's production.

Reagent	Supplier
Poly(DL-lactide) (PLA, Mw=75,000-120,000)	Sigma Chemical Company; REF: 531162
Dimethylformamide (DMF, 99.8% ACS grade)	Sigma Chemical Company; REF: 319937
Essential oil of Thymus vulgaris L. (thyme oil, red, Kosher) <i>p</i> -Cymene (96%) Thymol (Ph Eur) Vanillin (≥97%, FCC, FG)	Sigma Chemical Company; REF: W306401 Alfa Aesar; REF: ALFAA19226.AP1 BDH Prolabo; REF: 83558.180 Sigma Chemical Company; REF: W310700
Tween® 20, Tween® 80, Tergitol™ 15-S-9 Span® 85	Sigma Chemical Company; REF: P5927 Sigma Chemical Company; REF: P1754 Sigma Chemical Company; REF: 15S9 Sigma Chemical Company; REF: S7135
Octamethylcyclotetrasiloxane (OCMTS)	Merck Schuchardt OHG; REF: 814750
Pluronic® /F68 n-hexane (ACS grade) Ethanol absolute PA	Sigma Chemical Company; REF: 81112 Merck Schuchardt OHG; REF: 104374 PANREAC; REF : 121086.1212

3.2 Microcapsules production

3.2.1. Synthesis process and experimental procedures

In this work a process to encapsulate thyme oil using PLA as the wall material was developed according to the general scheme represented in Figure 3.1 plus a washing/storage step.

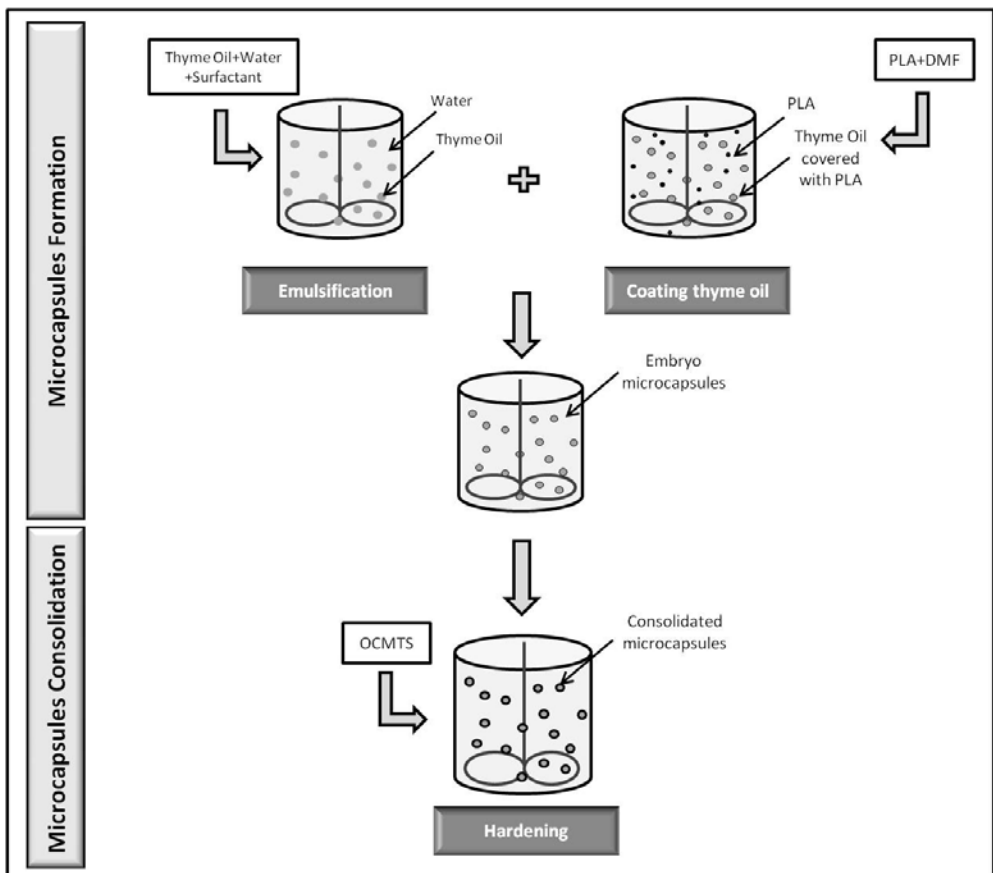


Figure 3.1. General process scheme used for the preparation of PLA microcapsules.

The used steps can be described briefly as follows:

Step I – Microcapsules Formation:

Firstly, an emulsion of thyme oil in water (o/w, with a ratio of 0.66%, v/v) stabilized with 1% (w/v) of a non-ionic surfactant, and a PLA solution in dimethylformamide (DMF) have been prepared. DMF is a good solvent for PLA and in addition is highly soluble in water.

Emulsification: The o/w emulsion was obtained by dispersion with an ultraturrax (IKA DI 25 Basic, yellow line) at 11,000 rpm during 90 seconds.

Coating core material: The PLA solution was added dropwise to the previously prepared o/w emulsion. The homogeneous solution of PLA in DMF, upon contact with water, promotes the precipitation of PLA around the thyme oil core. The encapsulation process has continued under moderate stirring (100 rpm) using an impeller stirrer in a batch reactor (IKA Model LR-2.ST) during one hour at room temperature.

Step II – Microcapsules Consolidation:

Hardening: The microcapsules formed were hardened by adding OCMTS and allowed to stand during one hour. OCMTS is a widely used hardening agent. It acts as nonsolvent for the PLA coacervate droplets thus promoting microcapsules solidification.

Step III – Washing and storage:

After hardening, the microcapsules were decanted and sequentially washed with Pluronic® F68 solution (0.1% w/w), an ethanol solution (30% v/v), and hexane. Ethanol and hexane have the role of, respectively, removing any remaining polar

and apolar compounds that weren't encapsulated. Pluronic F68, a surfactant, was added to keep the microcapsules solution stable during the washing process.

Finally, the microcapsules were freeze-dried during 40 hours and stored in powder form, or alternatively, stored directly in the Pluronic® F68 solution.

The synthesis procedure was repeated with different surfactant systems (simple surfactant or mixtures) added at the stage I – emulsification. The HLB (hydrophilic-lipophilic balance) value of the used surfactant system was comprised between 11 and 16.5 following the HLB recommendations for stabilizing an o/w emulsion.

The experimental system used for microcapsules production is presented in Figure 3.2 putting in evidence the different production steps.

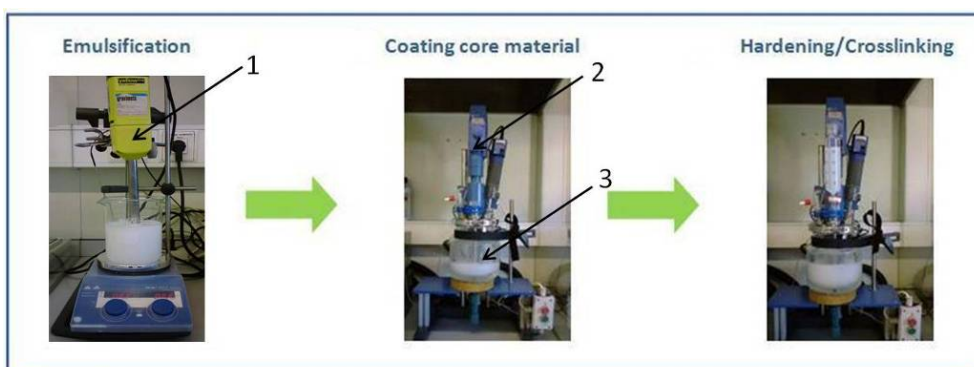


Figure 3.2. Experimental set-up for the thyme oil microcapsules production by coacervation: (1) ultraturrax (IKA DI 25 Basic); (2) overhead stirring drive and (3) reactor vessel.

The reactor system used was an IKA® LR – 2.ST system. This system is a modular miniplant reactor designed to simulate and optimize chemical reaction processes, as well as mixing, dispersion and homogenization processes at a model scale with a maximum volume of 2L. The reactor vessel can be heated up to 230 °C and the vacuum operation is possible up to 25 mbar. The system is composed of a reactor support (LR-2.ST type), a reactor vessel with a double-walled glass and a bottom

outlet valve (LR 2000.2 type), and a homogenization system equipped with a dispersion component type S 25 KV - 18 G. Data acquisition and control was performed with LABWORDSOFT software.

The freeze drying system (lyophilisation) used in the drying process of the produced microcapsules was a Cool Safe Freeze Drying equipment form *Scanvac*, and is shown in Figure 3.3. The freeze drying technique is a drying process where the solvent is frozen previous to drying, being thus sublimed. The solvent passed to the gas phase directly from the solid phase, below their melting point. This technique is intensively useful in dry foods, pharmaceutical or medical applications. The freeze drying process preserves a wide variety of products with minimal loss of their original physical qualities, since it keeps biological properties of proteins, and retains vitamins and bioactive compounds. Pressure can be reduced using a high vacuum pump where the vapour produced by sublimation is removed from the system by converting it into ice in a condenser, operating at very low temperatures, outside the freeze drying chamber.



Figure 3.3. Cool Safe Freeze Drying equipment (Scanvac, 2010).

The CoolSafe family of freeze dryers comprises of a large size range of condensers and a choice of either $-55\text{ }^{\circ}\text{C}$, $-95\text{ }^{\circ}\text{C}$, $-100\text{ }^{\circ}\text{C}$ or $-110\text{ }^{\circ}\text{C}$ temperatures. The temperature used in this work was -55°C (Scanvac, 2010).

3.3 Characterization techniques

3.3.1 Laser Dispersion - Size distribution and mean particle size of microcapsules

Particle size distribution of the produced microcapsules was analyzed by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman-Coulter) (Figure 3.4).

The Beckman Coulter LS 200 Series is a system of multifunctional particle characterization tools; the LS equipment uses a reverse Fourier optics incorporated in a patented binocular lens system and its technology is based on principles of light scattering. Its laser-based tools permit the analysis of particles without the risk of missing either the largest or the smallest particles in a sample. The LS equipment differs from other laser-based instruments because of its wide dynamic size range, number of size channels and sample measurement options. The allowed range of particle size is comprised between 0.375 μm - 2000 μm (Coulter, 2011). This equipment enables measures in volume and number.



Figure 3.4. LS™ 200 Series Laser Diffraction Particle Size Analyzer (Coulter, 2011).

3.3.2 Optical microscopy and Cryogenic Scanning Electron Microscopy (Cryo-SEM)

Microcapsules morphology was analysed by optical microscopy (Leica DM 2000 microscope equipped with the software Leica Application Suite Interactive measurement and transmitted light mode) and by cryogenic scanning electron microscopy (JEOL JSM-6301F/Inca energy 350/Gatan alto 2500).

Optical and electron microscopy (Scanning electron microscopy – SEM or cryogenic scanning electron microscopy – Cryo-SEM) involve the diffraction, reflection, or refraction of electromagnetic radiation/electron.

The Leica DM 2000 (Figure 3.5) microscope is used in biology, medicinal and clinical laboratories, and for general research microscopy applications. The microcapsules samples were analyzed using the transmitted light mode using bright field and dark field option. The bright field illumination provides the most uniform illumination of the sample, although under dark field, the inner circle area of the light cone is blocked, such that the sample is only illuminated by light that impinges on its surface at a glancing angle (Instruments, 2006). Through dark field option it is possible to distinguish the morphologic diversity in samples by colour difference.

The microcapsules were analysed, by optical microscopy, in solution after the production and dried after the drying process by lyophilisation.



Figure 3.5. Optical microscope, Leica DM 2000 (Instruments, 2006).

The Scanning Electron Microscope is a microscope that uses electrons rather than light to form an image (Figure 3.6). This type of microscope has a large depth of field, which allows a large amount of the sample to be in focus at once. With this equipment images of high resolution are obtained, which means that closely spaced features can be examined at a high magnification. The preparation of the samples is relatively easy since it only requires the sample to be conductive. In some cases, it is necessary to coat the sample with gold. Combination of higher magnification, larger depth of focus, greater resolution, and ease of sample observation makes the SEM one of the mostly used techniques in research areas (Klesel, 2006).



Figure 3.6. Scanning electron microscope JEOL FESEM JSM6301F associated with Cryo-SEM unit Gatan model Alto 2500 (Cemup, 2011).

In case of Cryo Scanning Electron Microscope (cryo-SEM; cryo =cold) images can be made of the surface of the frozen material. Figure 3.7 shows the scheme of the Cryo-SEM unit in the FESEM. Unlike other scanning microscopy methods Cryo-SEM offers the advantage of being able to promote an extremely rapid freezing of the analyzed samples, which preserves the original structure of microcapsules. When using cryo-fracture the samples break clean along weak edges without causing much malformation. As a result Cryo-SEM is a powerful tool to study microcapsules structure and formation. The microcapsules solution was analyzed

by Cryo-SEM after congealing the samples. The unit of Cryo-SEM used for preparation/transfer and observation of samples at low temperature (LN₂) is associated with the electron microscope JEOL FESEM JSM6301F.

The holder with frozen material is held under liquid nitrogen to be coupled to a rod and pulled back into a small cylindrical container. This procedure is done to transfer the sample to the high vacuum Cryo-SEM unit and prevent contamination with gas particles while sliding in the sample into the Cryo-chamber. The Cryo-chamber is equipped with a knife that can be handled from outside by means of a lever to fracture the sample for applications in which imaging of the surface of inner structures is aimed (Nijmegen, 2011).

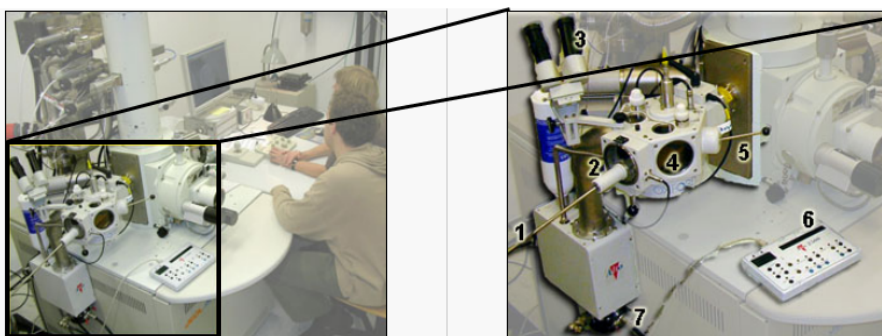


Figure 3.7. Parts of the Cryo-unit in the FESEM: 1) insertion rod, 2) cylindrical chamber for transfer of the sample from freezing unit to cryo-chamber, 3) binocular, 4) cryo-chamber proper containing breaking knife, gold palladium sputter, water decontamination sublimation unit, 5) lever to handle the fracture knife, 6) operation display and 7) supply of liquid nitrogen to the cryo-FESEM (Nijmegen, 2011)

3.3.3 Gas chromatography GC-FID/MS

Quantification of the encapsulated thyme oil was performed by gas chromatography GC/FID and the corresponding composition determined by GC/MS. The analyses were carried out using a Varian CP-3800 instrument equipped with split/splitless injector, two CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 μ m film thickness), a Varian FID detector and a Varian Saturn 2000 MS ion-trap mass spectrometer, controlled by the Saturn 2000 WS software (Figure 3.8). The oven temperature was isothermal at 50°C for 2 min, then increased from 50°C up to 200°C at 5°C/min and held at 200°C for 13 min. The injectors were set at 240°C, with a split ratio of 1:50 for FID and 1/200 for MS. The FID detector was maintained at 250°C. The sample volume injected was 0.1 μ L. The carrier gas was helium He N60, at a constant flow rate of 1 mL/min.

The flame ionization detector (FID) is a non-selective detector used in conjunction with gas chromatography. In gas chromatography GC-FID, detects analyses by measuring an electrical current generated by electrons from burning carbon particles in the sample. Gas chromatography separates the components of a mixture and mass spectroscopy (MS) characterizes each of the components individually. By combining the two techniques (GC-MS), it is possible to evaluate both qualitatively and quantitatively a solution containing a number of chemicals.



Figure 3.8. GC-FID Headspace equipment of LSRE laboratory with an automatic sampler coupled.

GC/MS analysis of material provided the separation of components that were identified using their mass spectra. The component identification was made by comparison of the obtained mass spectra with some available reference spectra using NIST98 spectral library, pure reference compounds (own laboratory library) and literature data. The composition of the samples was determined as the average of three GC injections.

3.4 References

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CHAPTER 4:

Microencapsulation of thyme oil by coacervation

“A person who never made a mistake never tried anything new. ”

[Albert Einstein]

The objective of this chapter is to develop a novel coacervation process to produce microcapsules of polylactide (PLA) containing thyme oil with potential application in cosmetics. The novelty of the developed approach consists on dissolving PLA in dimethylformamide (DMF) which is a good solvent for PLA but in addition has high solubility in water. Upon contact with water, the homogeneous solution of PLA in DMF, promote the precipitation of PLA around the thyme oil core. The produced microcapsules have bimodal particle size distributions in volume with a mean particle size of 40 μm . Microcapsules analysis by microscopy have confirmed the spherical shape, the rough surface, and allowed the estimation of the wall thickness around 5 μm . Quantification of the encapsulated thyme oil was performed by gas chromatography and pointed out for a preferential encapsulation of thyme oil apolar compounds.

Formulation evolution till the optimized one (final formulation) will be presented sequentially.

This chapter is based on the following publication:

Martins, I. M.; Rodrigues, S. N.; Barreiro, M. F.; Rodrigues, A. E. Microencapsulation of thyme oil by coacervation. *J. Microencapsulation* 26(8) (2009). 667-675.

4.1 Introduction

Microencapsulation is a technology that includes several processes to cover an active agent with a protective wall material. There are various industrial applications for microcapsules, such as carbonless paper, “scratch and sniff” fragrance sampling, “intelligent” textiles, controlled release of drugs, pesticides and cosmetics. A wide range of core materials have been encapsulated, including adhesives, agrochemicals, live cells, active enzymes, flavours, fragrances and pharmaceuticals (Ghosh, 2006). Many oils in the food and flavour categories have properties such as strong flavour and instability to oxidation, thus needing to be encapsulated in a core-shell material to reduced oxidative degradation, to control the release rate or even to improve shelf life of these materials (Lumsdon *et al.*, 2005). Microencapsulation techniques can be as diverse as coacervation (Jegat *et al.*, 2000; Soper *et al.*, 2000), atomization (Ascheri *et al.*, 2003), interfacial polymerisation (Hirech *et al.*, 2003; Mizuno *et al.*, 2005), spray drying (Bodmeier *et al.*, 1988) and “in situ” polymerisation (Bang *et al.*, 2005). The choice of the appropriate technique depends on the core material properties, the involved manufacturing conditions and the product end user requirements.

Coacervation offers many possibilities for the encapsulation of various types of active agents (Benoit *et al.*, 2001; Ferres *et al.*, 1999; Lumsdon *et al.*, 2005; Magdassi *et al.*, 1996; Schobel, 1986; Soper, 1996; Soper *et al.*, 2000; Whitaker *et al.*, 1991). The term coacervation was introduced in 1930 by Bungenberg de John and Kruyt for a process in which aqueous colloidal solutions were separated into two liquid phases, one rich in colloid (coacervate) and the other poor in colloid (Bungenberg De Jong, 1949; Jong *et al.*, 1930). According to International Union of Pure and Applied Chemistry (IUPAC), coacervation is defined as the separation of colloidal systems into two liquid phases. The general outline of the process consists in three steps that occur under continuous stirring (see Figure 4.1).

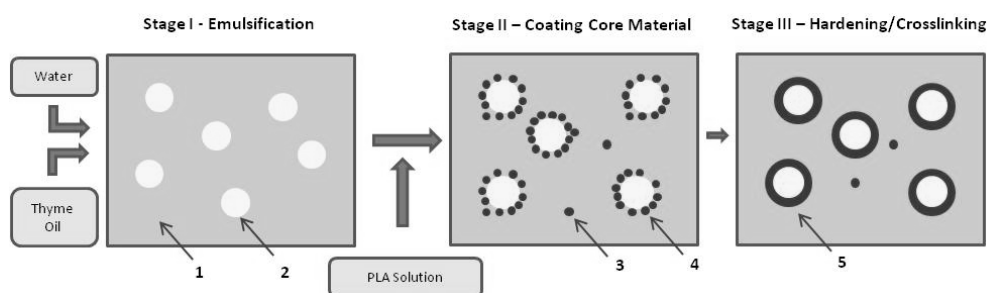


Figure 4.1. General process scheme for microcapsule preparation by coacervation. 1)water; 2) thyme oil; 3) PLA; 4) deposition the PLA coating upon thyme oil; 5) microcapsules.

The first step consists in the formation of an oil-in-water (o/w) emulsion (dispersion of the oil in a aqueous solution containing a surface-active hydrocolloid), the second comprises the formation of the coating (deposition the polymer coating upon the core material), and the last one is the stabilization of the coating (coating hardening, using thermal, crosslinking or desolvation techniques, to form self sustaining microcapsules) (Soest, 2007).

Coacervation in the aqueous phase can be classified into simple and complex, according to the involved phase separation mechanism. In simple coacervation, the polymer is salted out by the action of electrolytes, such as sodium sulphate, or desolvated by the addition of a water miscible non-solvent, such as ethanol, or by increasing/decreasing temperature. These conditions promote the macromolecule-macromolecule interactions in detriment of the macromolecule-solvent interactions. On the other hand, complex coacervation is essentially driven by the attractive forces of oppositely charged polymers (Gander *et al.*, 2006). The coexistence of a coacervate phase made of concentrated polyelectrolytes and a dilute equilibrium phase depends on pH, ionic strength and polyion concentrations (Ducel *et al.*, 2004). The coacervation in the aqueous phase can only be used to encapsulate insoluble material in water and on the other hand, the coacervation in

organic phase allows the encapsulation of hydrosoluble material, but requires the use of organic solvents (Kas, 2000).

Coacervation was the chosen technique to produce polylactide (PLA) microcapsules using *Thymus vulgaris* L. (thyme oil), an antioxidant and antimicrobial agent, as the core material.

PLA is an aliphatic polyester obtained from lactic acid by the fermentation of glucose or sucrose (Hong *et al.*, 2000), see Figure 4.2. Biodegradable microcapsules of PLA have received extensive attention as delivery systems for drug encapsulation. This type of biodegradable polymeric carriers can be hydrolyzed in the body to form products that are easily reabsorbed or eliminated (Huang *et al.*, 1997). The adjustable physicochemical proprieties of PLA such as swelling and biodegradation kinetics, or molecular interaction with potential embedded drugs, offer various possibilities in the design of controlled release systems (Blanco-Prieto *et al.*, 2004).

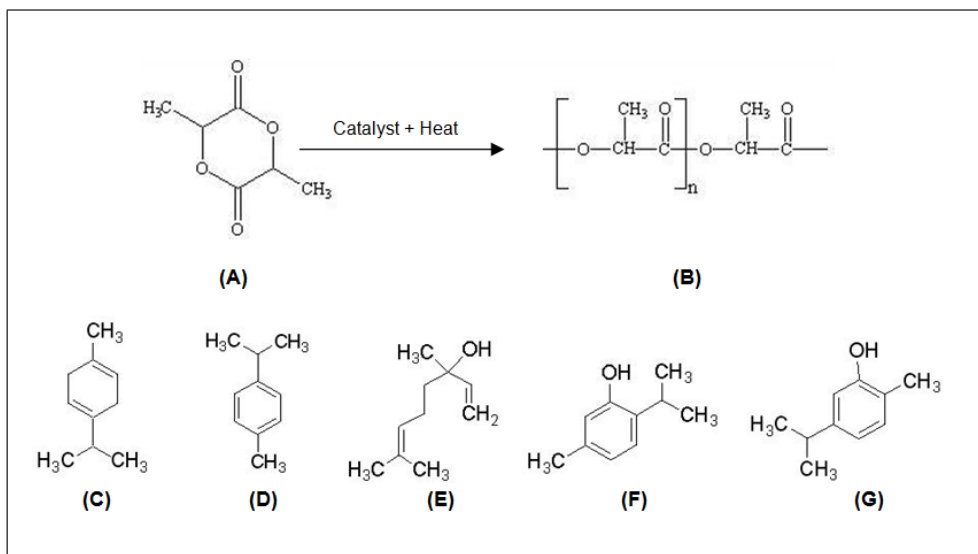


Figure 4.2. Chemical synthesis of polylactide ((A) Lactide and (B) Polylactide), and chemical structures of representative thyme oil components ((C) γ -Terpinene, (D) p-Cymene, (E) Linalool, (F) Thymol, and (G) Carvacrol).

The core material, thyme oil, is extracted from an aromatic plant of increasing economic importance in North America, Europe and North Africa, having an important and growing place in the world market (Naghdi Badi *et al.*, 2004). This essential oil is widely used in the flavour and food industries. Thyme oil has several components in its composition (see Figure 4.2) but its antimicrobial activity is mainly attributed to the presence of carvacrol, cinnamaldehyde, thymol, geraniol and eugenol among others (Sipailiene, 2006). As a pharmaceutical compound, thymol and carvacrol are used in mouthwashes, soaps and creams. Thyme oil itself is used in manufacture of perfumes and cosmetics.

The objective of this work is to develop a novel coacervation process to produce microcapsules of PLA to encapsulate thyme oil that will be used in cosmetics. PLA is soluble in organic solvents but insoluble in water. Generally, PLA is used to encapsulate water soluble active principles such as drugs, pesticides and dye-stuffs by coacervation, mainly by means of microspheres production or by using double emulsion techniques (o/w/o). However, the objective of this work is to encapsulate thyme oil, a water insoluble active principle that needs, in a first step, the preparation of an oil-in-water emulsion. The novelty of our process consists on dissolving PLA in dimethylformamide (DMF) which is a good solvent for PLA but in addition has high solubility in water. Upon contact with water, the homogeneous solution of PLA in DMF, promotes the precipitation of PLA around the thyme oil core. With this work a new, easy and executable method of coacervation was developed by introducing modifications on microencapsulation process that allow the encapsulation of an oily active principle by simply preparing an o/w emulsion. Control of size and wall thickness of microcapsules and encapsulation efficiency were studied.

4.2 Preliminary tests of microcapsules production

4.2.1 Chemical system

In this section studies concerning thyme oil microencapsulation by coacervation, using PLA as the wall material, have been performed. Several preliminary tests with the aim to inspect the morphology and particle size distribution of the produced microcapsules as a function of the used formulation and process steps were carried out. Table 4.1 illustrates the various chemical systems and composition of all formulations used.

The methodology for PLA microcapsules production by coacervation was based on the procedure described in *Hung et al.* (Huang *et al.*, 1997) and can be summarized in three steps:

- **Step I – Emulsification:** dispersion of the oil (core material) in a aqueous solution;
- **Step II - Coating core material:** deposition the polymer coating upon the core material;
- **Step III – Hardening:** addition of a nonsolvent thus promotes microcapsules solidification.

Table 4.1. Chemical systems and composition of compounds used in microcapsules formulation.

Formulations	Thyme oil (red)		Polymer Solvent		Aqueous Phase			Hardening Agent	
	Volume (mL)	mass (g)	Designation	Volume (mL)	Water Volume (mL)	Surfactant	mass (g)	Designation	Volume (mL)
Initial formulation 1	2.00	0.40000	Ethyl Acetate	16.0	20.0	-	-	-	-
Initial formulation 2	0.70	0.40000	Ethyl Acetate	16.0	20.0	-	-	-	-
Test 001	0.40	0.20000	Ethyl Acetate	5.0	50.0	Polyvinyl alcohol	0.840	-	-
Test 002	0.37	0.20093	Ethyl Acetate	7.0	80.0	Polyvinyl alcohol	1.344	-	-
Test 003	0.18	0.10768	Ethyl Acetate	2.8	30.0	Polyvinyl alcohol	0.504	-	-
Test 003G	0.18	0.10768	Ethyl Acetate	2.8	30.0	Polyvinyl alcohol	0.504	Glutaraldehyde (25%)	1.0
Test 004	0.18	0.10704	Ethyl Acetate	2.8	30.0	-	-	-	-
Test 005	2.027	0.11569	Dimethylsulfoxide	5.0	15.0	Polyvinyl alcohol	0.252	-	-
Test 005G	2.027	0.11569	Dimethylsulfoxide	5.0	15.0	Polyvinyl alcohol	0.252	Glutaraldehyde (25%)	1.0
Test 006	1.35	0.10111	Dimethylformamide	5.0	10.0	Polyvinyl alcohol	0.168	-	-
Test 006R	1.00	0.10119	Dimethylformamide	5.0	7.4	Polyvinyl alcohol	0.124	-	-

Table 4.1 (cont.). Chemical systems and composition of compounds used in microcapsules formulation.

Formulations	Thyme oil (red)		Polymer Solvent		Aqueous Phase			Hardening Agent	
	Volume (mL)	mass (g)	Designation	Volume (mL)	Water Volume (mL)	Surfactant	mass (g)	Designation	Volume (mL)
Test 006G	1.00	0.10119	Dimethylformamide	5.0	7.4	Polyvinyl alcohol	0.124	Glutaraldehyde (25%)	1.0
Test 007	0.75	0.30164	Dimethylformamide	15.0	5.6	Polyvinyl alcohol	0.940	-	-
Test 008	1.00	0.20650	Dimethylformamide	10.0	7.4	Polyvinyl alcohol	0.124	-	-
Test 009	0.20	0.10100	Dimethylformamide	5.0	20.0	Polyvinyl alcohol	0.336	Glutaraldehyde (25%)	1.0
Test 010	0.20	0.10097	Dimethylformamide	7.0	30.0	Polyvinyl alcohol	0.504	Octamethylcyclotetrasiloxane (OCMTS)	60.0
Test 011	0.20	0.10043	Dimethylformamide	7.0	30.0	Polyvinyl alcohol	0.504	Octamethylcyclotetrasiloxane (OCMTS)	0.35
Test 012	0.20	0.10271	Dimethylformamide	7.0	30.0	Tween 20	1.073	Octamethylcyclotetrasiloxane (OCMTS)	60.0
Test 012A	0.20	0.10266	Dimethylformamide	7.0	30.0	Tween 20	1.073	Octamethylcyclotetrasiloxane (OCMTS)	60.0
Test 013	0.20	0.10238	Dimethylformamide	7.0	30.0	Tween 20	1.073	Octamethylcyclotetrasiloxane (OCMTS)	60.0
Test 013R	0.20	0.10074	Dimethylformamide	7.0	30.0	Tween 20	1.073	Octamethylcyclotetrasiloxane (OCMTS)	60.0
Test 014	2.70	1.4800	Dimethylformamide	94.5	405.5	Tween 20	15.439	Octamethylcyclotetrasiloxane (OCMTS)	889.7

4.2.2 Characterization of produced microcapsules

Particle size distributions of the produced microcapsules were obtained by laser dispersion (Coulter LS230) based on the final reaction solution, and microcapsules morphology examined by optical microscopy (OM) and scanning electron microscopy (SEM). To make possible microcapsules analysis by SEM, textile samples were covered with the microcapsules solution and dried at room temperature. Figure 4.3 and 4.4 show the obtained particle size distributions, both in number and volume, for the formulation *Test002* and *Test003*, respectively. Figures 4.5 to 4.6 show the morphology of the produced PLA microcapsules. It can be seen for the shown samples that the particle size distribution in volume is bimodal whereas in number is unimodal. The mean particle size, in volume, of the produced microcapsules for formulation *Test002* was around 15 μm and for the formulations *Test003* and *Test003G* was 25 μm and 50 μm , respectively.

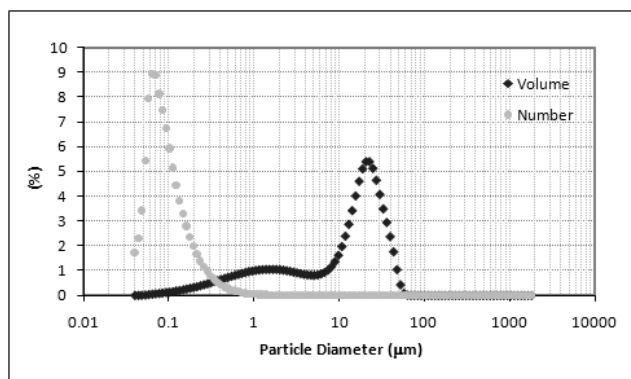


Figure 4.3. Differential particle size distribution in volume and number for thyme oil microcapsules obtained with formulation *Test002*.

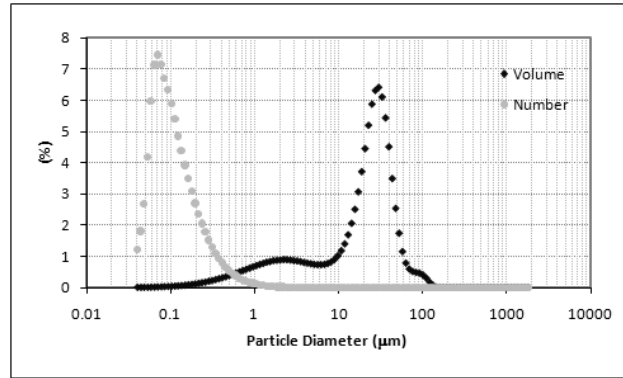


Figure 4.4. Differential particle size distribution in volume and number for thyme oil microcapsules obtained with formulation *Test003*.

Observing the SEM micrographs it can be seen the spherical shape of microcapsules, nevertheless some of them present deformations. With formulation *Test002*, small microcapsules in few number, were detected (Figure 4.5). In contrast, microcapsules produced with formulations *Test003* and *Test003G*, see Figures 4.6 and 4.7, have the aspect of “golf balls”. With *Test005G* and *Test006R* it is visible the existence of a residual material like a film, and small clusters of particles covering the fibers (Figure 4.8).

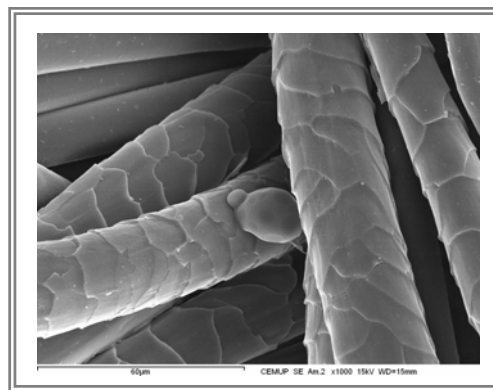


Figure 4.5. SEM micrograph of the impregnated textile with produced thyme oil microcapsules by formulation *Test002* with magnification of 1000x.

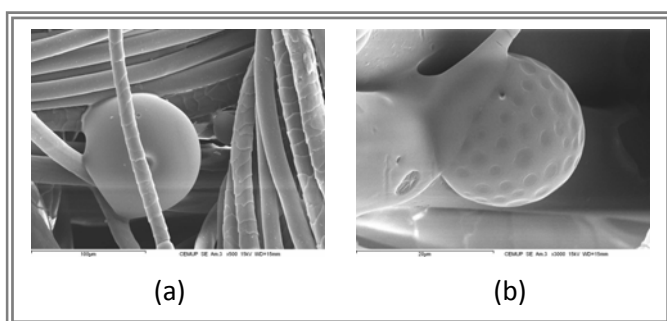


Figure 4.6. SEM micrograph of the impregnated textile with produced thyme oil microcapsules by formulation *Test003* with magnification of 500x (a) and 3000x (b).

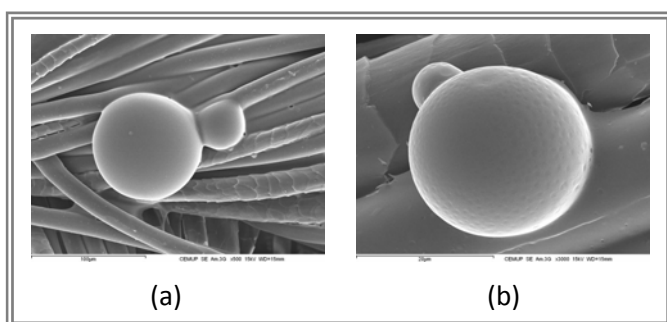


Figure 4.7. SEM micrograph of the impregnated textile with produced thyme oil microcapsules by formulation *Test003G* with magnification of 500x (a) and 3000x (b).

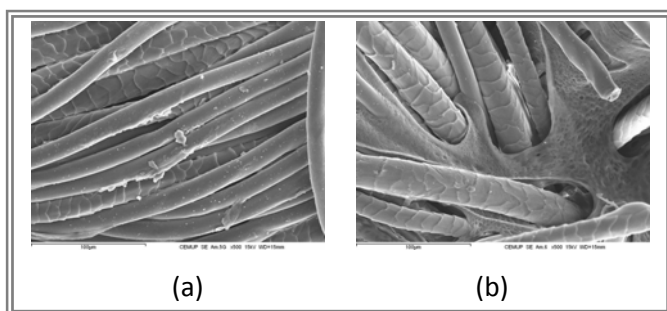


Figure 4.8. SEM micrograph of the impregnated textile with produced thyme oil microcapsules by formulation *Test005G* (a) and *Test006R* (b) with magnification of 500x.

Based on the performed analysis, it is possible to conclude that, formulations *Test003* and *Test003G*, have produced the best results. Thus, the following work focuses on the improvement of these formulations.

If the chosen polymer solvent does not have significant solubility in water, two liquid phases will be formed, and the polymer will remain dissolved in the organic phase hindering its precipitation, which is essential to cover the oil droplets and form the desired microcapsules. Therefore, under this assumption DMF works better than dichloromethane (DCM) or ethyl acetate (EtAc). Moreover, DMF is used as solvent in the synthesis of polyurethanes being not reactive with hydroxyls or isocyanates, i.e. it will be inert towards thyme oil. The high water solubility of DMF makes possible the contact of the polymer with water, i.e. promote its precipitation.

The amount and nature of hardening agent used in the microcapsules production and the surfactant ratio used to promote the stability of oil droplets will affect the consolidation of the produced microcapsules. Hence, the following formulations took into account these assumptions. The performed tests started to use octamethylcyclotetrasiloxane (OCMTS) as the crosslinking agent (hardener) and Tween 20 (nonionic surfactant), as the surfactant to stabilize the o/w emulsion.

The produced microcapsules using formulation *Test010* were analyzed by laser dispersion after production and storage. The particle size distribution in volume is presented in Figure 4.9. The microcapsules mean size was 61 μm , 39 μm and 15 μm , respectively for the analyses performed immediately after production (initial), one day and eight days after production. These evidences have shown that a problem of stability of the formed microcapsules exist, possible due to defective wall formation (poor deposition of the polymer within the oil droplets). An increase in the microcapsules volume fraction associated with small sizes was observed resulting in a reduction of the mean particle size of approximately 4x.

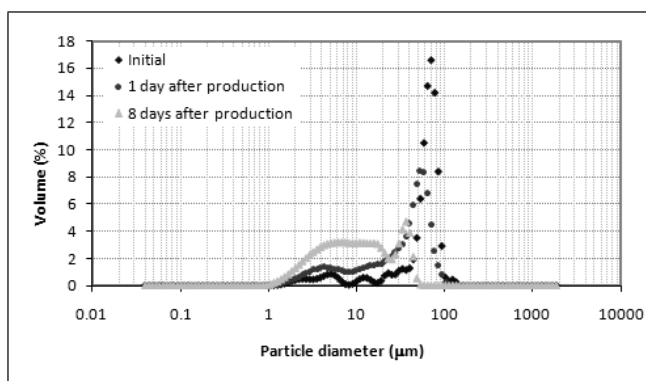


Figure 4.9. Time evolution of particle size distribution in volume for thyme oil microcapsules obtained with formulation *Test010*.

Figure 4.10 aims to characterize the stability of the produced microcapsules (formulation *test013*) face to successive washes and after 14 days of storage. It can be concluded that regardless of microcapsules washing process the behavior of the particle size distribution is unchanged. It can also be concluded that 14 days of storage did not change the mean particle size that remains constant and around 40.00 μm .

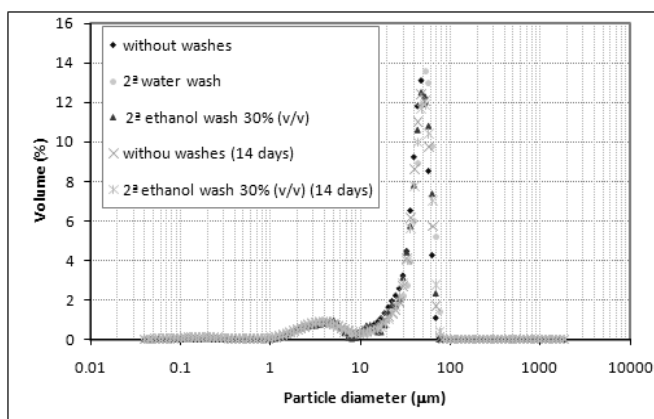


Figure 4.10. Particle size distribution in volume for thyme oil microcapsules obtained with formulation *Test013*, analyzing the effect of washes (two washing steps) and evolution over time.

Having in view testing the reproducibility of the process, *Test013* was repeated (*Test013R*) and the obtained particle size distributions are presented in figure 4.11. The obtained particle size distributions are quite similar thus confirming the reproducibility of the process.

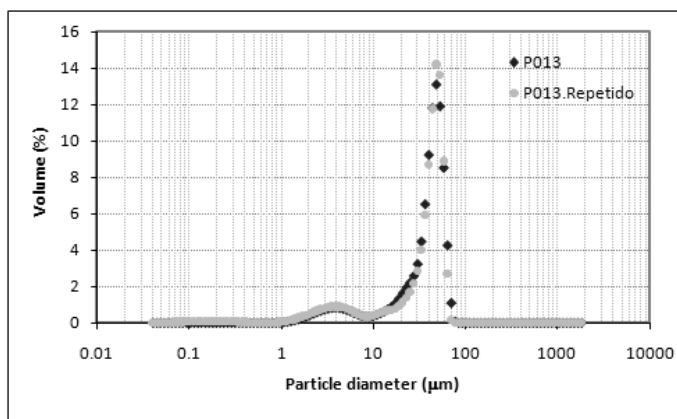


Figure 4.11. Particle size distribution in volume for thyme oil microcapsules obtained with formulation *Test013* and *Test013R*.

Optical microscopy (OM) was used to examine microcapsules morphology after being submitted to different washing processes. Figure 4.12 shows the effect observed on microcapsules proceeding from *Test013* as a function of the used washing procedure. It was observed that, independently of the used washing process, microcapsules do not modify their morphology. They keep their spherical and well defined shape and one can notice also the absence of agglomerates.

After several experiments the optimized formulation was set up. The details of this formulation (final formulation – formulation *Test014*) are described in next section, as well as, some of the performed characterizations.

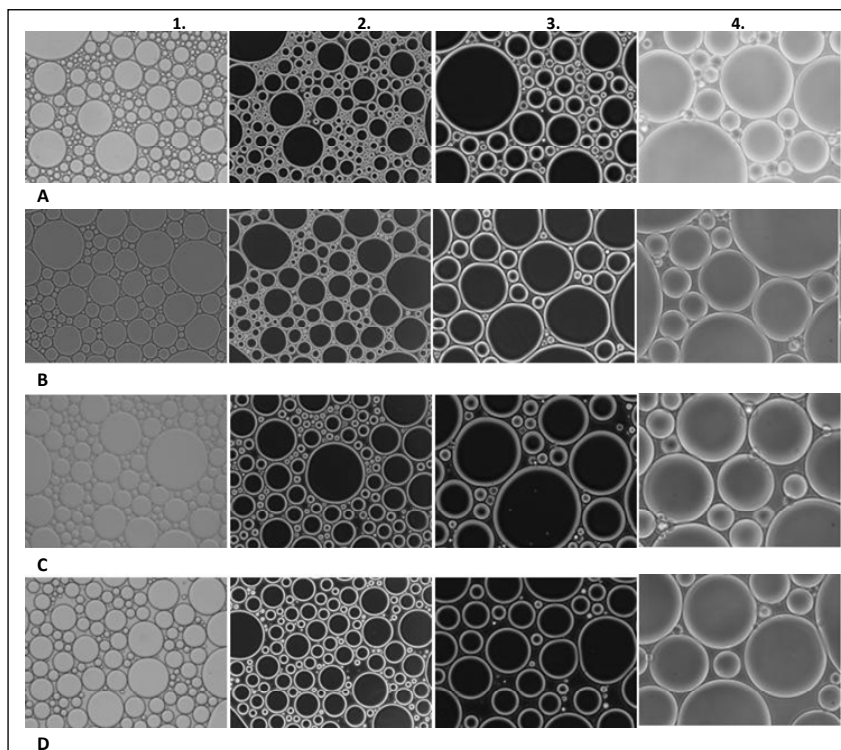


Figure 4.12. Optical micrographs of thyme oil microcapsules obtained from formulation *Test013* treated with different washing solutions. **A)** without washing; **B)** washing with deionized water; **C)** washing with ethanol solution of 30% (v/v), **D)** washing with ethanol solution of 50% (v/v). Images obtained using: **1.** magnification 100x in bright field, **2.** magnification 100x in contrast phase, **3.** magnification 200x in contrast phase and **4.** magnification contrast 400x in contrast phase.

4.3 Microcapsules final formulation

4.3.1 Materials and methods

4.3.1.1 Materials

Poly(DL-lactide) (PLA, Mw=75,000-120,000, inherent viscosity=0.55-0.75 dL/g) was used as the wall-forming material; dimethylformamide (DMF, 99.8% ACS grade) as the PLA solvent; essential oil of *Thymus vulgaris* L. (thyme oil, red, Kosher) as the core material; Pluronic®/F68 and Tween®20 as surfactants. All these reagents were obtained from Sigma Chemical Company (Germany). Octamethylcyclotetrasiloxane (OCMTS) and n-hexane (ACS grade) were purchased from Merck Schuchardt OHG (Germany).

4.3.1.2 Microcapsules preparation

The final microencapsulation process for thyme oil using PLA as the wall material was developed according to the general scheme represented in Figure 4.13. The following three steps can be described:

Step 1. Microcapsules formation: Firstly, a thyme oil emulsion in water (o/w ratio of 0.66%, v/v) stabilized with 1% (w/v) Tween® 20 (HLB of 16.7), and a PLA solution (V=94.5 mL) in dimethylformamide (DMF) (concentration of 15.7g/L) have been prepared. Thereafter, the PLA solution was dropwise to the previously prepared o/w emulsion. Upon contact with water, the homogeneous solution of PLA in DMF, promote the precipitation of PLA around the thyme oil core. The o/w emulsion was obtained by dispersion with an ultraturrax at 11,000 rpm during 90 seconds and the encapsulation process continued under stirring (100 rpm) using an impeller stirrer in a batch reactor for one hour using ambient temperature.

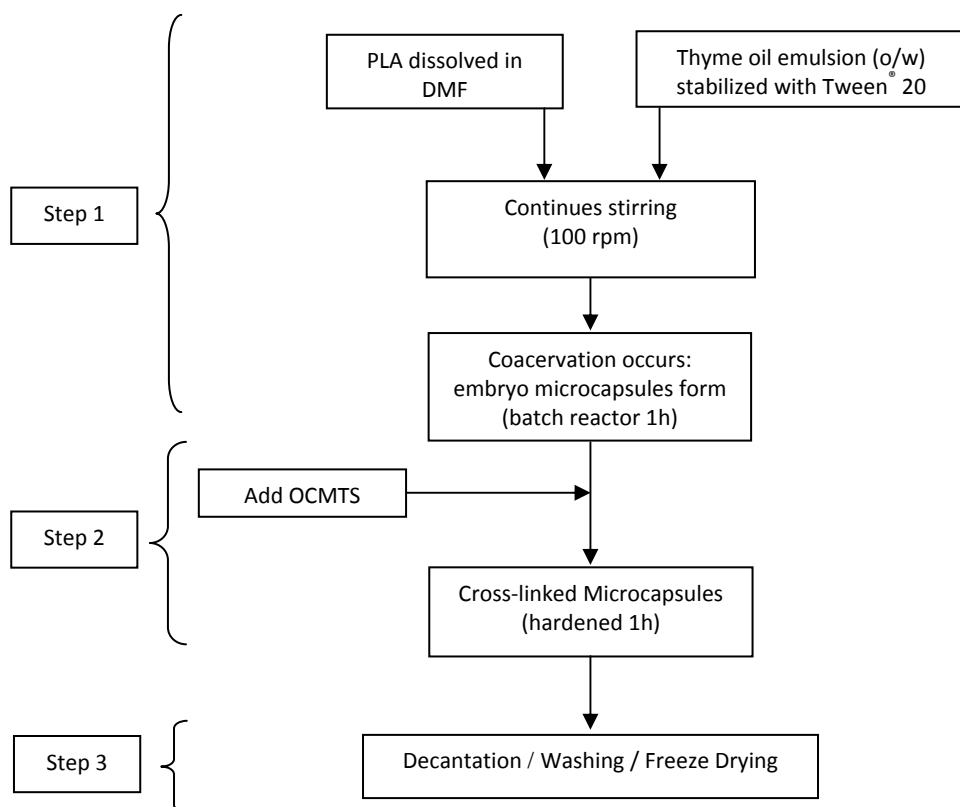


Figure 4.13. Process steps for microencapsulation of thyme oil by coacervation (Martins *et al.*, 2009).

Step 2. Microcapsules consolidation: The microcapsules formed were hardened by adding 60 mL of OCMTS and allowed to stand during one hour. OCMTS is a widely used hardening agent. It acts as nonsolvent for the PLA coacervate droplets thus promoting microcapsules solidification.

Step 3. Separation and washing: After hardening, the microcapsules were decanted and sequentially washed with Pluronic® F68 solution (0.1% w/w), an ethanol solution (30% v/v), and hexane. Ethanol and hexane have the role of, respectively, removing any remaining polar and apolar compounds that weren't

encapsulated. Pluronic F68, a surfactant, was added to keep the microcapsules solution stable during the washing process. Finally, the microcapsules were freeze-dried during 40 hours and stored in powder form.

4.3.1.3 Characterization techniques

Size distribution of microcapsules (Laser Dispersion)

Particle size distribution of the produced microcapsules was analyzed by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman-Coulter). The corresponding medium values in volume and number were determined.

Optical microscopy and Cryogenic Scanning Electron Microscopy (Cryo-SEM)

Microcapsules morphology was analysed by optical microscopy (Leica DM 2000 microscopy equipped with software Leica Application Suite Interactive measurement and with transmitted light mode) and by cryogenic scanning electron microscopy (JEOL JSM-6301F/Inca energy 350/Gatan alto 2500).

Gas chromatography GC-FID/MS

Quantification of the encapsulated thyme oil was performed by gas chromatography GC/FID and the corresponding composition determined by GC/MS. The analyses were carried out using a Varian CP-3800 instrument equipped with split/splitless injector, two CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 µm film thickness), a Varian FID detector and a Varian Saturn 2000 MS ion-trap mass spectrometer, controlled by the Saturn 2000 WS software. The oven temperature was isothermal at 50°C for 2 min, then increased from 50°C up to 200°C at 5°C/min and held at 200°C for 13 min. The injectors were set at 240°C, with a split ratio of 1:50 for FID and 1/200 for MS. The FID detector was maintained at 250°C. The sample volume injected was 0.1 µL. The carrier gas was helium He N60, at a constant flow rate of 1 mL/min.

The composition of thyme oil was expressed in percentage values determined from GC-FID peak areas in a base without solvent. The mass of encapsulated thyme oil has been calculated using a mass balance.

The individual components that characterize the nonencapsulated thyme oil were quantified by analysing the two phases obtained after microcapsules separation by decantation (aqueous phase and microcapsules rich phase). 1 ml of the aqueous phase and 1 ml of the microcapsules surrounding solution were collected using a syringe equipped with a 0.45 μm pore size filter and thereafter analysed by GC-FID. The mass of encapsulated oil was obtained by difference between the loaded original quantity and the nonencapsulated determined quantity.

The encapsulation efficiency (percentage of thyme oil present in microcapsules) was calculated based on the formula bellow.

$$\text{Encapsulation Efficiency (\%)} = \frac{m_{\text{total}} - m_{\text{out}}}{m_{\text{total}}} \times 100 \quad (4.1)$$

where m_{total} = amount of loaded essential oil (g) and m_{out} = amount of nonencapsulated essential oil (g).

4.3.2 Results and Discussion

4.3.2.1 Particle size distribution (Laser dispersion)

Figure 4.14 shows the experimentally measured particle size distributions, both in volume and in number, for the prepared PLA microcapsules. It was observed a bimodal distribution in volume with a mean particle size of 40 μm . In number the distribution was quite narrow and unimodal, with a mean particle size around 3 μm .

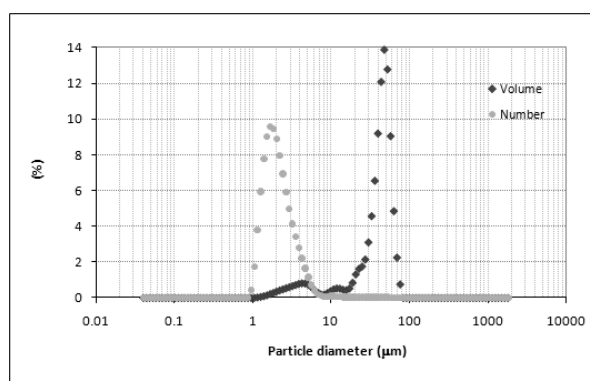


Figure 4.14. Particle size distribution of polylactide microcapsules with thyme oil. Distribution in volume and in number.

The same particle size distribution measurement was quantified both relative to the total number of particles and to the total volume of particles and it was observed that 99% by number of particles have diameters smaller than 10 μm (1% > 10 μm), but this represents 10% of the particles by volume (90% > 10 μm). This means that, even a large number of microcapsules have small size; most of the thyme oil was encapsulated in larger particles. Table 4.2 show microcapsule mean particle size obtained for three replicas of the experiment (batch 1 to 3). Although the obtained distributions have a wide dispersion, the results pointed out for a good reproducibility.

Table 4.2. Mean particle size in volume of microcapsules in three experiments.

Batch nº	Particle size
	(μm) (mean $\pm$ SD)
1	39.95 $\pm$ 19.73
2	38.55 $\pm$ 18.99
3	42.24 $\pm$ 17.94

4.3.2.2 Optical microscopy and Cryogenic Scanning Electron Microscopy (Cryo-SEM)

The analysis by optical microscopy had the objective to study the microcapsules morphology after the production (Figure 4.15) and after drying by lyophilisation (Figure 4.16). Figure 4.15 shows the aspect of the microcapsules in the bright field option at different magnifications. Microcapsules have spherical shape, with different sizes and one can notice also the absence of agglomerates. The analyses after freeze drying, Figure 4.16, have confirmed the spherical shape, the rough surface and allowed to estimate the wall thickness around $5\mu\text{m}$ by using Leica software tools. Additionally, it was observed two predominant sizes of microcapsules, compatible with a bimodal distribution and the absence of agglomerates.

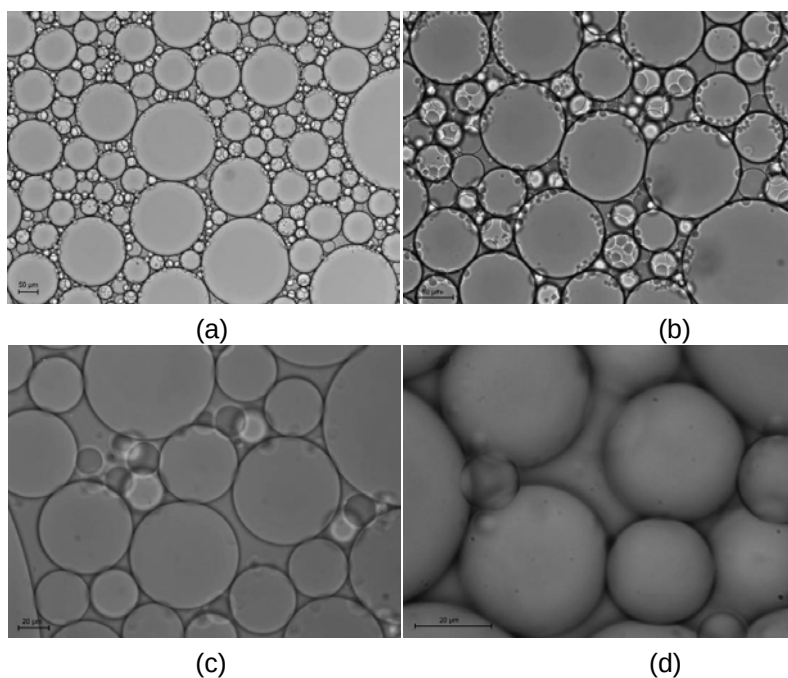


Figure 4.15. Optical microscopy of microcapsules solution after the production and without washing. Magnifications of images: a) 100x; b) 200x ; c) 400x; d) 1000x.

The wall thickness of microcapsule was additionally estimated using equation (4.2), to confirm the obtained value by microscopy.

$$d_s = (r_m - r_c) = \left[\left(\frac{w_s}{w_c} + 1 \right)^{1/3} - 1 \right] r_c \quad (4.2)$$

According to Ghosh (Ghosh, 2006), this equation represents the relationship between the wall thickness ($d_s = r_m - r_c = 5.24 \mu\text{m}$) where r_m is the outer radius of the microcapsule, r_c is the radius of the inner core and (w_s / w_c) is the ratio between shell material weight ($w_s = 0.10074 \text{ g}$) and core material weight ($w_c = 0.1832 \text{ g}$). The relationship between the wall thickness and the capsule diameter is linear when the ratio of $w_c / (w_s + w_c)$ is in the range of 0.50 to 0.95. A simplification was introduced assuming that the density of the core material (ρ_c) and wall material (ρ_s) were not significantly different ($\rho_c \approx \rho_s$). The obtained value was $5 \mu\text{m}$ which confirms the experimentally measured value.

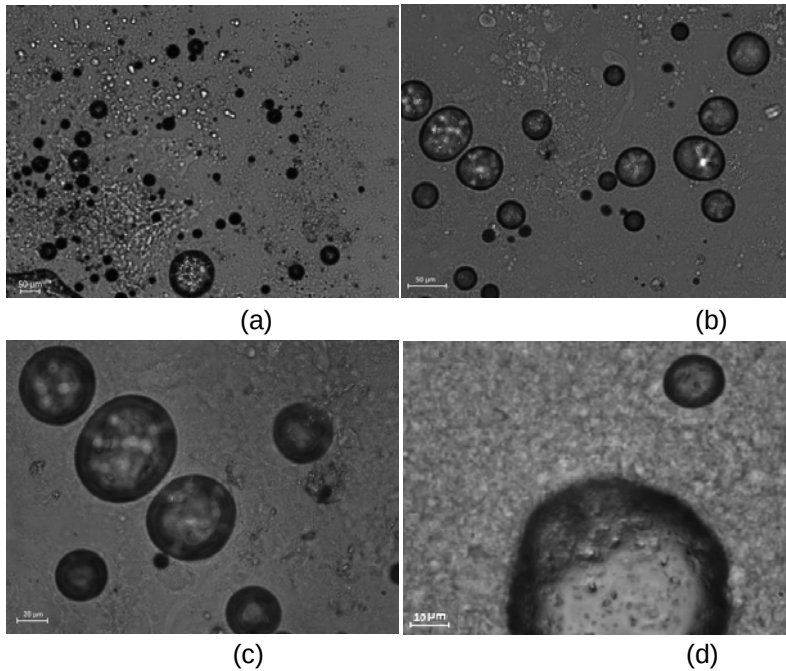


Figure 4.16. Optical microscopy of microcapsules after washing and freeze drying. Magnifications of images: a) 100x; b) 200x ; c) 400x; d) 1000x.

Unlike other microscopy methods Cryo-SEM offers the advantage of being able to promote an extremely rapid freezing of the analysed samples, which preserves the original structure of microcapsules. As a result Cryo-SEM is a powerful tool to study microcapsules structure and formation. Figure 4.17 shows the results obtained with the produced PLA microcapsules that confirmed the rough surface of microcapsules with some visible pinholes, cracks and pores.

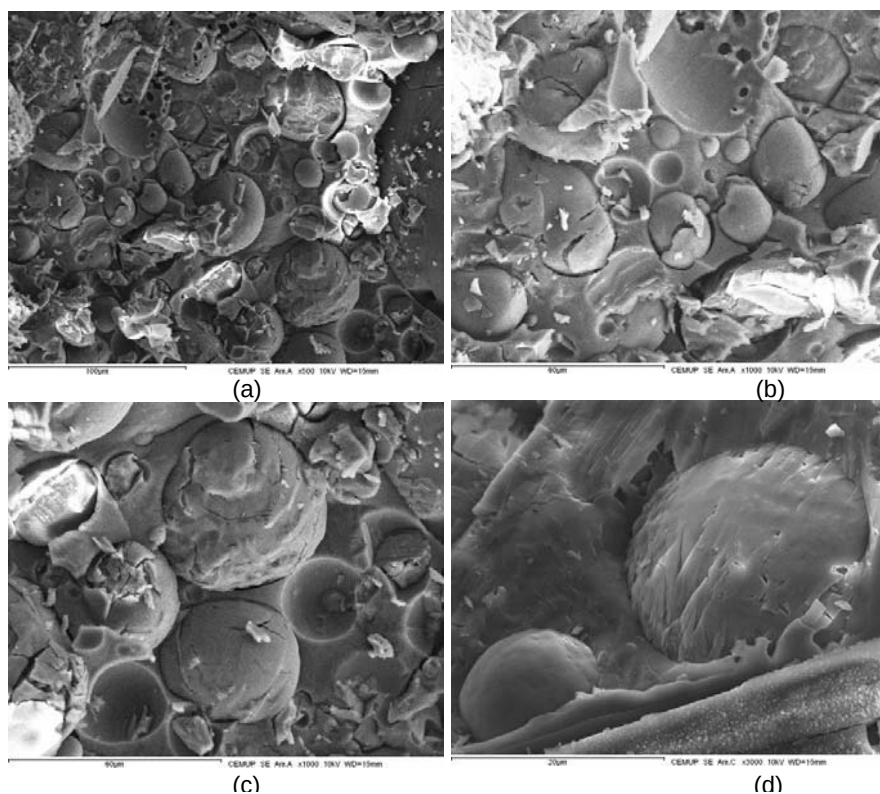


Figure 4.17. Cryo- SEM images of PLA microcapsules at different magnifications: (a) 500x, (b) and (c) 1000x and (d) 3000x.

4.3.2.3 Gas chromatography GC-FID/MS

The GC/MS analysis provided the separation and identification of thyme oil components. The oil composition was expressed in percentage values calculated directly from GC peak areas without the use of correction factors and in a solvent free basis. The component identification was made by comparison of the obtained mass spectra with some available reference spectra using NIST98 spectral library, pure reference compounds (own laboratory library) and literature data. The composition of the essential oil was determined as the average of three GC replicas.

The main composition of thyme oil is shown in Table 4.3. Phenols account for 54.6% of the essential oil, with a majority of thymol (47.7%). As can be seen from the chromatogram shown in Figure 4.18, thyme oil was characterized by a high percentage of the monoterpene phenols (mainly thymol) and derivatives (carvacrol). Thyme oil is also characterized by lower levels of monoterpene (terpinene and cymene amounting 37.8%) and oxygenated monoterpenes (7.6% linalool).

Table 4.3 Composition of the essential oil from *Thymus vulgaris* L. (thyme oil).

#	Main Components	Retention time (min)	Content* (%)
1	γ - Terpinene	11.5	6.2
2	p - Cymene	11.8	31.6
3	Linalool	18.6	7.6
4	Thymol	32.1	47.7
5	Carvacrol	32.6	6.9

* Calculated directly from GC peaks areas without use of correction factors.

Quantification of the nonencapsulated thyme oil was calculated based on GC-FID analysis and the mass of encapsulated thyme oil calculated using the mass balance.

The encapsulation efficiency (percentage of oil present in microcapsules) was obtained based on the individual thyme oil components peak analysis and uses the average of five injections.

Table 4.4 shows total, encapsulated and nonencapsulated masses for each thyme oil component. The encapsulation efficiency accounts for 30.5% of the loaded oil used in the encapsulation process. Table 4.4 shows that the apolar compounds, terpinene and cymene, have a higher percentage of encapsulation with values of 47.9% and 50.1%, respectively. On the other hand, the component with smaller percentage of encapsulation was carvacrol with 16.9%. Table 4.4 can also confirm that the amount of nonencapsulated mass was higher than the encapsulated.

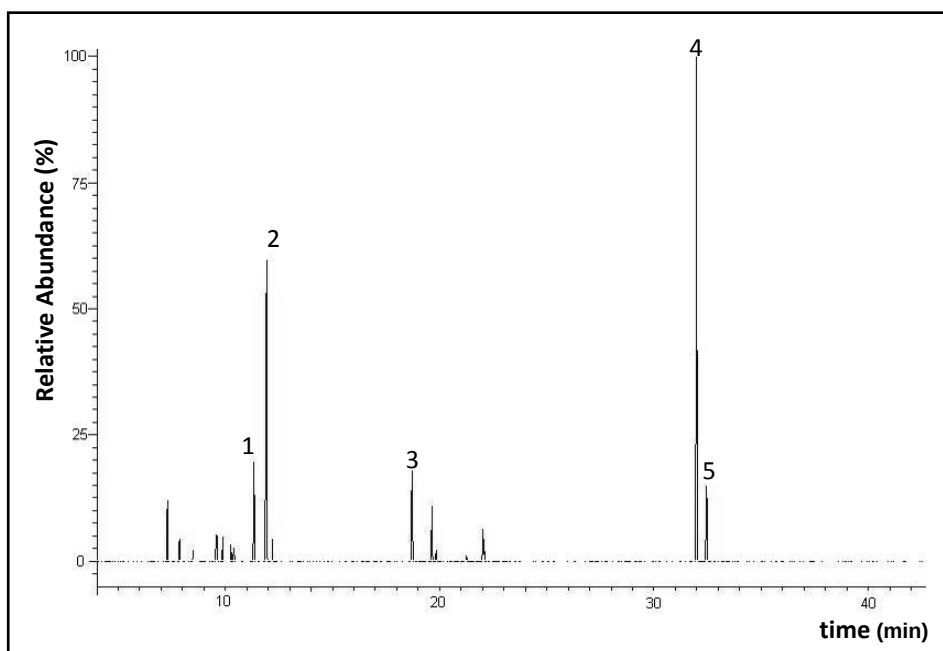


Figure 4.18. GC/MS chromatogram of thyme oil analysed on CP-Wax 52 CB bonded fused silica polar column. Identification numbers are according to table 1.

Table 4.4. Total, encapsulated and nonencapsulated masses discriminated by thyme oil component.

Component	mass _{total}	mass _{nonencapsulated} *	mass _{encapsulated} **	Encapsulation Efficiency (%)
	(g)	(g)	(g)	(mean $\pm$ SD)***
γ - Terpinene	0.155	0.081	0.074	47.91 $\pm$ 0.03
<i>p</i> - Cymene	0.781	0.390	0.391	50.08 $\pm$ 0.17
Linalool	0.189	0.134	0.055	28.95 $\pm$ 0.07
Thymol	1.179	0.973	0.206	17.43 $\pm$ 0.04
Carvacrol	0.170	0.141	0.029	16.84 $\pm$ 0.03
Total	2.474	1.719	0.755	30.49 $\pm$ 0.07

* - calculated based on GC-FID peak area

** - obtained by difference between mass_{total} and mass_{nonencapsulated}

*** - n = 5

The chromatograms obtained in the analysis of the two phases are shown in Figure 4.19 and allow inspecting the quality of the encapsulated oil. The outline of GC/FID spectra is corroborated by the analysis of the quantity of encapsulated and the non-encapsulated oil. The encapsulation efficiency can be related to the chosen type of surfactant used. Tween[®]20 (polyoxyethylene sorbitan monolaurate) is a non-ionic surfactant with a high value of hydrophil/lipophilic balance (HLB) (HLB of 16.7) widely used in biochemical applications. Since it contains no electrical charge, it is resistant to water hardness deactivation, thus enhancing the emulsion stability. The high HLB value of Tween[®]20 can favour the protection of the apolar compounds and thus favouring their preferential encapsulation. The hydrocarbon chain of the surfactant is drawn into the apolar components of the oil protecting them for encapsulation. Due to the high affinity of polar components with water part of them will not be protected thus will not be encapsulated.

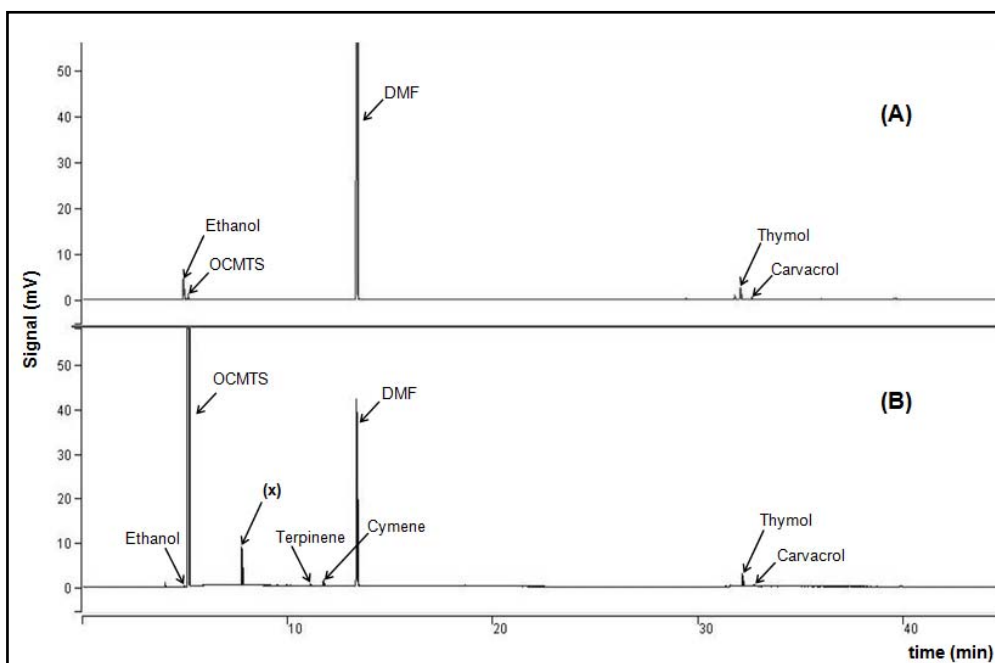


Figure 4.19. GC/FID chromatograms: aqueous phase (phase without microcapsules) (A); microcapsules surrounding phase (B). Peak (x) not identified (unknown compound).

4.3.3 Conclusions

PLA has been widely used in microencapsulation using coacervation processes with other active principles, such as hydrosoluble drugs, but not with oils. In this work a novel coacervation process to encapsulate oily principles with PLA was developed. Microcapsules of thyme oil have been produced by coacervation using PLA dissolved in DMF. This organic solvent is a good solvent for PLA and presents high solubility in water thus acts as a carrier to put PLA in contact with water thus promoting its precipitation around the thyme oil core. The hardening of the produced microcapsules was obtained with OCMTS.

Microcapsules particle size distributions were determined by laser dispersion. It was observed a bimodal distribution in volume with a mean particle size of 40 μm . Analysis by optical microscopy and by cryogenic scanning electron microscopy have confirmed the spherical shape, the rough surface with some visible pinholes, cracks or pores, and allowed to estimate the wall thickness around 5 μm . Moreover, it was observed two predominant sizes of microcapsules, compatible with a bimodal distribution and the absence of agglomerates.

Quantification of the encapsulated thyme oil was calculated based on GC-FID peak areas and the mass of encapsulated thyme oil has been calculated using a mass balance. The total percentage of phenols was 54.6%, with a major percentage of thymol (47.7%). The quality analysis of the encapsulated oil has shown that apolar compounds of thyme oil were preferentially encapsulated in detriment of the polar ones. The overall encapsulation efficiency of thyme oil was of 30.5%.

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CHAPTER 5:

PLA-based thyme oil microcapsules production: evaluation of surfactants

“Faith is taking the first step even when you don't see the whole staircase .”

[Martin Luther King, Jr.]

The objective of chapter five is to evaluate the effect of nonionic surfactants, comprising different hydrophilic-lipophilic balance (HLB) values, on thyme oil encapsulation efficiency, namely by considering polar and apolar compounds. Thus, Tween® 20, Tween® 80, Tergitol™ 15-S-9 and a combination of Tergitol™ 15-S-9 with Span® 85 have been used that comprise a HLB range between 11 and 16.5. For all the studied cases, microcapsules are spherical in shape and have bimodal particle size distribution with mean size between 30 and 40 µm. The amount of encapsulated thyme oil reaches a maximum of 65% for Tergitol™ 15-S-9 a polyglycol ether surfactant with a HLB value of 13.3. The results confirm the dependence of the encapsulation efficiency as result of the hydrophobic properties of the surfactants. Moreover, it was also confirmed a preferential encapsulation of thyme oil apolar compounds in detriment of polar ones.

This chapter is based on the following publication:

Martins, I. M.; Rodrigues, S. N.; Barreiro, M. F.; Rodrigues, A. E. (2010). Polylactide-based thyme oil microcapsules production: Evaluation of surfactants. *Industrial & Engineering Chemistry Research* 50(2): 898-904.

5.1. Introduction

The microencapsulation by coacervation is a widespread method, being investigated by numerous authors for a long time (Ferres *et al.*, 1999; Jong *et al.*, 1930; Lumsdon *et al.*, 2005; Magdassi *et al.*, 1996; Whitaker *et al.*, 1991). This technique offers many possibilities for the encapsulation of various types of active agents and it can be described as basically involving the separation of an aqueous colloidal solution into two liquid phases, one rich in the colloid (coacervate) and the other poor in the colloid. The general outline of the process consists of three steps that occur under continuous stirring: (1) formation of an oil-in-water (o/w) emulsion, (2) coating of the formed droplets and (3) stabilization of the coating to attain self sustainable microcapsules (Martins *et al.*, 2009).

The formation of microcapsules (size, shape and stability) is greatly affected by the conditions used in the o/w emulsion preparation, being particularly relevant the used surfactant (Guo *et al.*, 2005; Mohamed *et al.*, 2006; Yuan *et al.*, 2009). Surfactants play two main roles: one is to reduce interfacial tension between oil and aqueous phases allowing the formation of small droplets and the other is to prevent coalescence. The surfactant molecules will be positioned on the o/w interface forming a protective layer around the oil droplets (Magdassi *et al.*, 1996; Salaun *et al.*, 2009). These substances have an amphiphatic structure, i.e., they combine a long alkyl group, which is hydrophobic with a polar group (sometimes an ionic group), which is highly hydrophilic (Goddard, 1999). Mixing surfactants could be more efficient than use single ones and the enhanced stability is attributed to the formation of intermolecular complexes. During the encapsulation process of oils by coacervation the core material is readily covered by coacervates being the stability of the former emulsion improved by the use of surfactants (Ghosh, 2006 ; Katona *et al.*, 2010; Mayya *et al.*, 2003; Orafidiya *et al.*, 2002).

Thymus vulgaris, an herbaceous plant native from southern Europe, has been consumed for innumerable generations as culinary herbs or used for medicinal purposes. It is well recognized that the extracted essential oil, Thyme oil, has numerous therapeutic properties, such as, antirheumatic, antiseptic, antispasmodic, bactericidal, cicatrisant, diuretic, expectorant, insecticide, stimulant, tonic and vermifuge. Due to these attractive properties it is widely used in the flavour and food industries. Essential oils are in general complex mixtures with components susceptible to volatilization and/or oxidation. Moreover, they can't be used in its concentrated form since some of its components can irritate mucus membranes and cause skin irritation (Badi *et al.*, 2004; Hulzebos *et al.*, 2003; Sipailiene, 2006). Microencapsulation can help to overcome these constraints providing a way to protect the oil from evaporation and oxidation thus preserving its integrity. Additionally it enables a controlled release rate and could even help to mask its strong taste or smell.

In the previous chapter a novel coacervation process to produce polylactide (PLA - a biodegradable polymer which can be hydrolyzed in the body to form products that are easily reabsorbed or eliminated) microcapsules to encapsulate thyme oil (Martins *et al.*, 2009), has been reported. In this microencapsulation process the objective consists on the encapsulation of an oily active principle. Once the core material is a water insoluble active agent, an oil-in-water emulsion was prepared using a non-ionic surfactant. With this work we intend to improve the encapsulation efficiency of the microencapsulation process trying to enhance the stability of oil droplets by testing different nonionic surfactants.

According to Griffin (Griffin, 1949), surfactants can be identified from the empirical concept of HLB (hydrophilic-lipophilic balance) where hydrophilic refers to the fraction of the surfactant that is soluble in the aqueous phase and lipophilic refers to the oil soluble fraction of the surfactant. HLB scale values are in the range 1-20, and surfactants with low HLB value are appropriate to stabilize w/o emulsions whereas the ones with high HLB value will form o/w stable emulsions

(Magdassi *et al.*, 1996; Zhang *et al.*, 2008). The HLB value can be calculated by using the formula 5.1,

$$HLB = 20 \left(1 - \frac{S}{A} \right) \quad (5.1)$$

where, S is the saponification number of the ester (number of milligrams of potassium hydroxide required to saponify one gram of a given ester) and A is the acid number of the resulting acid (mass of potassium hydroxide in milligrams that is required to neutralize one gram of acid). This formula is satisfactory for non-ionic surfactants of various types. Nevertheless, non-ionic surfactants containing other components such as propylene oxide, butylene oxide, nitrogen or sulphur, exhibit a behavior which has not been related to their composition. For this kind of products, an experimental method must be used.

For surfactant mixtures, the final HLB value can be estimated considering that HLB has additive properties (Equation 5.2).

$$HLB = HLB_i \times f_i \quad (5.2)$$

where, f_i is the weight fraction of the surfactant i (Griffin, 1949; Pasquali *et al.*, 2008; Zhang *et al.*, 2008).

Nonionic surfactants, such as Tween®, Tergitol™ and Span® are widely used in pharmaceutical formulations as a result of its solubilization properties, reduction of surface and interfacial tension or wetting. These surfactants are used typically as emulsifying agents in the preparation of stable o/w pharmaceutical emulsions. For instance, they are used in the preparation of creams, emulsions and ointments for topical application. They may also be used as solubilizing agents for essential oils and oil-soluble vitamins, and as wetting agents in the formulation of oral and parenteral suspensions. Tween® and Span® are even further used in cosmetics and food products. Tween is a register trade mark of ICI Americas, Inc. Tween® 20 is a commercial name of polysorbate 20 and is a polysorbate surfactant. In the nomenclature of polysorbates, the numeric name following polysorbate refers to the lipophilic group. It is a polyoxyethylene sorbitol ester derivative of sorbitan

monolaurate and is distinguished from the other members in the Tween range by the length of the polyoxyethylene chain and the fatty acid ester moiety. Tween® 80 is a commercial name of polysorbate 80 is an emulsifier derived from polyethoxylated sorbitan and oleic acid, and is often used in foods. Polysorbate 80 is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers of ethylene oxide. Tergitol™ is trademark of The Dow Chemical Company (Dow). Tergitol™ 15-S-9, polyglycol ether, is a mixture of linear secondary alcohols reacted with ethylene oxide. Tergitol™ is a biodegradable nonionic surfactant, soluble in water and most polar organic solvents. Span® 85, sorbitan trioleate, is the triester of oleic acid and sorbitol and its monoanhydrides and dianhydrides. This surfactant is mainly used in medicine, cosmetics, textiles and paints as emulsifier and thickening agent. Span® is a registered trademark of Croda International PLC.

The aim of this chapter is to study the effect of different surfactants with hydrophilic-lipophilic balance (HLB) values from 11 to 16.5 on the microencapsulation process and evaluate the encapsulation efficiency of polar and apolar compounds of thyme oil. The required HLB for thyme oil encapsulation should correspond to the HLB value of the surfactant that provides the lowest interfacial tension between the oil and water phases. Different emulsions with thyme oil were prepared using the surfactants Tween® 20, Tween® 80, Tergitol™ 15-S-9 and a combination of Tergitol™ 15-S-9 with Span® 85. The criterion for the surfactant selection to prepare the oil/water emulsion takes into account the general correlation presented in literature which shows that for this type of emulsions the HLB values must be within the range from 8 to 18 (Atlas, 1973). This study was performed trying to cover the range typically recommended in the literature. Since essential oils are complex mixtures of components (both polar and apolar) the use of surfactant combinations might be more effective than using a single one; it adds complementary properties of both surfactants resulting in

intermediate HLB values. Microcapsules size, morphology and encapsulation efficiency were studied as a function of the used surfactant.

5.2. Materials and methods

5.2.1 Materials

The used reagents for the preparation of polylactide microcapsules were: Poly(DL-lactide) (PLA, Mw=75,000-120,000, inherent viscosity=0.55-0.75 dL/g) as the wall-forming material; dimethylformamide (DMF, 99.8% ACS grade) as the PLA solvent; essential oil of *Thymus vulgaris* L. (thyme oil, red, Kosher) as the core material; Tween®20, Tween® 80, Tergitol™ 15-S-9 and a combination of Tergitol™ 15-S-9 with Span® 85 as the nonionic surfactants used to stabilize the o/w emulsion; Pluronic® F68 is a surfactant used to keep the microcapsules solution stable during the washing process. Figure 5.1 shows the chemical structure of the used surfactants. All these reagents were obtained from Sigma Chemical Company (Germany). Octamethylcyclotetrasiloxane (OCMTS) was used as hardening agent and n-hexane (ACS grade) was used as the washing solvent. These reagents were purchased from Merck Schuchardt OHG (Germany).

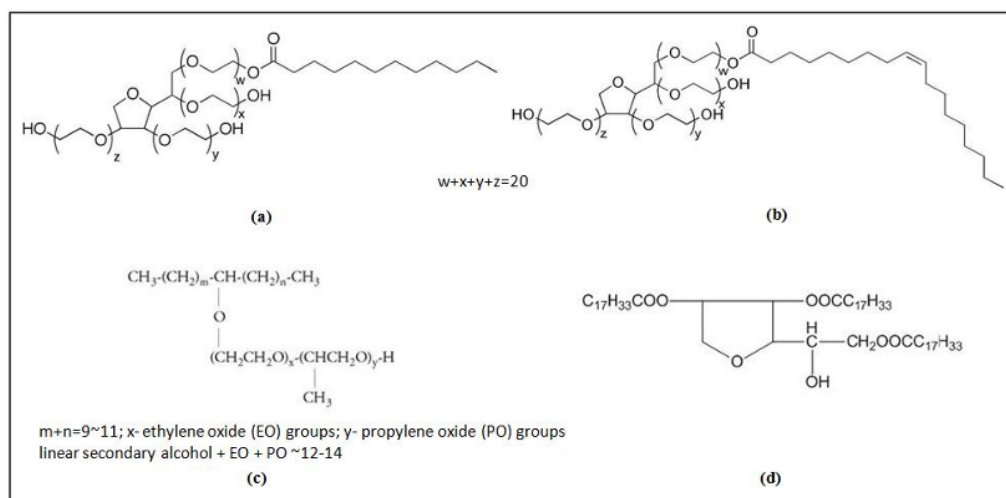


Figure 5.1. Chemical structure of: (a) Tween® 20 (HLB=16.5); (b) Tween® 80 (HLB=15.0), (c)Tergitol™ (HLB=13.3) and (d)Span® 85(HLB=1.8).

5.2.2 Microcapsules preparation

Microcapsules of PLA containing thyme oil were prepared according to the procedure developed in the previous chapter and reported in Martins *et al.* (Martins *et al.*, 2009). The corresponding procedure is summarized schematically in Figure 5.2. Firstly, the o/w emulsion was obtained by dispersing a chosen amount of thyme oil in water with a nonionic surfactant using an ultraturrax (IKA DI 25 Basic) at 11,000 rpm during 90 seconds (Step I – emulsification). Thereafter, the emulsion was transferred to a batch reactor (IKA Model LR-2.ST) and the PLA in DMF solution was added dropwise to the previously prepared thyme oil emulsion. Upon contact with water PLA precipitated around the thyme oil core (step II - coating core material). The encapsulation process continued under stirring for one hour at room temperature. The microcapsules formed were hardened by adding a hardening agent, OCMTS, and allowed to stand during one hour (step III – hardening). After hardening, the microcapsules were decanted and sequentially washed with Pluronic® F68 solution, an ethanol solution and finally hexane. The procedure was repeated with different types of surfactants/surfactant mixtures added at the stage I – emulsification, as described in Table 5.1. The HLB value of the surfactants used was comprised between 11 and 16.5 since we intend to stabilize an o/w emulsion.

5.2.3 Characterization techniques

Size distribution of microcapsules (Laser Dispersion)

The particle size distribution of the produced microcapsules was analyzed by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman-Coulter). The corresponding medium values in volume and number were determined.

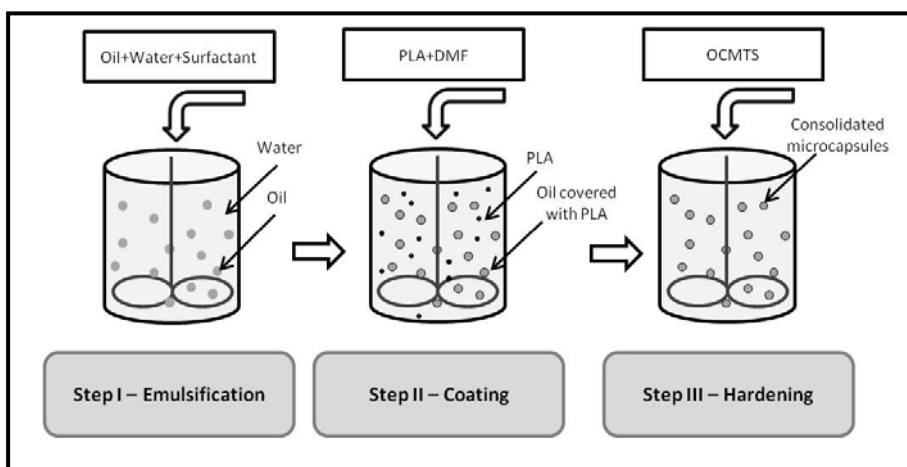


Figure 5.2. General process scheme for the preparation of thyme oil microcapsules with biodegradable polymer - PLA.

Optical microscopy

Microcapsules in solution form and after freeze-dried were analysed by optical microscopy using a Leica DM 2000 microscope equipped with software Leica Application Suite Interactive measurement and with transmitted light mode.

Gas chromatography GC-FID/MS

Quantification of the encapsulated thyme oil was performed by gas chromatography GC/FID. The analyses were carried out using a Varian CP-3800 instrument equipped with split/splitless injector, two CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 μ m film thickness) and a Varian FID detector controlled by the Saturn 2000 WS software. The oven temperature was isothermal at 50°C for 2 min, then increased from 50°C up to 200°C at 5°C/min and held at 200°C for 13 min. The injectors were set at 240°C, with a split ratio of 1:50 for FID and 1/200 for MS. The FID detector was maintained at 250°C. The sample volume injected was 0.1 μ L. The carrier gas was helium He N60, at a constant flow rate of 1 mL/min.

In order to analyze the influence of the used surfactant in the thyme oil encapsulation efficiency, this parameter (encapsulation efficiency - percentage of thyme oil present in PLA microcapsules) was calculated based on the methodology described previously in chapter 4, equation 4.1 (*Martins et al., 2009*) .

$$\text{Encapsulation Efficiency (\%)} = \frac{m_{\text{total}} - m_{\text{out}}}{m_{\text{total}}} \times 100 \quad (4.1)$$

where m_{total} = amount of loaded essential oil (g) and m_{out} = amount of nonencapsulated essential oil (g).

The individual components that characterize the nonencapsulated thyme oil were quantified by analysing the two phases obtained after microcapsules separation by decantation (aqueous phase and microcapsules rich phase). One millilitre of the aqueous phase and one ml of the microcapsules surrounding solution were collected using a syringe equipped with a 0.45 μm pore size filter and thereafter analysed by GC-FID. The mass of encapsulated thyme oil has been calculated using a mass balance. This mass was obtained by difference between the loaded original quantity and the nonencapsulated determined quantity.

5.3. Results and discussion

5.3.1. Particle size distribution (Laser dispersion)

Figure 5.3 shows the experimentally measured particle size distributions, both in volume and in number, for PLA microcapsules prepared with four different kinds of surfactants (Tween[®] 20, Tween[®] 80, Tergitol[™] 15-S-9 and the mixture 80 %Tergitol[™] 15-S-9 + 20% Span[®] 85). Table 5.1 shows the obtained microcapsule mean particle size as a function of the used surfactant.

Table 5.1. HLB values of surfactants and surfactant mixtures, mean particle size in volume of microcapsules and microcapsules wall thickness for each type of surfactants.

Surfactant System	%	HLB value	Particle size (μm)	
			(mean $\pm$ SD)	Wall thickness (μm)
Tween® 20	100	16.5 ^a	42.24 $\pm$ 17.94	3.06
Tween® 80	100	15.5 ^a	32.94 $\pm$ 16.96	2.30
Tergitol™ 15-S-9	100	13.3 ^b	29.30 $\pm$ 18.37	2.01
Tergitol™ 15-S-9 + Span® 85	80+20	11.0 ^c	32.52 $\pm$ 17.24	2.34

^afrom Ponzetto et al (Ponzetto, 2003)

^bfrom Dow (Unioncarbide, 2001)

^cobtained by calculation

HLB value for Span® 85 = 1.8 (Rabiskova *et al.*, 1998)

A mean particle size of 40 μm in volume was obtained with Tween® 20 while the value of 30 μm was obtained with the other used surfactants/mixtures (Tween® 80, Tergitol™ 15-S-9 and the mixture 80 %Tergitol™ 15-S-9 + 20% Span® 85). The distributions in volume for all the studied formulations have showed a similar distribution pattern, i.e., a bimodal distribution and pointed out that the use of Tergitol™ 15-S-9 generates smaller particles. The corresponding distributions in number were quite narrow and unimodal in shape. For the mixture 80% Tergitol™ 15-S-9 + 20% Span® 85 it can be observed that the curve shifts to the left showing an increase, in number, of smaller microcapsules. It is predicted that this mixture of surfactants has stabilized oil droplets with a lower resistance to the stirring conditions imposed by the ultraturrax (lower surface tension between oil and water, which favors the emulsion dispersion) and consequently smaller droplets were obtained.

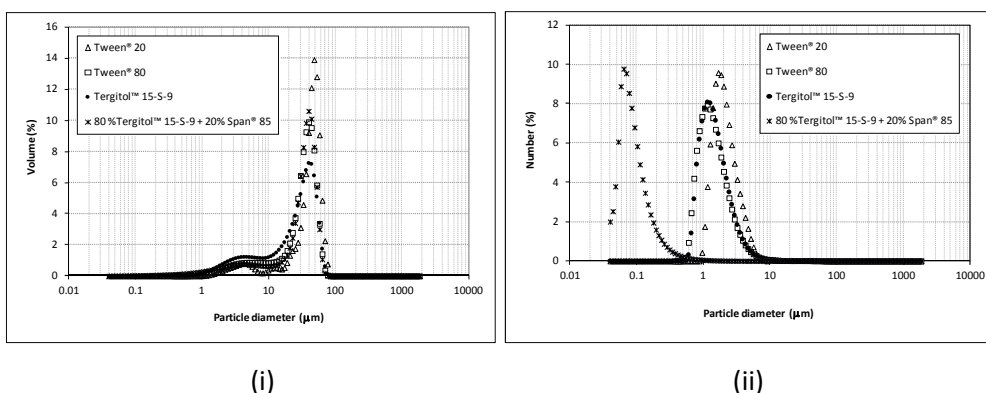


Figure 5.3. Particle size distribution of polylactide microcapsules with thyme oil for different surfactant systems and after washing the microcapsules. Distribution in volume (i) and in number (ii).

5.3.2 Optical microscopy

Optical microscopy images of the microcapsules are shown in Figure 5.4. The pictures have been taken at different magnifications and immediately after the microcapsules production, without washing. All figures show that the droplets of thyme oil have been individually encapsulated as spherical particles with size distribution consistent with a bimodal distribution, and one can notice also the absence of agglomerates. The wall thickness of microcapsule was estimate using equation (4.2) and the obtained value confirmed by microscopy.

By optical microscopy, an optimized image of the microcapsules morphology was firstly obtained using exposure adjustments. Through dark field option it was possible to distinguish the polymer membrane around the oil core by colour difference. Thereafter measurement annotation tools were added to images allowing microcapsules wall thickness estimation around 2-3μm. Figure 5.4 shows the observed microcapsules solution using the dark field option with a formulation using Tergitol™ 15-S-9 as surfactant. In these images it was observed the thyme oil core entrapped in a PLA shell of a fairly constant thickness.

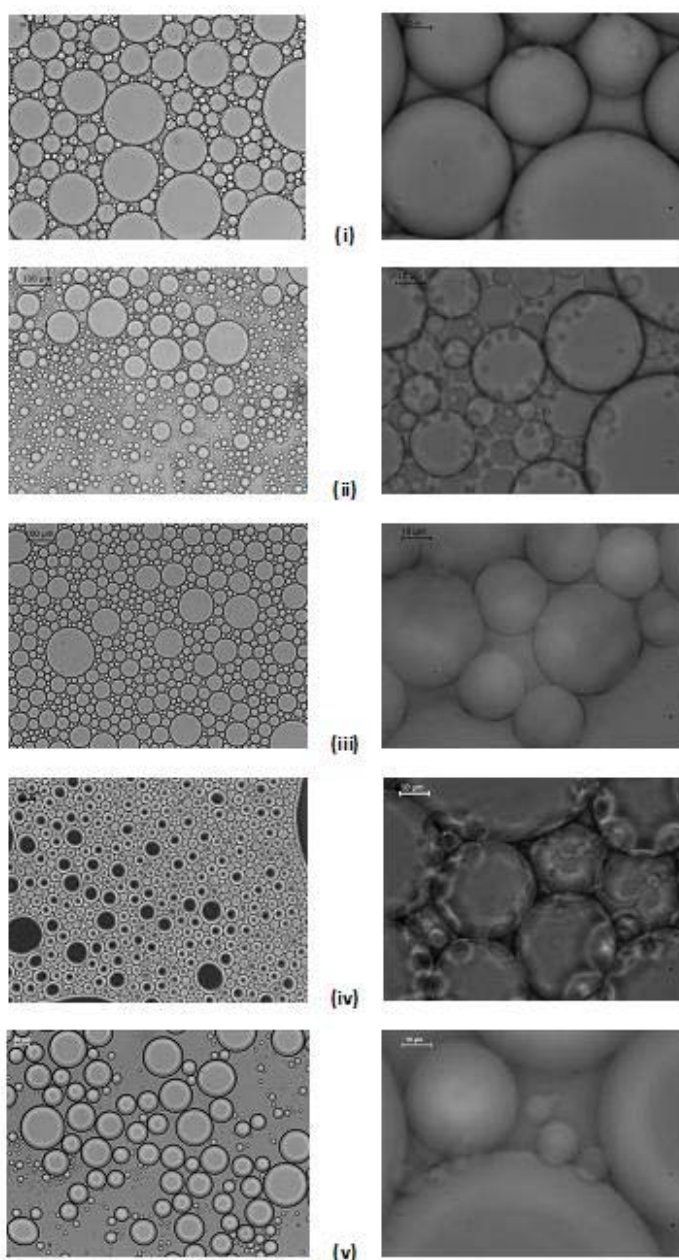


Figure 5.4. Optical microscopy of microcapsules solution after the production and without washing using: (i) Tween® 20; (ii) Tween® 80; (iii) Tergitol™ 15-S-9; (iv) Tergitol™ 15-S-9 (in dark field option) and (v) 80 %Tergitol™ 15-S-9 + 20% Span® 85 as surfactants. Magnification of images: 100x (on the left) and 1000x (on the right).

Therefore, the wall thickness values obtained using equation (4.2) are in good agreement with those obtained using the Leica software tools. Table 5.1 shows microcapsules wall thickness for all the used types of surfactants (Tween[®] 20, Tween[®] 80, Tergitol[™] 15-S-9 and the mixture 80 %Tergitol[™] 15-S-9 + 20% Span[®] 85) as determined by equation (4.2).

5.3.3. Gas chromatography GC-FID/MS

Since thyme oil includes several compounds with polar groups (thymol, carvacrol and linalool representing, approximately, 62.2% of the total thyme oil) it can present to some extent a “water loving” or polar character. This solubility in water is reported as negligible, i.e., thyme oil has a more prominent lipophilic – “oil loving” or non-polar character, so the recommended surfactant must have a medium HLB number (in the range 8-18) (Hlb System, 1973). Figure 5.5 shows the effect of using different surfactant systems with HLB values from 11 to 16.5 on the encapsulation efficiency of thyme oil. It was observed that when surfactants with HLB values higher than 15.0 (Tween[®] 20 and Tween[®] 80) were used, the amount of encapsulated thyme oil was low and around 30-40%. The larger is the hydrophobic chain of surfactant the lower is the surface tension at the o/w interface and consequently it becomes easier to form the emulsion. Nevertheless, thyme oil presents both polar and apolar compounds so these properties does not favor the efficiency of encapsulation. On the other hand, a significant increase of the oil content in the microcapsules, around 65%, was found when Tergitol[™] 15-S-9 with HLB value of 13.3 was used. Tergitol[™] 15-S-9 is a mixture of linear secondary alcohols reacted with ethylene oxide; its features include formation of gels over a narrow concentration range, rapid dissolution even in cold water, fast foam collapse rates and compatibility with a wide range of solvents. These characteristics favor the encapsulation of both compounds of thyme oil. It could be concluded that when using Tergitol 15-S-9 as surfactant in oil/water emulsion preparation smaller and more stable oil droplets are obtained.

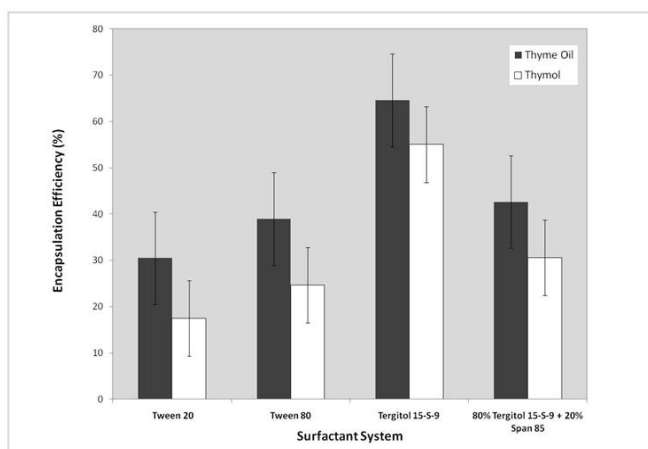


Figure 5.5. Percentage of encapsulation efficiency for total thyme oil and thymol using different surfactant systems.

According to *Capan et al* (*Capan et al., 1999*) poor emulsion stability leads to lower encapsulation efficiency values being this effect allied to surface interfacial behaviour (*Mohamed et al., 2006*).

The percentage of encapsulated thyme oil decreased to values around 40%, when using the combination of surfactants containing 80 %Tergitol™ 15-S-9 + 20% Span® 85. Within the context of the performed study it can be deduced that the mixture of surfactants used was not efficient as the single surfactants tested. This mixture is obtained through surfactants with quite different HLB values and chemical nature and consequently can influence negatively the interfacial tension in o/w emulsion decreasing the encapsulation efficiency.

The system using Tergitol™ 15-S-9 as surfactant gives rise to the higher encapsulation efficiency value. This result confirms that total encapsulation efficiency depends on the individual encapsulation of polar and apolar components of thyme oil that contribute with different amounts for the global value. Thus, if we consider only the systems using pure surfactants an increase of efficiency with the HLB decrease is observed. Nevertheless, when using the

mixture combining two surfactants of different HLB (80 %Tergitol™ 15-S-9 + 20% Span® 85) a clear cut off tendency is observed.

From Figure 5.6 we can notice that the apolar compounds of thyme oil were preferentially encapsulated in detriment of the polar ones for all surfactant systems studied. With Tergitol™ 15-S-9 it was obtained 80% of encapsulation for the apolar compounds while for the polar compounds only 54% was achieved. Figure 6 also shows the encapsulation efficiency ratio of polar *versus* apolar compounds. It was observed that for the surfactants Tergitol 15-S-9 (HLB=13.3) and Tween® 80 (HLB=15.0) the determined ratio was similar (around 0.7).

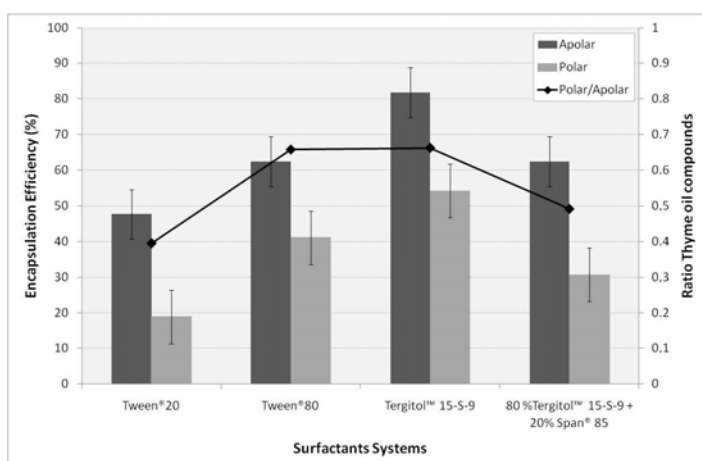


Figure 5.6. Values of encapsulation efficiency of apolar and polar compounds of thyme oil and encapsulation efficiency ratio apolar/polar for all surfactant system.

Furthermore, table 5.2 shows the total encapsulated and nonencapsulated masses for each thyme oil component using Tergitol™ 15-S-9. The encapsulation efficiency (percentage of thyme oil present in microcapsules) accounts for 65% of the loaded oil used in the encapsulation process and the encapsulated percentage of thyme oil apolar compounds around 80%. The increased polarity affords stronger oil-polymer interactions, thereby improving thyme oil encapsulation. These results confirm the dependence of thyme oil encapsulation with the HLB

value of surfactant; they show that encapsulation efficiency might have a large range value depending of the type of surfactant used on microencapsulation process as was previously confirmed by other authors (Mayya *et al.*, 2003; Mohamed *et al.*, 2006).

Table 5.2. Total, encapsulated and nonencapsulated masses discriminated by thyme oil component using Tergitol™ 15-S-9 as surfactant.

Components	mass _{total} (g)	mass _{nonencapsulated} [*] (g)	mass _{encapsulated} ^{**} (g)	Encapsulation Efficiency (%) (mean + SD) ^{***}
γ - Terpinene	0,011	0,003	0,009	75,13 ± 0,01
<i>p</i> - Cymene	0,058	0,010	0,049	83,08 ± 0,00
Linalool	0,014	0,007	0,007	49,38 ± 0,00
Thymol	0,088	0,040	0,049	55,04 ± 0,01
Carvacrol	0,013	0,006	0,007	53,61 ± 0,00
Apolar ^a	0,070	0,013	0,057	81,78 ± 0,01
Polar ^b	0,115	0,053	0,062	54,19 ± 0,01
Total	0,185	0,065	0,119	64,62 ± 0,02

* - calculated based on GC-FID peak area

** - obtained by difference between mass_{total} and mass_{nonencapsulated}

*** - n = 5

a – Apolar components of thyme oil: γ – Terpinene and *p* - Cymene

b – Polar components of thyme oil: Linalool, Thymol and Carvacrol

Since apolar compounds of thyme oil are preferentially encapsulated it means that the polar ones are not so protected within the capsule remaining in the microcapsules surrounding phase. Taking into account that thymol is the compound with higher percentage in thyme oil composition (47.7%) it means that it will be the more detected in the final application.

5.4. Conclusions

In this chapter the effect of using different surfactants systems in particle size distribution, morphology and yield of encapsulation of thyme oil in PLA microcapsules was investigated. Microcapsules particle size measured by laser dispersion have shown a bimodal distribution in volume with mean particle size around 40 μm for Tween® 20 and around 30 μm for Tween® 80, Tergitol™ 15-S-9

and for the mixture 80 %Tergitol™ 15-S-9 + 20% Span® 85. Analysis by optical microscopy confirmed the spherical morphology for all microcapsules produced and the existence of two predominant sizes, compatible with a bimodal distribution.

Quantification of the encapsulated oil was calculated based on GC-FID peak area. The apolar compounds of thyme oil were preferentially encapsulated in detriment of the polar ones and the encapsulation efficiency of thyme oil was higher when using Tergitol™ 15-S-9 (around 65%).

In conclusion, this work shows that the type of used surfactant can influence the yield of encapsulation of thyme oil in PLA microcapsules prepared by coacervation.

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CHAPTER 6:

Release of thyme oil from polylactide microcapsules

“The miracle is not that we do this work, but that we are happy to do it. “

[Mother Teresa in Calcutta]

Microencapsulation has numerous advantages over conventional applications of flavors or fragrances, being the release behaviour an issue of interest. Thus, in this chapter, thyme oil release rate through the polylactide (PLA) microcapsules wall was studied. The results showed that the release rate of thymol is faster in the first hour, remaining almost constant in the subsequent days. Moreover, it was observed that the release of the polar compounds of thyme oil is faster than the apolar ones. The diffusion coefficient in first hour of release was $1.39 \times 10^{-15} \text{ m}^2/\text{s}$ for thymol and $5.21 \times 10^{-17} \text{ m}^2/\text{s}$ for p-cymene. However, the diffusion is slower considering a period of 5 days, with diffusion coefficients of $3.81 \times 10^{-17} \text{ m}^2/\text{s}$ for thymol and $1.43 \times 10^{-18} \text{ m}^2/\text{s}$ for cymene. The diffusion of the thyme oil across the PLA membrane was dependent on the morphological characteristics of the microcapsules.

This chapter is based on the following publication:

Martins, I. M.; Rodrigues, S. N.; Barreiro, M. F.; Rodrigues, A. E. (2011). Release of thyme oil from polylactide microcapsules. *Industrial & Engineering Chemistry Research* 50(24): 13752-13761

6.1 Introduction

New product development is an essential goal for modern society and encapsulation has grown over the years (Costa *et al.*, 2006; Rodrigues *et al.*, 2009; Rodriguez Romero *et al.*, 2007; Sanchez-Silva *et al.*, 2010). With the variety of techniques available, many different types of liquid cores can be encapsulated (Yow *et al.*, 2006). Several flavours have been encapsulated, usually in solid matrices and often by spray drying; the most frequently used technique despite some limitations due to the relatively high temperature used, even though other delivery systems and encapsulation techniques, such as coacervation, are also commercially being used (Moretti *et al.*, 2002; Ubbink *et al.*, 2001). Many oils in food and flavour categories have special properties and thus it is necessary to encapsulate them in a core-shell membrane (Rodrigues *et al.*, 2008). The encapsulation of oils and flavours are very important to protect the volatilization of compounds from evaporation and to prevent oxidation during storage (Lumsdon *et al.*, 2005).

The incorporation of essential oils, perfumes, deodorants, moisturizers and other active agents in polymers for the purpose of controlled release over a certain period of time has been a question of considerable research in recent years (Calkin *et al.*, 1994; Costa *et al.*, 2008; Gumi *et al.*, 2009; Oliveira *et al.*, 2006; Peña *et al.*, 2009; Peppas *et al.*, 1996; Peppas *et al.*, 1997; Thies, 1996). In this work the core material used was thyme oil. This essential oil is extracted from an aromatic plant (*Thymus vulgaris* L.) of increasing economic importance in North America, Europe and North Africa, having an important and growing place in the world market. The essential oil of *Thymus vulgaris* L. is not only widely used in manufacture of perfumes and cosmetics but also in flavour and food industries. Thyme oil has many compounds in this constitution but the antimicrobial activity is mainly attributed to the presence of carvacrol, cinnamaldehyde, thymol,

geraniol and eugenol. As a pharmaceutical compound, thymol and carvacrol are used in mouthwashes, soaps and creams (Martins *et al.*, 2009). This essential oil has a complex mixture of components susceptible to volatilization and it can't be used in its concentrated form since some of its components can irritate mucus membranes and cause skin irritation. Therefore, microencapsulation by coacervation helps to overcome these constraints and additionally allows a controlled release rate and even help to mask its strong taste or smell (Martins *et al.*, 2010).

Controlled release technologies are used to deliver compounds such as drugs, pesticides, fragrances or flavours at prescribed rates, together with improved efficacy, safety and convenience (Romero-Cano *et al.*, 2002). Nowadays, core-shell microcapsules have been investigated extensively for utilization in a controlled release system, especially in drug delivery, where the polymeric wall acts as a permeable element that can determine the release behaviour of the core materials (Guo *et al.*, 2005). Polymeric wall properties, such as, thickness, flexibility and mobility, water-uptake and swelling behaviour, extent of plasticization, or interactions between polymer and active agent will affect the diffusion rates, and therefore, the oil release behaviour (Wischke *et al.*, 2008).

Despite several systems proposed, biodegradable polymers have emerged as potential candidates for the development of carriers for targeting compounds to specific sites in the body. These kinds of polymers are usually biocompatible, non-antigenic and highly hydrophilic in nature, thus hydrophilic compounds can be easily incorporated into them (Nimesh *et al.*, 2006). During the last years, numerous processes for drug encapsulation have been developed that currently use aliphatic polyesters, such as poly(lactic acid) (PLA) and copolymers of lactic and glycolic acids that are well known biodegradable polymers. The biodegradability of these polymers can be manipulated by incorporating a variety of reactive groups such as ethers, anhydrides, carbonate, amides, ureas and urethanes in their main chain (Chandy *et al.*, 2002; Del Valle *et al.*, 2009; Pálíncó-

Biró *et al.*, 2001; Wischke *et al.*, 2008).

According to Del Valle *et al.* (Del Valle *et al.*, 2009) diffusion of active agents occurs when a drug or oil passes through the polymer that forms the controlled release device. There are different classifications for primarily diffusion in controlled delivery systems: (a) reservoir system, where the active agent is retained in a central compartment and surrounded by a polymeric membrane through which it must diffuse, thus controlling the rate of delivery, and (b) monolithic systems, where there is no local separation between the active agent reservoir and the release rate controlling wall (Siepmann *et al.*, 2008). A schematic of these systems is shown in Figure 6.1.

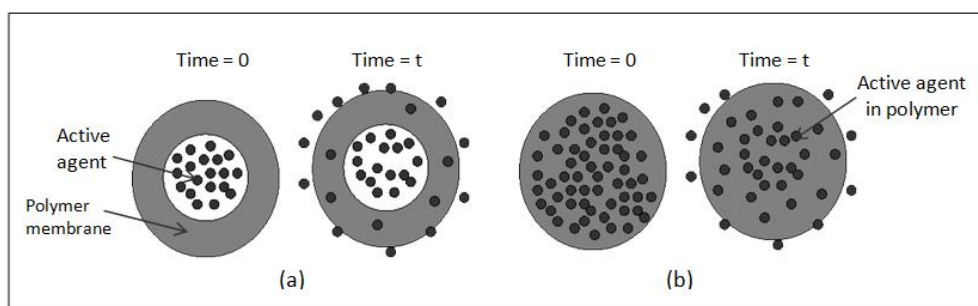


Figure 6.1. Mechanisms for active agent release: (a) reservoir system and (b) monolithic system.

Nevertheless, the release of the active agent from the delivery systems can be classified based on other mechanisms, such as, erosion (the product gradually dissolves in the membrane wall), diffusion (the oil diffuses out of the delivery system), extraction (mechanical forces during chewing or processing enlarge oil diffusion area) and burst (a reservoir system ruptures under influence of mechanical or osmotic forces)(Ubbink *et al.*, 2001).

Several diffusion models have been proposed in the literature to describe the release of an active agent from microcapsules (Borgquist *et al.*, 2004; Cryer *et al.*, 2009; Gumi *et al.*, 2009; Kwok *et al.*, 1991; Lü *et al.*, 2000; Marucci *et al.*, 2008; Muschert *et al.*, 2009; Sanna Passino *et al.*, 2004; Tavera *et al.*, 2009). Analysis and comparison of diffusion mechanisms according to microcapsule's geometries and

materials can provide the needed information to understand the mass transfer behaviour in such systems.

This work follows the work described in the previous chapters and already reported (Martins *et al.*, 2009; Martins *et al.*, 2011) where a coacervation method was developed and optimized in terms of surfactant type. Taking into account the potential applications in various fields such as the cosmetic, fragrance and food, the understanding of the release behaviour of the oil itself and its individual components is of crucial interest. As so, in this chapter, a diffusion model for thyme oil compounds across the polymeric shell was developed allowing determining the corresponding diffusion coefficients and thus describing the release behaviour with time. The developed model can be applied to other single-layer microcapsule systems. The release of thyme oil was investigated by using microcapsules in solution by analysing the first days period after production. Calculated and experimental diffusion profiles of oil components across the polymeric membrane have been compared. Control of microcapsules size and wall thickness, as well as, of encapsulation efficiency was also performed.

6.2. Materials and methods

6.2.1 Materials

The reagents used for the preparation by coacervation of polylactide microcapsules were: Poly(DL-lactide) (PLA, product number: 531162, Mw=75,000-120,000, inherent viscosity=0.55-0.75 dL/g) as the wall-forming material; dimethylformamide (DMF, product number: 319937, 99.8% ACS grade) as the PLA solvent; essential oil of *Thymus vulgaris* L. (thyme oil, product number: W306401, red, Kosher) as the core material; Tergitol™ 15-S-9 as the nonionic surfactant used to stabilize the o/w emulsion; Pluronic® F68 (product number: 81112) is a surfactant used to keep the microcapsules solution stable during the washing

process. All these reagents were obtained from Sigma Chemical Company (Germany). Octamethylcyclotetrasiloxane (OCMTS, product number: 8.14750.0250) was used as hardening agent; it acts as nonsolvent for the PLA coacervate droplets thus promoting microcapsules solidification, n-hexane (product number: 1.04367.2500, ACS grade) and ethanol (product number: 1.00983.2511, ACS grade) were used as washing solvent, these reagents were purchased from Merck Schuchardt OHG (Germany).

6.2.2 Microcapsule preparation

In this work thyme oil release studies were performed using microcapsules solution. The process to encapsulate thyme oil using PLA as the wall material was made according to the general scheme represented in Figure 6.2 and is described in more detail in chapter 4. However, in this study Tergitol™ 15-S-9 was the used nonionic surfactant to stabilize the o/w emulsion, as it gave better encapsulation efficiency (Martins *et al.*, 2010). The whole procedure of microcapsules production and storage was performed at room temperature.

6.2.3 Release study of thyme oil in microcapsules solution

The release studies of thyme oil were performed by using the produced microcapsules solution. After hardening with OCMTS, the microcapsules were decanted and sequentially washed with Pluronic® F68 solution, an ethanol solution and finally hexane. After washing, the microcapsules solution was immediately confined in a closely sealed bottle and placed in a water bath at room temperature and the first sample (1 mL) of microcapsules surrounding phase was collected (initial time). At predetermined time intervals samples were subsequently collected. The microcapsule surrounding phase was collected using a syringe equipped with a 0.45 µm pore size filter (Sartorius - cellulose acetate

filter) to separate free thyme oil from the loaded microcapsules (see Figure 6.2).

The concentration of free thyme oil in the filtrate was determined by GC-FID chromatography as described in more detail in section 6.2.3.

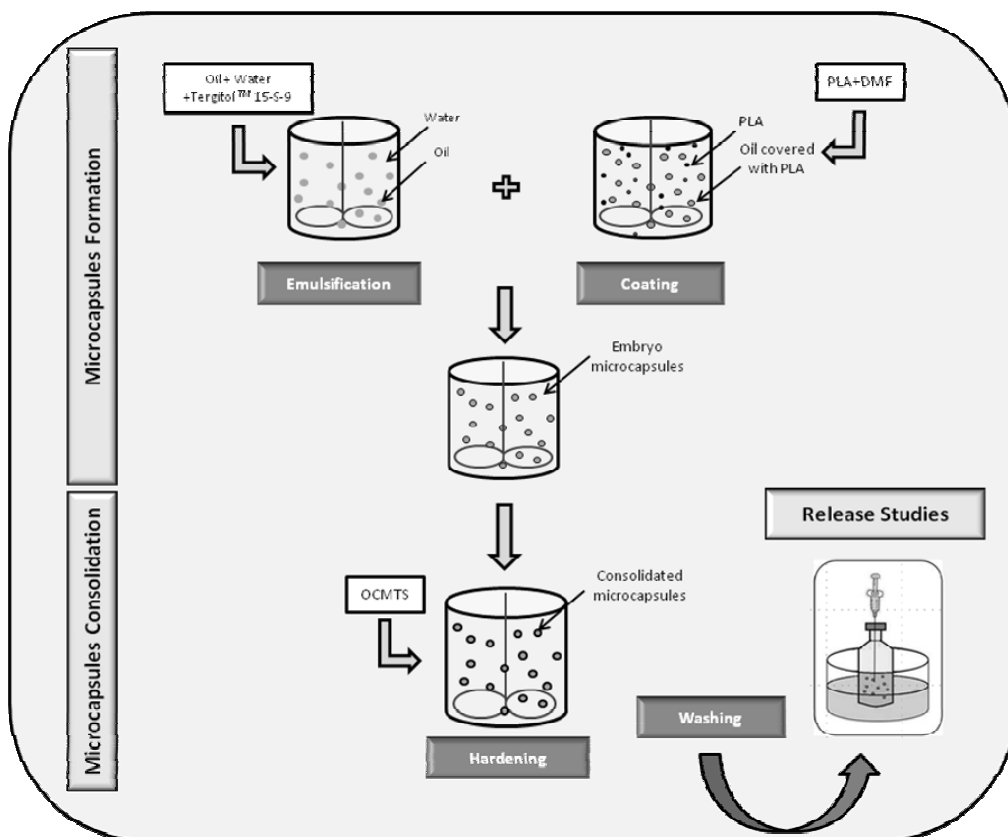


Figure 6.2. Process steps for microencapsulation of thyme oil by coacervation technique and for release studies.

6.2.4 Characterization techniques

Size distribution of microcapsules (Laser Diffraction)

The microcapsule particle size distribution was measured by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman-Coulter). The corresponding average values in volume and number were determined.

Optical microscopy

The microcapsules were analyzed by optical microscopy in transmitted light mode using a Leica DM 2000 microscope equipped with Leica Application Suite Interactive measurement software.

Gas chromatography GC-FID/MS

Quantification of the encapsulated thyme oil was performed by gas chromatography GC/FID. The analyses were carried out using a Varian CP-3800 instrument equipped with split/splitless injector, two CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 μ m film thickness) and a Varian FID detector controlled by the Saturn 2000 WS software. The oven temperature was isothermal at 50°C for 2 min, then increased from 50°C up to 200°C at 5°C/min and held at 200°C for 13 min. The injectors were set at 240°C, with a split ratio of 1:50 for FID and 1/200 for MS. The FID detector was maintained at 250°C. The sample volume injected was 0.1 μ L. The carrier gas was helium He N60, at a constant flow rate of 1 mL/min.

6.3. Analytical model for thyme oil release

The type of microcapsules considered in this study consists of a liquid core (thyme oil) coated with a permeable membrane (polymer-PLA). According to Fick's first law several factors can control the oil diffusion across the microcapsule membrane: the permeability, the available diffusion area and the concentration gradient across the membrane (Crank, 1975).

PLA microcapsules present a structure of a single-layer sphere with inner and outer radius $r_c < r_p$, which is assumed to be unchanged over the time (Figure 6.3). This assumption does not consider the volume changes due to the polymer matrix degradation or swelling effects in the capsule.

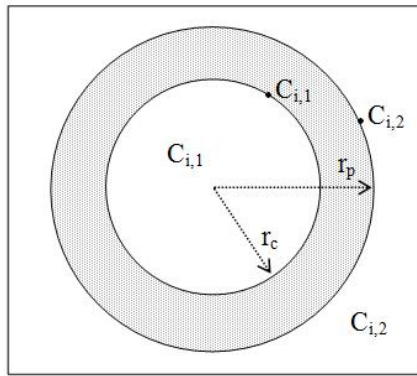


Figure 6.3. Schematic representation of a microencapsulated particle.

The developed release model is represented in Figure 6.4 and considers the following assumptions:

- (i) Thyme oil composition in the microcapsule core is homogeneous (the components are well-mixed); therefore the concentration of thyme oil components in core is uniform (no concentration gradients exist);
- (ii) The bulk solution is well-mixed; therefore the concentration of thyme oil in bulk solution is uniform (no concentration gradients exist);
- (iii) All capsules are of identical size, each capsule contains at any time the same amount of oil;
- (iv) Diffusion occurs from the inner to the outside of the microcapsule in a non-steady state ($C_{i,1} > C_{i,2}$) and capsule membrane offers the main resistance to oil diffusion;
- (v) The oil concentration profiles are uniform in both inner core and bulk solutions but variable at the core/wall interface;
- (vi) The amount of oil in the shell can be considered negligible in terms of the total mass balance.

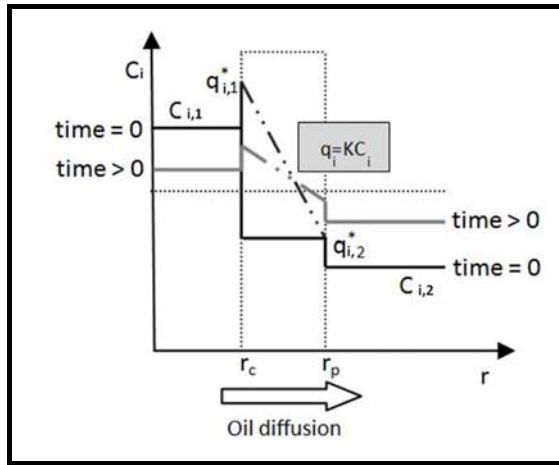


Figure 6.4. Thyme oil concentration profile: diffusion of thyme oil from core solution to outside of microcapsule.

For each thyme oil component present in the core compartment, a mass balance can be written as follows:

$$\frac{dm_{i,1}}{dt} = V_1 \frac{dC_{i,1}}{dt} \quad (6.1)$$

and in the outer solution

$$\frac{dm_{i,2}}{dt} = V_2 \frac{dC_{i,2}}{dt} \quad (6.2)$$

From Eqs 6.1 and 6.2, since $-dm_{i,1} = dm_{i,2}$, it results that:

$$m_{i,1}(t) = m_{i,1}^0 + m_{i,2}^0 - m_{i,2}(t) \quad (6.3)$$

where, $m_{i,1}^0$ is the initial oil mass in microcapsules core; $m_{i,2}^0$ is the oil mass in the surrounding solution; V_1 is the core volume and V_2 is the solution volume outside microcapsules.

The rate of change of thyme oil in the microcapsule core can be related to the oil concentration gradient at the core-polymer interface, according to Fick's first law of diffusion.

$$\frac{dm_{i,1}}{dt} = A_c D_i \left. \frac{\partial q_i}{\partial r} \right|_{r=r_c} \quad (6.4)$$

In the case of a limited volume, well-mixed bulk (outside microcapsules) solution, the bulk thyme oil concentration, $C_{i,2}$, changes with the diffusion of oil to the outside of microcapsules. By applying Fick's law of diffusion, the rate of solute outward the wall from the bulk solution can be expressed as:

$$\frac{dm_{i,2}}{dt} = -A_p D_i \left. \frac{\partial q_i}{\partial r} \right|_{r=r_p} \quad (6.5)$$

The mass balance in a volume element of the polymeric wall of the microcapsule, in transient state, valid between radius r_c and r_p can be described by the equation:

$$\frac{\partial q_i(r,t)}{\partial t} = D_i \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial q_i}{\partial r} \right) \quad (6.6)$$

After averaging over the shell volume, i.e., multiplying both sides of Equation (6.6) by $r^2 dr$ and integrating between r_c e r_p ,

$$\frac{d \langle q_i(t) \rangle}{dt} = \frac{3}{r_p^3 - r_c^3} D_i \left[r_p^2 \left. \frac{\partial q_i(t)}{\partial r} \right|_{r_p} - r_c^2 \left. \frac{\partial q_i(t)}{\partial r} \right|_{r_c} \right] \quad (6.7)$$

where the average oil adsorbed concentration is:

$$\langle q_i \rangle = \frac{\int_{r_c}^{r_p} r^2 q_i(r) dr}{\int_{r_c}^{r_p} r^2 dr} \quad (6.8)$$

In the above equations A_c is the inside surface area of microcapsule, A_p is the outside surface area of microcapsule, q_i is the oil adsorbed concentration in the wall, D_i is the oil diffusion across membrane, r is the radial position, r_c is the core radius, r_p is the microcapsule radius and $\frac{\partial q_i}{\partial r}$ is the oil adsorbed concentration gradient. At the interfaces oil/wall $r = r_c$ and $r = r_p$ we assume adsorption equilibrium $q_{i,1}^* = Kc_{i,1}$ and $q_{i,2}^* = Kc_{i,2}$.

Equations (6.3), (6.4), (6.5) and (6.7) provide the mathematical model for the thyme oil balance in the core solution, bulk solution and the polymer membrane respectively.

Assuming a linear profile between the radius r_c and r_p ; this is only valid if the wall thickness $(r_p - r_c)$ of the microcapsules is much smaller than the particle radius,

$$\text{i.e., } \frac{(r_p - r_c)}{r_p} \ll 1$$

$$q_i(r, t) = q_{i,1}^*(t) - \frac{q_{i,1}^*(t) - q_{i,2}^*(t)}{r_p - r_c} (r - r_c) \quad (6.9)$$

From Equations 6.8 and 6.9, it results that:

$$\langle q_i \rangle = \left(\frac{q_{i,1}^*(t)r_p - q_{i,2}^*(t)r_c}{r_p - r_c} \right) - \frac{3}{4} \frac{q_{i,1}^*(t) - q_{i,2}^*(t)}{r_p - r_c} \frac{r_p^4 - r_c^4}{r_p^3 - r_c^3} \quad (6.10)$$

By calculating $\frac{\partial q_i}{\partial r}$ at $r=r_p$ and $r=r_c$ from Equation (6.9) and replacing those values, together with $\langle q_i \rangle$ from Equation (6.10), in Equation (6.7) we obtain a ordinary differential equation (ODE) in the q variables $q_{i,1}^*$ and $q_{i,2}^*$ which are related with $m_{i,2}$ by $q_{i,2}^* = Kc_{i,2} = \frac{K}{V_2}m_{i,2}$ and $q_{i,1}^* = Kc_{i,1} = \frac{K}{V_1}m_{i,1} = \frac{K}{V_1}(m_{i,1}^0 + m_{i,2}^0 - m_{i,2})$.

The final ODE in $m_{i,2}(t)$ leads after integration to Equation (6.11).

$$m_{i,2}(t) = m_{i,2}^{eq} + (m_{i,2}^0 - m_{i,2}^{eq}) e^{-Dt / \left[\frac{r_p(r_p^3 - r_c^3)}{3(r_p^2 - r_c^2)} - \frac{r_p^2 + r_c^2}{4} - \varepsilon_1 \frac{r_p^3 - r_c^3}{3(r_p + r_c)} \right]} \quad (6.11)$$

where $\varepsilon_1 = \frac{V_1}{V_1 + V_2}$ is the fraction of total volume occupied by oil in microcapsules

core and the final steady-state value is $m_{i,2}^{eq} = (1 - \varepsilon_1)(m_{i,1}^0 + m_{i,2}^0)$.

6.4. Results and discussion

6.4.1. Particle size distribution (Laser dispersion)

Figure 6.5 shows the experimentally measured particle size distributions, both in volume and in number, for the PLA microcapsules used in the release studies. A bimodal distribution in volume was observed with a mean particle size of 36 μm . In number the distribution was quite narrow and unimodal, with a mean particle size around 2 μm . Moreover, it was observed that 99% of particles in number have diameters smaller than 10 μm (1% > 10 μm), but this represents 18% of the particles in volume (82% > 10 μm). This means that, even though a large number of microcapsules have small size; most of the thyme oil was encapsulated in larger particles. Mean particle size, obtained for three replicas of the assay (batch 1 to 3), is shown in Table 6.1. Even the obtained distributions have extensive dispersion, the results pointed out for a good reproducibility.

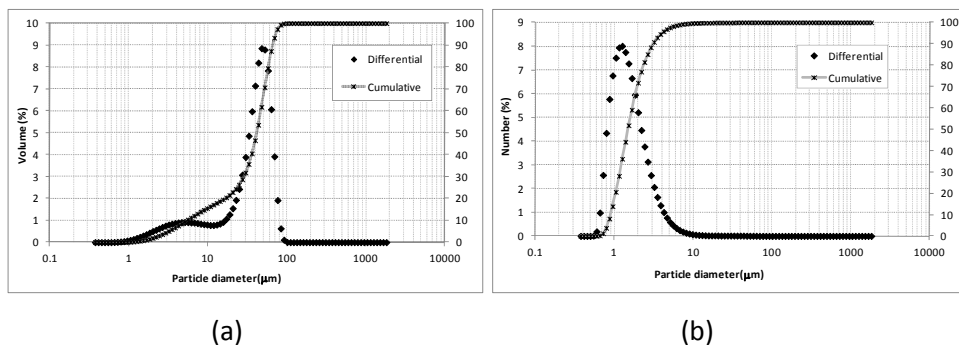


Figure 6.5. Particle size distribution of polylactide microcapsules with thyme oil. Distribution in volume (a) and in number (b).

Table 6.1. Mean particle size in volume of microcapsules in three experiments.

Batch	Particle size (μm)
	(mean $\pm$ SD)
1	39.19 $\pm$ 21.50
2	37.43 $\pm$ 20.00
3	31.59 $\pm$ 17.67

6.4.2 Optical microscopy

Optical microscopy images of the obtained PLA microcapsules are shown in Figure 6.6. The existence of microcapsules is clearly visible. Optical microscopy highlighted the presence of microcapsules in solution, as well as, their morphology. Figure 6.7 shows that microcapsules have spherical shape, with different sizes and one can notice also the absence of agglomerates. The observed size heterogeneity is a direct consequence of the used dispersion technique (ultraturrax). The optical analyses allowed estimating the wall thickness around $2\mu\text{m}$, by using the Leica software tools. Through dark field option it was possible to distinguish the polymeric membrane around the oil by colour gradient difference (Martins *et al.*, 2010). The wall thickness of microcapsules was also confirmed by Equation (4.2). It was also observed that two predominant sizes of microcapsules were present which is compatible with a bimodal distribution.

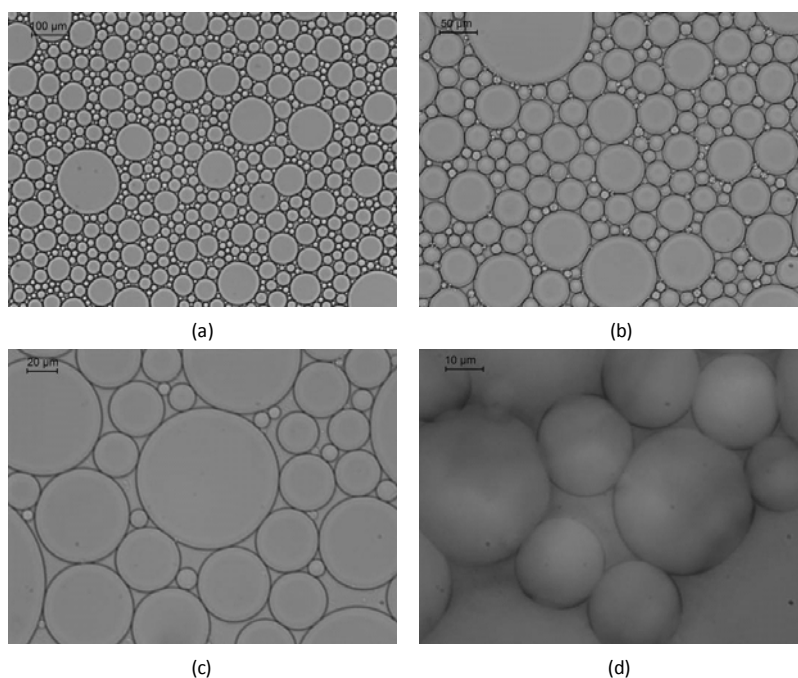


Figure 6.6. Optical microscopy of PLA microcapsules solution after the production and without washing. Magnification of images: (a)100x; (b)200x; (c)400x and (d)1000x.

6.4.3 Release study

The experimental data for oil release in the first hour is shown in Figure 6.7 for the analyzed individual components (γ -terpinene, *p*-cymene, linalool, thymol and carvacrol). Furthermore, Figure 6.8 refers to the release profile of thymol in microcapsules solution of PLA for the first five days. This figure shows that the release rate of thymol is faster in first hour and that it becomes constant over the next 5 days period.

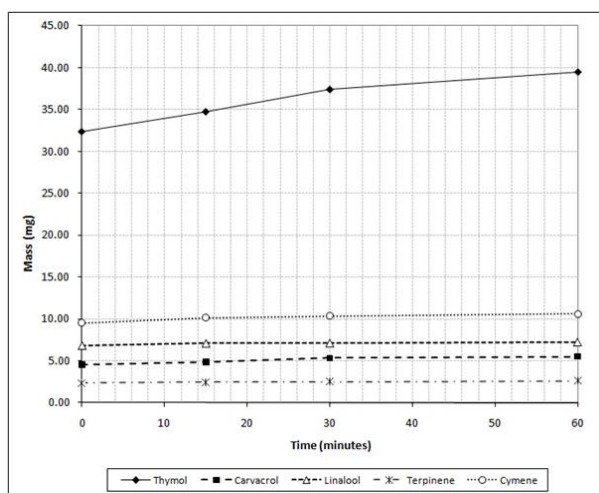


Figure 6.7. Experimental data for release of thyme oil in microcapsules solution of PLA in first hour.

According to the literature, the release of the active agent through PLA polymer matrices is mainly controlled by oil diffusion through the lipophilic matrices in first hours, crossing over to a regime controlled by the matrix degradation (Moritera *et al.*, 1991). The latter mechanism depends on the molecular weight of the polymer used in the microcapsule wall. PLA is a linear polymer and the overall mobility of its chain will decrease with increasing molecular weight (Mw). Therefore the use of a low Mw polymer will allow a faster diffusion of the active agent through the polymer matrix. For PLA polymers with low molecular weight the hydrolytic degradation can start in a few days. For diffusion of the active agent the oil needs to pass to the microcapsule surface, either through the polymer matrix or through water-filled pores (Hora *et al.*, 1990).

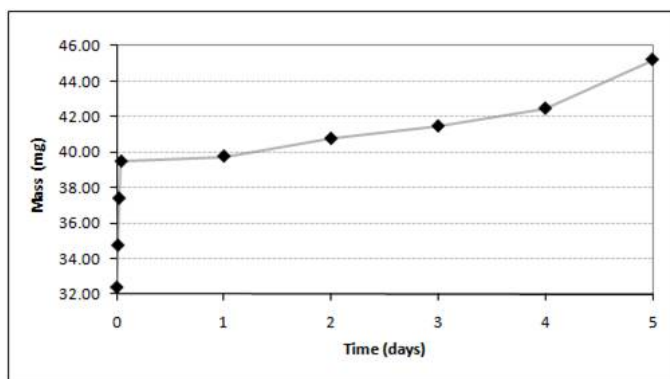


Figure 6.8. Experimental data for release of thymol in microcapsules solution of PLA for first five days.

The diffusion behavior observed in Figure 6.8 could be confirmed by the results presented in Table 6.2 and 6.3. Table 6.2 shows the chemical composition of thyme oil, the encapsulation efficiency and the mass released for each component of thyme oil in first 5 days.

The encapsulation efficiency (percentage of thyme oil present in the PLA microcapsules) of each thyme oil component was calculated based on the methodology described in previous chapters. Accordingly:

$$\text{Encapsulation Efficiency (\%)} = \frac{m_{\text{total}} - m_{\text{out}}}{m_{\text{total}}} \times 100 \quad (4.1)$$

where m_{total} = amount of loaded thyme oil (g) is the total amount of thyme oil dispersed in water in emulsification step of the encapsulation process and m_{out} = amount of nonencapsulated thyme oil (g).

Thyme oil contains a high percentage of phenolic polar compounds (62.2 %), among which thymol prevails (47.7%). The used coacervation process gave a high encapsulation efficiency, around 64.6% (80% for the apolar compounds, while for the polar compounds only 54% was achieved).

Table 6.2. Total, encapsulated and released masses and Encapsulation Efficiency (EE) discriminated by each component of thyme oil in microcapsules solution of PLA.

Component	ENCAPSULATION				RELEASE			
	Content (%)	mass _{total} (mg)	mass _{encapsulated} (mg)	EE (%)	mass _{in core initial} * (mg)	mass _{out initial} * (mg)	Δm released _{1 day} * (mg)	Δm released _{5 days} * (mg)
γ - Terpinene	6.2	11.458	8.608	75.13	6.271	2.337	0.363	0.756
<i>p</i> - Cymene	31.6	58.397	48.516	83.08	38.972	9.544	1.155	2.563
Linalool	7.6	14.045	6.935	49.38	0.145	6.790	—	—
Thymol	47.7	88.150	48.513	55.04	16.154	32.359	7.394	12.840
Carvacrol	6.9	12.751	6.836	53.61	2.314	4.522	1.083	1.806
Total	100	184.800	119.409	64.62	63.857	55.552	10.644	19.747

* Calculated

+ Experimental

The release of thyme oil polar compounds is faster than the apolar ones (Table 6.3), and after five days it appears that 63.8% of thyme oil is released. In the case of thymol and carvacrol more than 80% of the encapsulated oil was released during the first day. It was also observed that the release rate of linalool seems to be higher than 100% immediately after the first day; which could be possibly attributed to quantification errors when determining the nonencapsulated oil based on GC-FID peak analysis. The initial condition for the release experiment clearly shows that the loss of encapsulated polar components was higher than for apolar components. Also, the rate of release follows the same trend with faster diffusion at short times because of high concentration gradient and slower diffusion at longer times, which is typical for this type of microcapsules (reservoir systems)(Del Valle *et al.*, 2009).

To evaluate the diffusion differences between polar and apolar thyme oil components, a comparative study between experimental and theoretical results obtained using the model referenced in Section 6.3 was performed. Thymol and *p*-cymene were chosen as representative of the polar and nonpolar components of thyme oil, respectively. Figure 6.8 shows the release kinetics for thymol during the

first hour of release and Figure 6.9 shows the release kinetics during a five days period.

Table 6.3. Percentage of oil release of first five days discriminated by each thyme oil component in microcapsules solution of PLA.

	Release (%)		
	initial	1 day	5 days
γ - Terpinene	27.2	31.4	35.9
<i>p</i> - Cymene	19.7	22.1	25.0
Linalool*	97.9	—	—
Thymol	66.7	81.9	93.2
Carvacrol	66.1	82.0	92.6
Total	46.5	55.4	63.1

*The release of linalool was not followed since at time zero almost all encapsulated linalool was already in solution outside microcapsules.

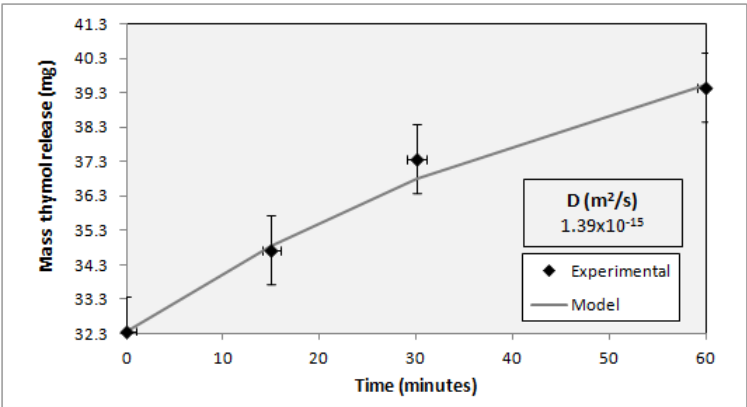


Figure 6.9. Comparison between experimental and model results for thymol released from PLA microcapsules solution in first hour.

$$m_1^0 = 16.154 \text{ mg}; m_2^0 = 32.359 \text{ mg}; V_1 = 6.38 \times 10^{-6} \text{ m}^3; V_2 = 6.28 \times 10^{-5} \text{ m}^3; m_2^{eq} = 44.039 \text{ mg}.$$

It was observed through Figure 6.9 that the diffusion coefficient was $1.39 \times 10^{-15} \text{ m}^2/\text{s}$ for thymol, the polar component. For the apolar component, *p*-cymene, the diffusion coefficient for the first hour of release was $5.21 \times 10^{-17} \text{ m}^2/\text{s}$, which is lower than that obtained for thymol. This behaviour is in accordance with the previously

observed by Wischke and Schwendeman, where the release differences are attributed to the distinct hydrophobic characteristics of the two compounds (Wischke *et al.*, 2008).

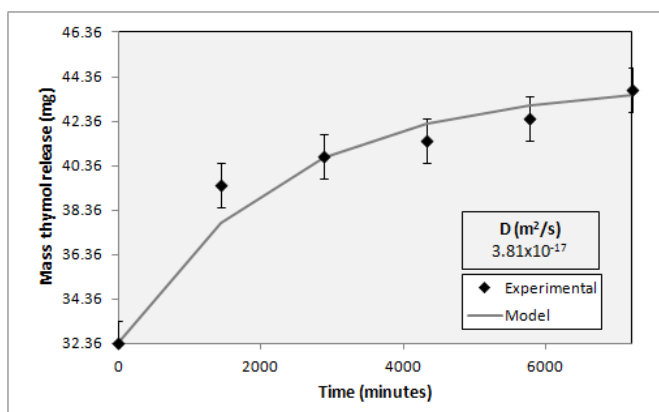


Figure 6.10. Comparison between experimental and model results for thymol released from PLA microcapsules solution for 5 days.

$$m_1^0 = 16.154 \text{ mg}; m_2^0 = 32.359 \text{ mg}; V_1 = 6.38 \times 10^{-6} \text{ m}^3; V_2 = 6.28 \times 10^{-5} \text{ m}^3; m_2^{eq} = 44.039 \text{ mg}.$$

As observed in Figure 6.8 the diffusion was slower after the first hour of release. The diffusion coefficient estimated over a 5 days period was $3.81 \times 10^{-17} \text{ m}^2/\text{s}$ for thymol and $1.43 \times 10^{-18} \text{ m}^2/\text{s}$ for p-cymene, as shown in Figure 6.10. This release difference can be attributed to the lipophilic solubility of some oil components (hydrophobic components of oil). The lipophilic components of thyme oil could become more homogeneously distributed in the PLA matrix which can be considered as lipophilic (Moritera *et al.*, 1991).

Lipophilic substances interact among themselves and with other lipophilic substances mainly through London dispersion forces; these species are not able to form hydrogen bonds and have large o/w partition coefficients. On contrary, the oil polar compounds have the capacity to form hydrogen bonds with water, DMF

and ethanol (the release medium of microcapsules) and thereafter release through the PLA wall to the solution surrounding microcapsules (Wischke *et al.*, 2008).

6.5. Conclusions

The objective of the present study was to evaluate the release rate of thyme oil through PLA microcapsules produced by a coacervation process followed by hardening with OCMTS. The average size of the microcapsules, as determined by the Laser Diffraction Particle Size Analyser measurements, was 36 μm with bimodal distribution in volume and quite narrow distribution in number. Analysis by optical microscopy showed spherical particles and allowed an estimate of the wall thickness of 2 μm . Two predominant sizes of microcapsules, compatible with a bimodal distribution were observed, as well as, a total absence of agglomerates. The release of thymol and cymene from the PLA microcapsules can be explained by a diffusion mechanism, as the developed model was found to be in good agreement with the experimental measurements, both for the first hour of release and along a five days period. For the first hour of release, the diffusion coefficient was $1.39 \times 10^{-15} \text{ m}^2/\text{s}$ for thymol and $5.21 \times 10^{-17} \text{ m}^2/\text{s}$ for cymene. For a 5 days period of release, $3.81 \times 10^{-17} \text{ m}^2/\text{s}$ for thymol and $1.43 \times 10^{-18} \text{ m}^2/\text{s}$ for cymene, was determined. These differences can be ascribed to the distinct lipophilic solubility of the analysed thyme oil components and the obtained rather small diffusion coefficient values interpreted in terms of the very dense polymer matrix, which might constitute a significant impeditive effect. The used encapsulation process originates higher encapsulation efficiency for apolar compounds of thyme oil, and release studies pointed out for a release rate of the polar ones. The developed model can be extended to other single-layer microcapsule systems.

6.6. References

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CHAPTER 7:

Release studies of vanillin, thymol and *p*-cymene from polylactide microcapsules

“Nearly everything you do is of no importance, but it is important that you do it.”

[Mahatma Gandhi]

In seventh chapter, “Release studies of vanillin, thymol and *p*-cymene from polylactide microcapsules”, studies aiming at characterize the release of vanillin, thymol and *p*-cymene as model core materials across the PLA membrane are presented. The microcapsules were obtained by the coacervation process developed in chapter 4 and have shown a spherical shape with mean particle sizes of 21 μm for vanillin, 25 μm for thymol and 37 μm for *p*-cymene. Quantification of the encapsulated model compounds was performed by gas chromatography and pointed out that all the vanillin was entrapped in microcapsules. Vanillin release from the polylactide microcapsules has been monitored along with time, but no amount was detected in the outside solution of microcapsules. Nevertheless, the results have shown that the release of thymol and *p*-cymene is faster in the first hour keeping almost constant in the subsequent days. The diffusion coefficient in the first hour of release was $1.99 \times 10^{-16} \text{ m}^2/\text{s}$ for thymol and $4.34 \times 10^{-16} \text{ m}^2/\text{s}$ for *p*-cymene. However, the diffusion is slower if considering a period of 5 days with the diffusion coefficients of $3.34 \times 10^{-19} \text{ m}^2/\text{s}$ for thymol and $3.45 \times 10^{-18} \text{ m}^2/\text{s}$ for cymene.

7.1 Introduction

The encapsulation of active compounds, such as essential oils has become a very attractive process in food, cosmetic and pharmaceutical industries (Baranauskienė *et al.*, 2007; Dowding *et al.*, 2004; Peppas *et al.*, 1997). The microencapsulation is an useful technique to protect an active agent and provide its controlled release (Baranauskienė *et al.*, 2007). Many oils have properties such as strong flavour and instability to oxidation, therefore encapsulation can provide protection from oxidation caused by heat, light and humidity. On the other hand, the encapsulated compounds are easy to handle and can stay stable in case of prolonged storage (Peña *et al.*, 2012). The release behaviour of oils entrapped in the microcapsules is an important issue and governs the desired industrial applications. Nevertheless, the controlled release of the active agents is still a challenge for some industries (Gumi *et al.*, 2009; Peña *et al.*, 2012). In fact, it is important to access the controlled release of active agents through the microcapsules polymeric wall in order to develop devices suitable for delivering drugs, pesticides, fragrances or flavours at prescribed rates, together with improved efficacy, safety and convenience. In general terms, oil release from microcapsules depends on the diffusion coefficient value across the polymeric matrix, from the size and shape of the oil molecule, as well as, from the polarity of the matrix (Romero-Cano *et al.*, 2002).

Taking into account the previous work, where release studies were performed with thyme oil, in this chapter the release of vanillin, thymol and *p*-cymene, used as model core materials, was performed. Vanillin is an organic compound with the molecular formula $C_8H_8O_3$ (4-hydroxy-3-methoxybenzaldehyde, see Figure 7.1) being the major constituent of vanilla (Araújo *et al.*, 2010). Vanillin is one of the most used flavouring materials in food industry and also as a fragrance in the perfumery industry (Araújo *et al.*, 2010; Walton *et al.*, 2003; Walton *et al.*, 2000;

Zabkova *et al.*, 2006). It has antioxidant and antimicrobial properties thus presenting a high potential to be used as a food preservative. It is also used as an intermediate in the chemical and pharmaceutical industries for the production of herbicides, antifoaming agents or drugs (Zabkova *et al.*, 2006). Thymol and *p*-cymene are constituents of thyme oil and represent the polar and apolar compounds of oil, respectively. Thyme oil is usually used as antimicrobial and antiseptic agent, in food packing and fragrance for soaps or detergents.

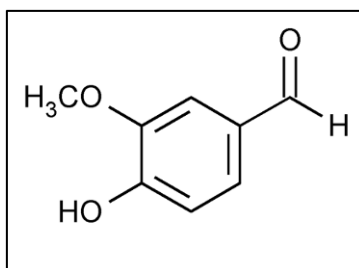


Figure 7.1. Chemical structure of vanillin.

In this work experimental data concerning vanillin, thymol and *p*-cymene release from the PLA microcapsules, obtained by coacervation as previously described, is presented. The release study was performed in solution during the five days period subsequent to its production. Experimental and calculated diffusion profiles of the model compounds across the polymeric membrane were analyzed and compared. Size, wall thickness and encapsulation efficiency of the used microcapsules were also determined.

7.2. Materials and methods

7.2.1 Materials

The reagents used for the preparation of polylactide microcapsules were: Poly(DL-lactide) as the wall-forming material; dimethylformamide as the polymer solvent; vanillin ($\geq 97\%$, FCC, FG - Sigma Chemical Company), Thymol (Ph Eur - BDH Prolabo) and *p*-Cymene (96% - Alfa Aesar) as the core materials. Since thymol and vanillin were in powder form it was necessary to use olive oil (B&T Technology; $\rho = 0.920 \text{ g/cm}^3$; used in cosmetics, pharmaceuticals and soaps) as solvent of these oils. Tergitol™ 15-S-9 was used as the nonionic surfactant to stabilize the o/w emulsion and Pluronic® F68 (product number: 81112) as a surfactant to keep the microcapsules solution stable during the washing process. Octamethylcyclotetrasiloxane was used as hardening agent; n-hexane (product number: 1.04367.2500, ACS grade) and ethanol (product number: 1.00983.2511, ACS grade) were used as washing solvent, these reagents were purchased from Merck Schuchardt OHG (Germany).

7.2.2 Microcapsules preparation and release study of model compounds

Release studies of vanillin, thymol and *p*-cymene were performed using microcapsules in solution. The process to encapsulate the oil using PLA as the wall material was made according to the general scheme represented in Figure 7.2 where Tergitol™ 15-S-9 was used as the nonionic surfactant to stabilize the o/w emulsion because it gave better encapsulation efficiency according to the previous presented studies. The whole procedure for microcapsules production and storage was performed at room temperature. The methodology for PLA microcapsules production can be summarized in following steps.

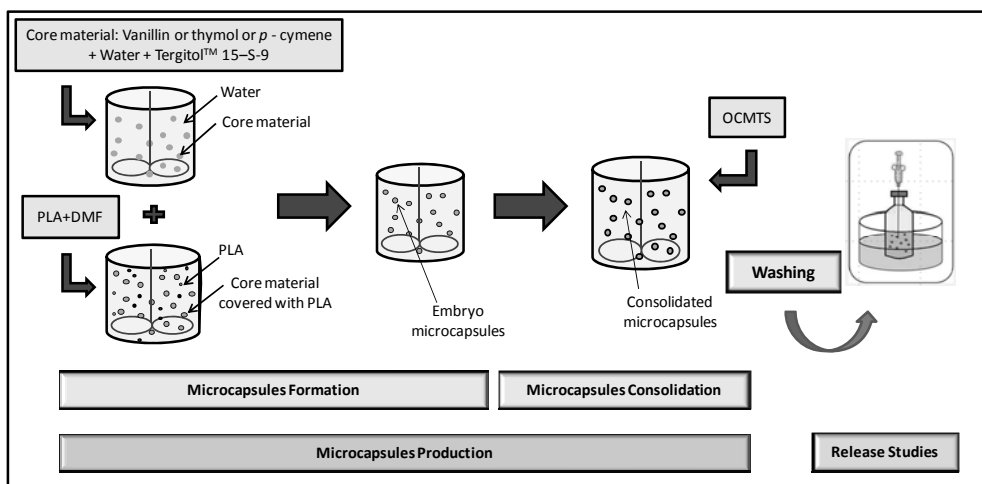


Figure 7.2. General process scheme for the preparation of microcapsules with PLA and the release studies.

Firstly, an oil emulsion in water stabilized with Tergitol 15-S-9 (HLB of 13.3), and a PLA solution in dimethylformamide (DMF) have been prepared. For vanillin and thymol it was necessary to previously prepare an oil solution with olive oil, since these compounds were in powder form. Thereafter, the PLA solution was added dropwise to the previously prepared o/w emulsion.

Upon contact with water, the homogeneous solution of PLA in DMF, promotes the precipitation of PLA around the oil core. The o/w emulsion was obtained by dispersion with an ultraturrax during 90 seconds and the encapsulation process continued under stirring using an impeller stirrer in a batch reactor for one hour using ambient temperature.

The microcapsules formed were hardened by adding OCMTS and allowed to stand during one hour. OCMTS is a widely used hardening agent. After hardening with OCMTS, the microcapsules were decanted and sequentially washed. After washing, microcapsules solution was immediately kept in a closely sealed bottle and placed in a water bath at room temperature and was collected the first sample (1 mL) of microcapsules surrounding phase (initial time). At predetermined

intervals of time, samples were collected. The microcapsule surrounding phase was collected using a syringe equipped with a 0.45 μm pore size filter (Sartorius - cellulose acetate filter) to separate free core material from the loaded microcapsules (see Figure 7.2).

The concentration of free core material in the filtrate was determined by GC-FID chromatography as described in more detail in section 7.2.3.

Table 7.1 illustrates the various chemical systems and composition of all formulations used.

Table 7.1. Chemical systems and composition of compounds used in microcapsules formulation.

Formulations	Active agent		Polymer	Polymer Solvent	Aqueous Phase		Hardening Agent
	Model Compound	Mass (mg)	PLA Mass (g)	DMF Volume (mL)	Water Volume (mL)	Tergito™15-S-9 Volume (mL)	OCMTS Volume (mL)
A	<i>p</i> -cymene	171.40	1.4153	94.5	405.5	14.1	890
B	Vanillin*	16.46	1.4726	94.5	405.5	14.1	890
C	Thymol*	130.48	1.4781	94.5	405.5	14.1	890

*Compounds dissolved in olive oil: $C_{\text{Vanillin}} = 6.0975 \text{ g/L}$; $C_{\text{Thymol}} = 48.8125 \text{ g/L}$

7.2.3 Characterization techniques

Size distribution of microcapsules (Laser Diffraction)

The microcapsule particle size distribution was measured by laser dispersion using a Laser Diffraction Particle Size Analyser LS 230 (Beckman-Coulter). The corresponding average values in volume and number were determined.

Optical microscopy

The microcapsules were analysed by optical microscopy in transmitted light mode using a Leica DM 2000 microscope equipped with Leica Application Suite Interactive measurement software.

Gas chromatography GC-FID/MS

Quantification of the encapsulated core material was performed by gas chromatography GC/FID. The analyses were carried out using a Varian CP-3800 instrument equipped with split/splitless injector, two CP-Wax 52 CB bonded fused silica polar columns (50 m x 0.25 mm, 0.2 μ m film thickness) and a Varian FID detector controlled by the Saturn 2000 WS software. The oven temperature was isothermal at 50°C for 2 min, then increased from 50°C up to 200°C at 5°C/min and held at 200°C for 13 min. The injectors were set at 240°C, with a split ratio of 1:50 for FID and 1/200 for MS. The FID detector was maintained at 250°C. The sample volume injected was 0.1 μ L. The carrier gas was helium He N60, at a constant flow rate of 1 mL/min.

The nonencapsulated core material was quantified by analysing the two phases obtained after microcapsules separation by decantation (aqueous phase and microcapsules rich phase). 1 ml of the aqueous phase and 1 ml of the microcapsules surrounding solution were collected using a syringe equipped with a filter and thereafter analysed by GC-FID. The mass of encapsulated core material was obtained by difference between the loaded original quantity and the nonencapsulated determined quantity. The encapsulation efficiency (percentage of core material present in microcapsules) was calculated based on the encapsulation efficiency formula (equation 4.1) described in previous chapters.

7.3 Analytical model for vanillin, thymol and p-cymene release

The type of microcapsules considered in this study consists of a liquid core (vanillin or thymol dissolved in olive oil and *p*-cymene alone) coated with a permeable membrane (PLA polymer). PLA microcapsules present a structure of a single-layer sphere with inner and outer radius $r_c < r_p$, which is assumed unchanged over the time. Taking into account all the considerations previously

described in chapter 6, the model for release of thymol and cymene is represented by the final equation 6.11.

$$m_{i,2}(t) = m_{i,2}^{eq} + (m_{i,2}^0 - m_{i,2}^{eq}) e^{-Dt / \left(\frac{r_p(r_p^3 - r_c^3)}{3(r_p^2 - r_c^2)} + \frac{r_p^2 + r_c^2}{4} - \varepsilon_1 \frac{r_p^3 - r_c^3}{3(r_p + r_c)} \right)} \quad (6.11)$$

where, $\varepsilon_1 = \frac{V_1}{V_1 + V_2}$ is the fraction of total volume occupied by the core material (vanillin or thymol dissolved in olive oil, *p*-cymene); V_1 is the core volume and V_2 is the volume of the solution outside microcapsules; the final steady-state value is $m_{i,2}^{eq} = (1 - \varepsilon_1)(m_{i,1}^0 + m_{i,2}^0)$, $m_{i,1}^0$ is the initial mass of model compound in microcapsules core and $m_{i,2}^0$ is the mass of oil in the surrounding solution.

7.4 Results and discussion

7.4.1. Particle size distribution (Laser dispersion)

Figure 7.3 shows the experimentally measured particle size distributions, both in volume and in number, for the PLA microcapsules prepared with vanillin. It was observed a slight bimodal distribution in volume with a mean particle size of 21.38 μm . On the other hand, in number, the distribution was quite narrow and unimodal, with a mean particle size around 2 μm . Nevertheless, it was observed (see cumulative trace in Figure 7.3) that 99% of particles in number have diameters smaller than 10 μm (1% > 10 μm), but this represents 30% of particles in volume. This means that even though a large number of microcapsules have small size, most of the vanillin was encapsulated in large particles.

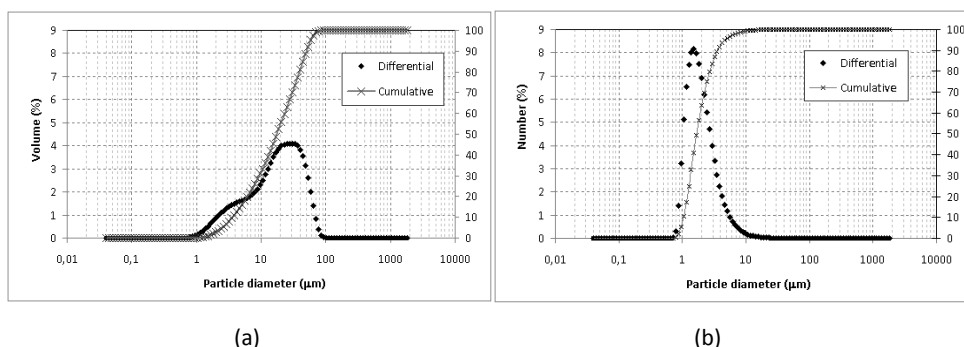


Figure 7.3. Particle size distribution of PLA microcapsules containing vanillin after production and washing. Distribution in volume (a) and in number (b).

Figure 7.4 shows the experimentally measured particle size distributions, both in volume and in number, for the PLA microcapsules prepared with thymol. It was observed a bimodal distribution in volume for thymol, with a mean particle size of 25.49 μm , whereas in number the distribution was quite narrow and unimodal in shape, with a mean particle size around 2 μm . Summarizing, it was observed (see cumulative trace in Figure 7.4) that 99% of particles in number have diameters smaller than 10 μm (1% > 10 μm), but this represents only 20% of particles in volume. This means that even though a large number of microcapsules have small size, most of the thymol was encapsulated in large particles.

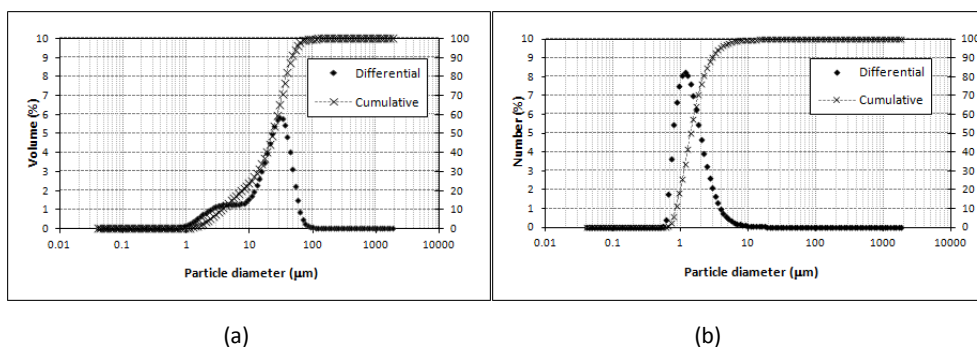


Figure 7.4. Distribution in volume and in number obtained for polylactide microcapsules prepared with thymol: differential (a) and cumulative (b).

Figure 7.5 shows the experimentally measured particle size distributions, both in volume and in number, for the PLA microcapsules prepared with *p*-cymene. A bimodal distribution in volume was observed with a mean particle size of 37 μm . In number the distribution was quite narrow and unimodal, with a mean particle size around 2 μm . A more detailed analysis reveal that, in volume, 85% of particles have diameters higher than 10 μm (15% <10 μm).

Mean particle size of the PLA microcapsules obtained for vanillin, thymol and *p*-cymene, as well as the corresponding wall thickness are shown in Table 7.2.

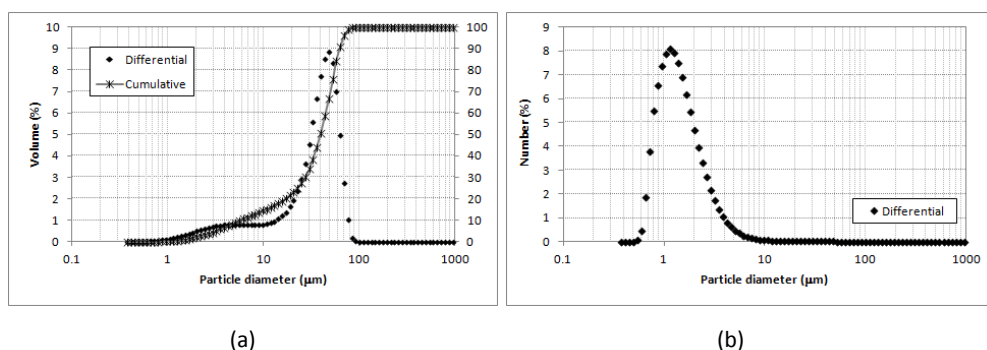


Figure 7.5. Distribution in volume and in number obtained for polylactide microcapsules prepared with *p*-cymene: differential (a) and cumulative (b).

Table 7.2. Mean particle size in volume and wall thickness of polylactide microcapsules obtained with vanillin, thymol and *p*-cymene.

Core material	Mean Particle size (μm)	Wall thickness (μm)
Vanillin	21.38	1.53
Thymol	25.49	1.77
<i>p</i> - Cymene	37.33	2.17

7.4.2 Optical microscopy

The analysis by optical microscopy had the objective to inspect microcapsule's morphology after production and washing steps (Figures 7.6 to 7.9). All figures show that the droplets of vanillin, thymol and *p*-cymene have been individually encapsulated as spherical particles with size distribution consistent with a bimodal distribution. Using optical microscopy, an optimized image of microcapsules morphology was obtained by exposure adjustments.

Figure 7.6 shows the aspect of the vanillin microcapsules in the bright field option at different magnifications. Microcapsules have spherical shape, with different sizes and one can notice also the absence of agglomerates. Figure 7.7 shows the vanillin microcapsules solution using the dark field option, in these images it was observed the vanillin core entrapped in a PLA shell of a fairly constant thickness. Through dark field option it was possible to distinguish the polymer membrane around the oil by color difference. Thereafter, measurement annotation tools were added to images allowing vanillin microcapsules wall thickness estimation around 2 μm .

The shape and morphology of thymol microcapsules are shown in Figure 7.8. The optical microphotograph was taken using bright field option at different magnifications, and it shows many microcapsules with diameters smaller than 10 μm . Moreover, they present a spherical shape and confirm a bimodal size distribution. Figure 7.9 shows the thymol microcapsules solution using the dark field option. In these images a fairly constant microcapsule thickness can be noticed that measurement annotation tools allowed estimating as around 2 μm .

Figure 7.10 shows *p* - Cymene microcapsules in the bright field option at different magnifications. It can be observed that microcapsules have a quite regular shape with a wide distribution of sizes, which confirm the bimodal size distribution of microcapsules, and one can notice also the absence of agglomerates.

The wall thickness of microcapsule was also confirmed by Gosh equation (4.2). This equation represents the relationship between the wall thicknesses and the microcapsule diameter. Table 7.1 shows wall thickness for vanillin and thymol microcapsules as determined by equation (4.1). These values are in good agreement with those obtained using the Leica software tools.

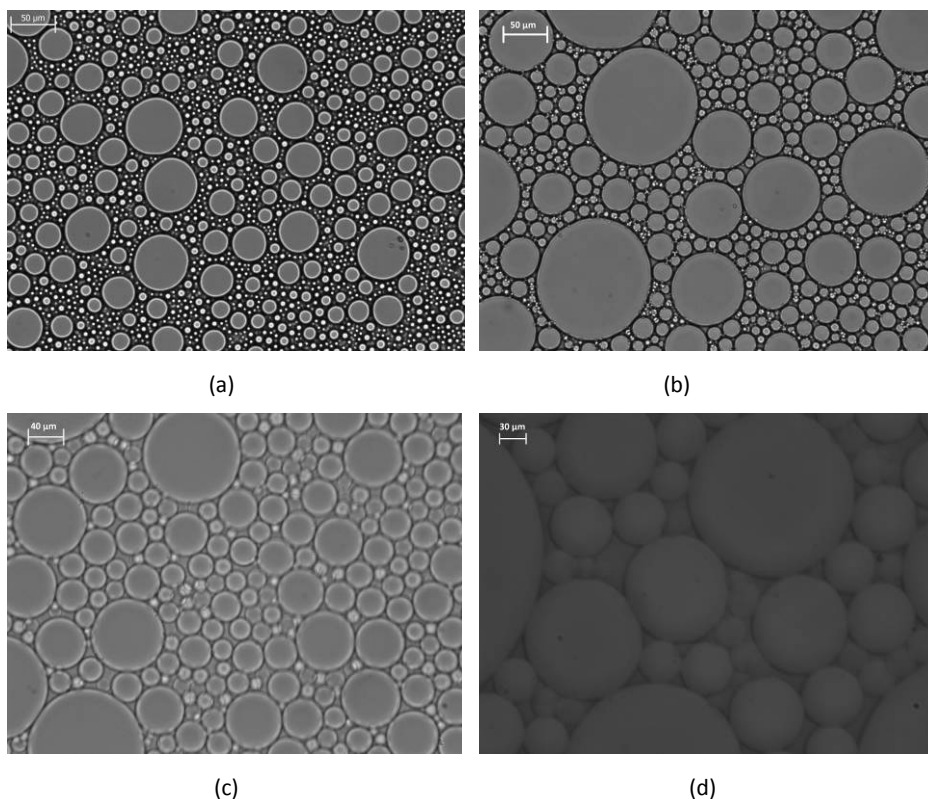


Figure 7.6. Optical microscopy of microcapsules solution of vanillin after the production and washing. Magnifications of images: a) 100x; b) 200x ; c) 400x; d) 1000x.

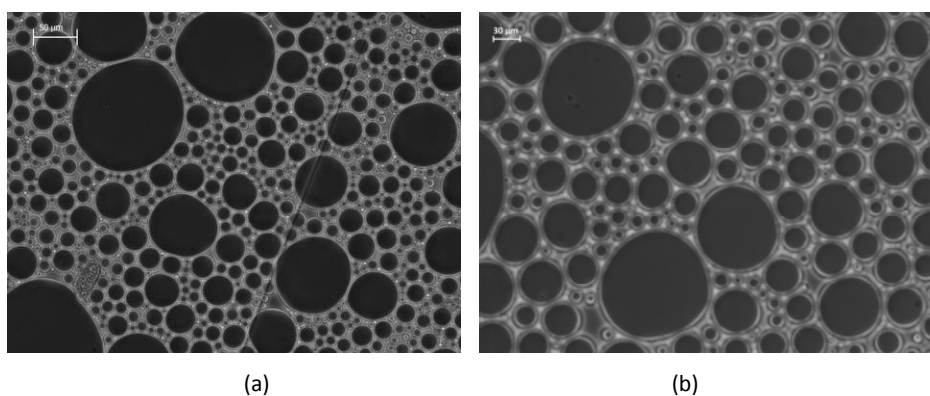


Figure 7.7. Optical microscopy of microcapsules solution of vanillin after the production and washing; images with dark field option. Magnifications of images: a) 200x ; b) 400x.

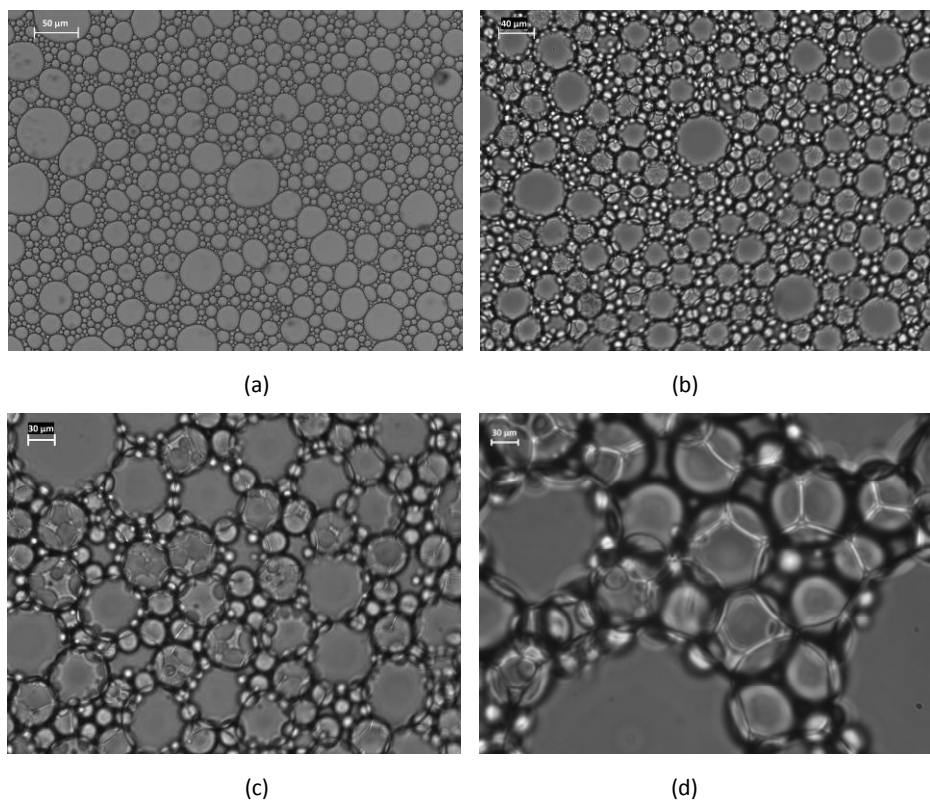


Figure 7.8. Optical microscopy of microcapsules solution of thymol after the production and washing. Magnifications of images: a) 100x ; b) 200x ; c) 400x ; d) 1000x.

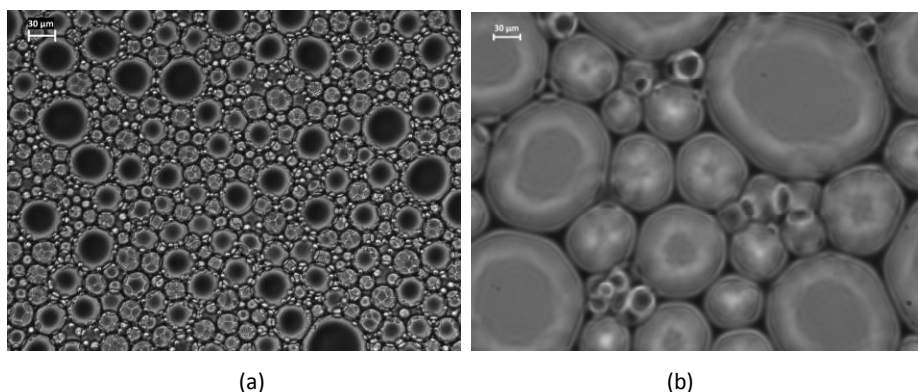


Figure 7.9. Optical microscopy of microcapsules solution of thymol after the production and washing; images with dark field option. Magnifications of images: a) 200x ; b) 1000x.

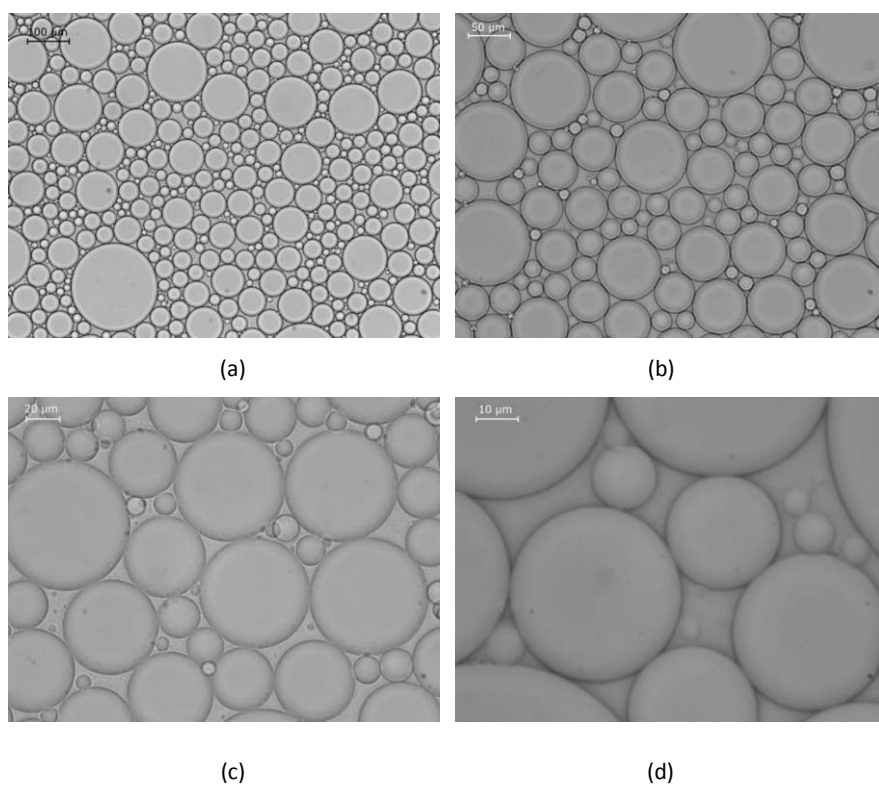


Figure 7.10. Optical microscopy of microcapsules solution of *p*-cymene after the production and washing. Magnifications of images: a) 100x; b) 200x ; c) 400x; d) 1000x.

7.4.3 Release study

In this work the release of vanillin, thymol and *p*-cymene through the PLA wall was studied using microcapsules in solution. A model to describe diffusion of model compounds across the polymer wall was also considered. Nevertheless, before analyzing the release of active principles it was necessary to characterize olive oil by GC/MS in order to verify that vanillin and thymol were not included in its composition (Figure 7.11). The component identification was made by comparison of the obtained mass spectra with some available reference spectra using NIST98 spectral library, pure reference compounds (own laboratory library) and literature data.

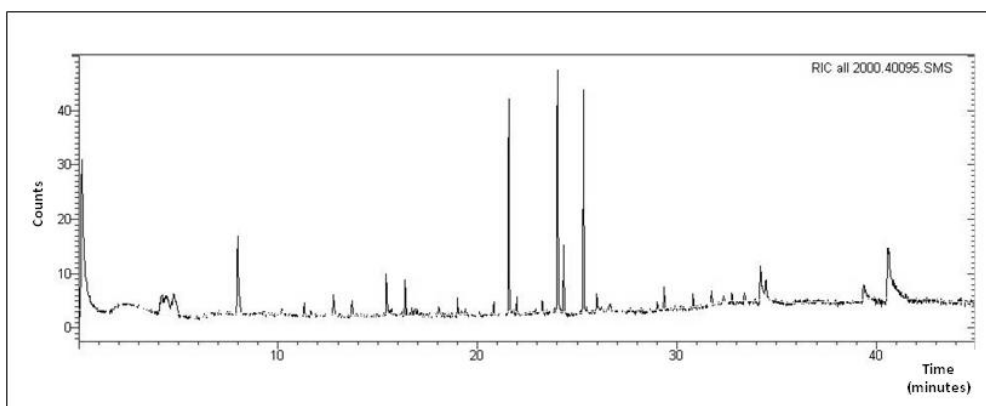


Figure 7.11. GC/MS chromatogram of olive oil analyzed on CP-Wax 52 CB bonded fused silica polar column. Identification numbers are according to table 7.2.

Under the same conditions, vanillin and thymol have retention time of 41.5 and 32.1 minutes respectively, and these peaks are not detected in the olive oil GC/MS chromatogram. Thus, analysis of the GC/MS chromatogram corroborates that vanillin and thymol are not present in olive oil. Quantification of the

nonencapsulated model compound was calculated based on GC-FID analysis and the mass of encapsulated oil calculated using the mass balance.

In the release studies with vanillin it could be observed that no amount of vanillin was detected in the outside microcapsules solution. It indicates that probably all the vanillin was encapsulated and stayed entrapped. Moreover, the used amount of vanillin is very small, due to its low solubility in olive oil and in all the microencapsulation process the dilution coefficient is very high. In fact, the used solvent (olive oil) to dissolve the core material (vanillin) has to be good solvent for vanillin and not present solubility in water. This condition allows the formation of the emulsion and promotes the precipitation of the polymer around the oil. However, the release of the olive oil from the microcapsules was detected by the presence of small representative peaks.

The release profiles of thymol and *p*-cymene in microcapsules solution of PLA along the first five days is shown in Figures 7.12 and 7.13. Furthermore, the experimental data for thymol release considering the first hour and subsequent days after production is shown in Figures 7.14 and 7.15. Figure 7.12 and Figure 7.13 show that the release of thymol and *p*-cymene is faster in first hour becoming constant over the subsequent five days period. The release tendency presents a rapid increase within the first hour of experiment, followed by an equilibrium period with slow increase. The diffusion behavior observed in Figures 7.12 and 7.13 is confirmed by the results presented in table 7.3 and 7.4. Table 7.3 shows the encapsulation efficiency and the mass released of thymol and *p*-cymene along the 5 days period after production.

The used coacervation process gave similar encapsulation efficiency as previously studied, around 55% for thymol and 83% for *p*-cymene. However, Table 7.4 indicates a slower release rate for thymol when it is used as model core material.

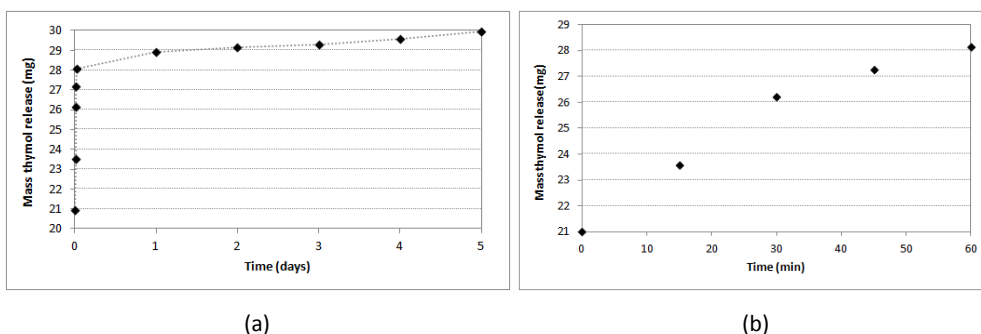


Figure 7.12. Experimental data for thymol release: (a) for the first five days and (b) in first hour.

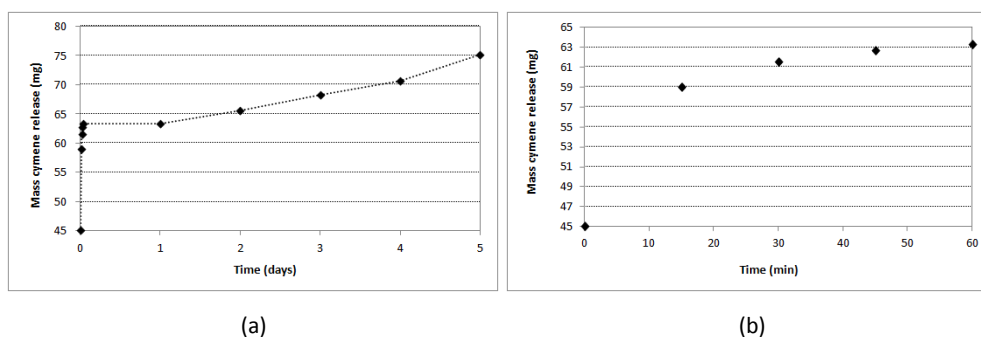


Figure 7.13. Experimental data for p-cymene release: (a) for the first five days and (b) in first hour.

It was observed that only 40% of the encapsulated thymol was released, while for *p*-cymene it was observed that 44 % of the encapsulated oil was released during the first day. This behavior is probably due to the fact that thymol was dissolved in olive oil which makes its diffusion slower. For the diffusion of the core material from the inner to the outer of microcapsule the oil needs to pass to the microcapsule surface, either through the polymer matrix or through water-filled pores (Hora *et al.*, 1990). Thus, the lipophilic components of olive oil could become more homogenously distributed in the polymer matrix which can be considered as lipophilic and interact with themselves (Martins *et al.*, 2011; Moritera *et al.*, 1991; Wischke *et al.*, 2008).

Table 7.3. Total, encapsulated and released masses and Encapsulation Efficiency (EE) discriminated to thymol and *p*-cymene in microcapsules solution of PLA.

Component	ENCAPSULATION		EE (%)	INITIAL CONDITION		RELEASE	
	mass _{total} (mg)	mass _{encapsulated} (mg)		mass _{in core initial} [*] (mg)	mass _{out initial} [*] (mg)	Δm released _{1 day} [*] (mg)	Δm released _{5 days} [*] (mg)
Thymol	130.480	71.790	55.02	50.861	20.929	7.968	9.017
<i>p</i> - Cymene	171.400	143.840	83.92	99.283	44.557	18.318	30.101

^{*} Calculated

^{*} Experimental

To evaluate the diffusion differences between thymol and *p*-cymene components, a comparative study between the experimental and theoretical results obtained using the model referenced in section 7.3 was performed. Figure 7.14 shows the release kinetics for thymol during the first hour of release and Figure 7.15 shows the release kinetics during a five days period.

Table 7.4. Percentage of oil release along the first five days discriminated by thymol and *p*-cymene in microcapsules solution of PLA.

Core material	Release (%)	
	1 day	5 days
Thymol	39.7	41.7
<i>p</i> -cymene	43.7	51.9

It was observed through Figure 7.14 that the diffusion coefficient was $1.99 \times 10^{-16} \text{ m}^2/\text{s}$ for thymol and the developed model was found to be in good agreement with the experimental measurements. For the apolar component, *p*-cymene, the diffusion coefficient during the first hour of release was $4.34 \times 10^{-16} \text{ m}^2/\text{s}$. As observed in Figure 7.15 the diffusion becomes slower after the first hour of release. The diffusion coefficient estimated considering a 5 days period was $3.34 \times 10^{-19} \text{ m}^2/\text{s}$ for thymol, as shown in Figure 7.15 and $3.45 \times 10^{-18} \text{ m}^2/\text{s}$ for *p*-cymene.

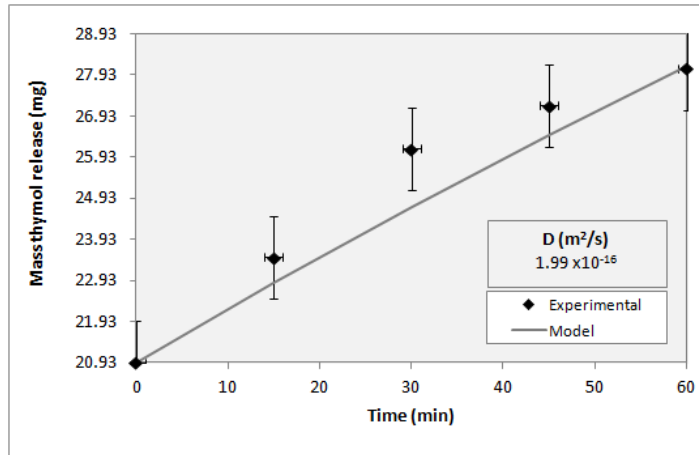


Figure 7.14. Comparison between experimental and model results for thymol released from PLA microcapsules in first hour.

$$m_1^0 = 50.861 \text{ mg}; m_2^0 = 20.929 \text{ mg}; V_1 = 9.79 \times 10^{-5} \text{ m}^3; V_2 = 9.50 \times 10^{-4} \text{ m}^3; m_2^{eq} = 65.086 \text{ mg}.$$

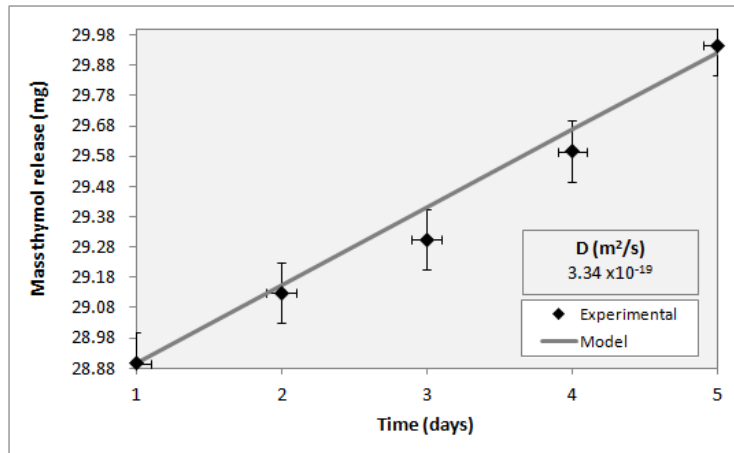


Figure 7.15. Comparison between experimental and model results for thymol released from PLA microcapsules for 5 days.

$$m_1^0 = 42.893 \text{ mg}; m_2^0 = 28.897 \text{ mg}; V_1 = 9.79 \times 10^{-5} \text{ m}^3; V_2 = 9.50 \times 10^{-4} \text{ m}^3; m_2^{eq} = 65.086 \text{ mg}.$$

7.5 Conclusions

The objective of the present study was to evaluate the release rate of vanillin, thymol and *p*-cymene through PLA microcapsules produced by a coacervation process followed by hardening with OCMTS. The average size of the microcapsules, as determined by the Laser Diffraction Particle Size Analyser measurements, was 21.38 μm for vanillin, 25.49 μm for thymol and 37.33 μm for *p*-cymene with bimodal distribution in volume and quite narrow distribution in number in all cases. Analysis by optical microscopy showed spherical particles and allowed to estimate a wall thickness of 1.53 μm for vanillin, 1.77 μm for thymol and 2.17 μm for *p*-cymene. Two predominant sizes of microcapsules, compatible with a bimodal distribution were observed, as well as, a total absence of agglomerates. The used encapsulation process originates a similar value of encapsulation efficiency, when compared with previous studies, for thymol and *p*-cymene (55% and 83%, respectively) and certainly all vanillin were encapsulated. The release studies pointed out for a slower release rate for thymol when used as single core material. The release of oils from the PLA microcapsules can be explained by a diffusion mechanism and the developed model was found to be in good agreement with the experimental measurements for the first hour of release. The diffusion coefficient was $1.99 \times 10^{-16} \text{ m}^2/\text{s}$ for thymol and $4.34 \times 10^{-16} \text{ m}^2/\text{s}$ for *p*-cymene in first hour of release and was $3.34 \times 10^{-19} \text{ m}^2/\text{s}$ for thymol and $3.45 \times 10^{-18} \text{ m}^2/\text{s}$ for *p*-cymene in period of 5 days of release.

7.6 References

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CHAPTER 8:

Conclusions and Future work

“There are only two ways to live your life. One is as though nothing is a miracle.
The other is as though everything is a miracle.”

[Albert Einstein]

In this work a novel coacervation technique for the microencapsulation of thyme oil with a biodegradable polymer (polylactide - PLA) was developed. Generally PLA has been used for the microencapsulation of hidrosoluble active principles, but not with oils. The novelty of the here developed process consists on dissolving PLA in dimethylformamide (DMF). This organic solvent is a good solvent for PLA and presents simultaneously high solubility in water; therefore it acts as a carrier to put PLA in contact with water thus promoting its precipitation around the thyme oil core. Particle size distributions were determined by laser dispersion and it was observed a bimodal distribution in volume with a mean particle size of 40 μm . Analysis by optical microscopy and by cryogenic scanning electron microscopy have confirmed the spherical shape, the rough surface with some visible pinholes, cracks or pores, and allowed to estimate the wall thickness around 5 μm . It was also detected two predominant sizes of microcapsules, compatible with a bimodal distribution and the absence of agglomerates confirmed.

Quantification of the encapsulated thyme oil was calculated based on GC-FID peak areas and the total percentage of phenols was 54.6%, with a major percentage of thymol (47.7%). The qualitative analysis of the encapsulated oil has showed that apolar compounds of thyme oil were preferentially encapsulated in detriment of the polar ones. The overall encapsulation efficiency of thyme oil was of 30.5%.

The effect of using different surfactants systems in the particle size distribution, morphology and thyme oil encapsulation yield was also investigated. It was concluded that when using Tergitol™ 15-S-9, a surfactant with a HLB of 13, an encapsulation yield of around 65% was obtained. The particle size analysis showed a bimodal distribution in volume with mean particle size around 30 μm for Tween® 80, Tergitol™ 15-S-9 and for the mixture 80 %Tergitol™ 15-S-9 + 20% Span® 85. Analysis by optical microscopy confirmed the spherical shape for all the produced microcapsules plus two predominant sizes, compatible with a bimodal distribution.

The release rate of thyme oil through the PLA microcapsules wall was evaluated and thymol and *p*-cymene chosen as representative of its polar and nonpolar components, respectively. It could be concluded that the release might be explained by a diffusion mechanism. The developed model was found to be in good agreement with the experimental measurements, both for the first hour of release and along a five days period. For the first hour of release, the diffusion coefficient was $1.39 \times 10^{-15} \text{ m}^2/\text{s}$ for thymol and $5.21 \times 10^{-17} \text{ m}^2/\text{s}$ for cymene. For a 5 days period of release, $3.81 \times 10^{-17} \text{ m}^2/\text{s}$ for thymol and $1.43 \times 10^{-18} \text{ m}^2/\text{s}$ for cymene, was determined. These differences can be ascribed to the distinct lipophilic solubility of the analysed thyme oil components and the obtained rather small diffusion coefficient values interpreted in terms of the very dense polymer matrix, which might constitute a significant hindrance effect. The developed diffusion model could be extended to other single-layer microcapsule systems.

The release of vanillin, thymol and *p*-cymene as model core materials through the PLA membrane was also studied and the diffusion coefficients for thymol and *p*-cymene were in accordance with the ones previously obtained. In what concerns the vanillin studies, no amount of vanillin was detected in the surrounded microcapsules solution, i.e., no release was observed pointed out that the vanillin stayed entrapped in the produced microcapsules.

In summary, a new, easy and executable method of coacervation was validated for the encapsulation of an oily active principle starting with the preparation of an o/w emulsion. Modifications to the former developed microencapsulation process, by testing different surfactants, allowed increasing the encapsulation efficiency of thyme oil and understand its effect on the encapsulation of polar and nonpolar components of the oil.

Scientific advances towards the development of microencapsulation processes are an imperative goal for the conception of innovative products and can become an asset for the creation of added-value products.

Suggestions for future work:

In order to extend and complement the results obtained in this investigation it is recommended:

1) Optimization of the microcapsules production.

The optimization of the process to produce PLA microcapsules needs a cyclic work of synthesis, analyses and characterization of microcapsules. The parameters that might be intensely studied should be the oil/polymer molar ratio, the amount of hardening agent, the effect of change of polymer solvent and the effect of stirring on microcapsules size. The consolidation and washing steps of microcapsules production, as well as the molar ratio of oil/water should be improved to obtain a better productivity of microcapsules per reactor volume.

2) Produce microcapsules in a microreactor.

Produce PLA microcapsules in a flat microreactor constituted of two half pieces in which we have channels for the feed of reactants and at fixed distances mixing chambers. This basic unit is then repeated. This is in line with new microchannel technologies as those developed by LSRE and Fluidinova (NetMix) which has been successfully applied and patented for hydroxyapatite nanoparticles continuous production and that is been already used for production and commercialization. Other possibilities are the T-Jet mixers (also available at LSRE) or other commercial prototypes as those from Velocys (USA).

3) Test the developed process with other biodegradable polymers, such as polylactide-co-glycolide (PLGA) and Polyglycolide (PGA), and perform the microcapsules characterization (physico-chemical, morphological and release behaviour).

4) Improve the technique used for the study of release of thyme oil through the PLA microcapsules. Develop a new method for studying the release of oil, that is less subject to interference from external factors and determine the influence of microcapsules wall characteristics, such as thickness, porosity and type of polymer on the release of active agent.

5) Determine the antimicrobial activity of the produced microcapsules. Thyme oil is an essential oil with antimicrobial characteristics and it would be interesting to assess their ability against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and a yeast.

6) Incorporate the microcapsules in final products, such as creams and soaps, and evaluate its performance. This is an important step to promote the production of microcapsules at a larger scale enabling their introduction in an industrial process.

APPENDICES

Appendix A – Core Materials Safety Data Sheets

A1 – Thyme oil

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Thyme oil

Product Number: W306401

Brand: Aldrich

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Thyme oil; Thymus vulgaris

Formula: C₁₀H₁₄O

Molecular Weight: 150,2 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form - liquid

Colour - colourless

Safety data

pH - no data available

Melting point: no data available

Boiling point: - 190 °C

Flash point: 60 °C - closed cup

Density: 0,916 g/cm³ at 25 °C

Water solubility - no data available

4. HAZARDS IDENTIFICATION

Symbol	  GH502, GH505
Signal word	Danger
Hazard statements	H226-H314
Precautionary statements	P280-P305 + P351 + P338-P310
Personal Protective Equipment	Face shields, full face respirator (US), Gloves, Goggles, multi purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	C
Risk Statements	34
Safety Statements	26-36/37/39-45
RIDADR	UN 2924 3/PG 3
WGK Germany	2
RTECS	XP2000000
Flash Point(F)	140 °F
Flash Point(C)	60 °C

A2 – Thymol

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Thymol

Product Number: 83558.180

Brand: BDH Prolabo

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: 5-Methyl-2-isopropylphenol; 5-Methyl-2-(1-methylethyl)phenol

Formula: C₁₀H₁₄O

Molecular Weight: 150,22 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form crystalline

Colour colourless

Safety data

pH 7 at 1 g/l

Melting point: 48 - 51 °C - lit.

Boiling point :232 °C - lit.

Vapour pressure: 1 hPa at 64 °C

Density: 0,965 g/cm³ at 25 °C

Water solubility no data available

4. HAZARDS IDENTIFICATION

Symbol	 GH505, GH507, GH509
Signal word	Danger
Hazard statements	H302-H314-H411
Precautionary statements	P273-P280-P305 + P351 + P338-P310
Personal Protective Equipment	dust mask type N95 (US), Eyeshields, Faceshields, full-face particle respirator type N100 (US), Gloves, respirator cartridge type N100 (US), type P1 (EN143) respirator filter, type P3 (EN 143) respirator cartridges
Hazard Codes	C, H
Risk Statements	22-34-51/53
Safety Statements	26-28-36/37/39-45-61
RIDADR	UN 3261 8/PG 3
WGK Germany	2
RTECS	XP2275000
Flash Point(F)	230 °F
Flash Point(C)	110 °C

A3 – Cymene

1. IDENTIFICATION OF THE SUBSTANCE

Product name: *p*-Cymene (96%)

Product Number: ALFAA19226.AP1

Brand: Alfa Aesar

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: *p*-Cymene; *p*-Isopropyltoluene; Dolcymene

Formula: C₁₀H₁₄

Molecular Weight: 134.22 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Safety data

Boiling Point (°C): 176 - 178

Melting Point (°C): -68

Refractive index: 1.489 - 1.491

4. HAZARDS IDENTIFICATION

Symbol	  GH502, GH507
Signal word	Warning
Hazard statements	H226-H315-H319-H335
Precautionary statements	P261-P305 + P351 + P338
Personal Protective Equipment	Eyeshields, Faceshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	Xi
Risk Statements	10-36/37/38
Safety Statements	26-36
RIDADR	UN 2046 3/PG 3
WGK Germany	2
RTECS	GZ5950000
Flash Point(F)	116.6 °F
Flash Point(C)	47 °C

A4 – Vanillin

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Vanillin

Product Number: W310700

Brand: Aldrich

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: 4-Hydroxy-3-methoxybenzaldehyde

Formula: C₈H₈O₃

Molecular Weight: 152,15 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: solid

Colour: light yellow

Safety data

Melting point/range: 81 - 83 °C - lit.

Initial boiling point: 170 °C at 20 hPa - lit.

Flash point: 153 °C - closed cup

Vapour pressure: 1 hPa at 107 °C; < 0,01 hPa at 25 °C; 0,0022 hPa at 25 °C

Relative density: 1.056 g/cm³ at 20 °C

Water solubility: 10 g/l at 25 °C - slightly soluble

4. HAZARDS IDENTIFICATION

Symbol	 GHS07
Signal word	Warning
Hazard statements	H302-H317-H319
Precautionary statements	P280-P305 + P351 + P338
Personal Protective Equipment	dust mask type N95 (US), Eyeshields, Gloves
Hazard Codes	Xn
Risk Statements	22
WGK Germany	1
RTECS	YW5775000
Flash Point(F)	307,4 °F
Flash Point(C)	153 °C

Appendix B – Wall Material Safety Data Sheet

B1 – Polylactide

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Poly(D,L-lactide),
inherent viscosity 0.55-0.75 dL/g (lit.)

Product Number: 531162

Brand: Aldrich

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Poly(D,L-lactide)

Formula: (C₃H₄O₂)_n

Molecular Weight: average Mw 75,000-120,000

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: Crystals

Colour: White to Dark Yellow

Safety data

Melting point/range: 262 °C

Tin (Sn) ≤200 ppm

Inherent viscosity: 0.55-0.75 dL/g (lit.)

Transition temp: Tg 32.9 °C

4. HAZARDS IDENTIFICATION

Personal Protective Equipment	Eyeshields, Gloves, type N95 (US), type P1 (EN143) respirator filter
WGK Germany	3

B2 - Dimethylformamide

1. IDENTIFICATION OF THE SUBSTANCE

Product name: N,N-Dimethylformamide

Product Number: 319937

Brand: Sigma-Aldrich

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: DMF

Formula: C₃H₇NO

Molecular Weight: 73,09 g/mol.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: liquid, clear

Colour: colourless

Safety data

pH: 6,7

Melting point/range: 61 °C - lit.

Boiling point: 153 °C - lit.

Flash point 58 °C - closed cup

Vapour pressure: 3,60 hPa at 20 °C; 5,16 hPa at 25 °C

Vapour density: 2,52 - (Air = 1.0)

Relative density :0,944 g/mL

Water solubility: completely miscible

4. HAZARDS IDENTIFICATION

Symbol	 GH502, GH507, GH508
Signal word	Danger
Hazard statements	H226-H312-H319-H332-H360D
Precautionary statements	P201-P280-P305 + P351 + P338-P308 + P313
Personal Protective Equipment	Eyeshields, Faceshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	T
Risk Statements	61-20/21-36
Safety Statements	63-46
RIDADR	UN 2265 3/PG 3
WGK Germany	1
RTECS	1.02100000
Flash Point(F)	136.4 °F
Flash Point(C)	58 °C

Appendix C – Hardening Agent Safety Data Sheet

C1 – Octamethyltetrasiloxane

1. IDENTIFICATION OF THE SUBSTANCE

Product name:

Product Number:

Brand:

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Octamethylcyclotetrasiloxane

Formula: C₈H₂₄O₄Si₄

Molecular Weight: 296.62 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: liquid

Colour: colourless

Safety data

Melting point/range: 17 - 18 °C - lit.

Initial boiling point and boiling range: 175 - 176 °C - lit.

Flash point: 56 °C - closed cup

Vapour density: 10,24 - (Air = 1.0)

Relative density: 0,956 g/mL at 25 °C

4. HAZARDS IDENTIFICATION

Symbol	 GHS08
Signal word	Warning
Hazard statements	H361f-H413
Precautionary statements	P281
Personal Protective Equipment	Eyeshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	Xn
Risk Statements	53-62
Safety Statements	36/37-46-51-61
RIDADR	UN 1993 3/PG 3
WGK Germany	3
RTECS	GZ4397000
Flash Point(F)	132.8 °F
Flash Point(C)	56 °C

Appendix D – Surfactants Safety Data Sheets

D1 – Tween®20

1. IDENTIFICATION OF THE SUBSTANCE

Product name: TWEEN® 20

Product Number: P5927

Brand: Sigma

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: polyethylene glycol sorbitan monolaurate, Polyoxyethylenesorbitan monolaurate

Formula: C58H114O26

Molecular Weight: mol wt ~1228

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: viscous liquid

Safety data

pH: 7

Boiling point: 100 °C

Flash point > 110,00

Vapour pressure: < 1,33 hPa

Density: 1,095 g/mL

Water solubility: soluble

4. HAZARDS IDENTIFICATION

Personal Protective Equipment	Eyeshields, Gloves
WGK Germany	1
RTECS	TR7400000
Flash Point(F)	>230 °F
Flash Point(C)	>110 °C

D2 – Tergitol™ 15-S-9

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Tergitol®

Product Number: 15S9

Brand: Sigma

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Secondary Alcohol Ethoxylate

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance Pale: yellow liquid

Safety data

Actives, wt% 100

Cloud Point: 60 °C

pH, 1% aq solution 7.1

Viscosity at 25°C (77°F), cP 60

Density at 20°C (68°F), g/mL 1.006

Flash Pt, Closed Cup, ASTM D93 193°C 380°F

Soluble in water

4. HAZARDS IDENTIFICATION

Symbol	 GHS09
Hazard statements	H411
Precautionary statements	P273
Personal Protective Equipment	Eyeshields, Gloves, half-mask respirator (EU), half-mask respirator (US), multi-purpose combination respirator cartridge (US)
Hazard Codes	Xn,II
Risk Statements	22-38-41-51/53
Safety Statements	26-39-61
RIDADR	UN 3082 9/PG 3
WGK Germany	3
Flash Point(F)	379.4 °F
Flash Point(C)	193 °C

D3 – Tween® 80

1. IDENTIFICATION OF THE SUBSTANCE

Product name: TWEEN® 80

Product Number: P1754

Brand: Sigma-Aldrich

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Polyethylene glycol sorbitan monooleate; Polyoxyethylenesorbitan monooleate;

Polysorbate 80

Molecular Weight: average mol wt 1310

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: viscous liquid

Colour: yellow

Safety data

Boiling point: 100 °C

Flash point: 113 °C - closed cup

Vapour pressure: < 1 hPa at 20 °C

Density: 1,064 g/cm³

Water solubility: soluble

4. HAZARDS IDENTIFICATION

Personal Protective Equipment	Eyeshields, Gloves
WGK Germany	1
RTECS	WG2932500
Flash Point(F)	235.4 °F
Flash Point(C)	113 °C

D4 – Span® 85

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Span® 85

Product Number: S7135

Brand: Sigma

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Sorbitane trioleate

Formula: C60H108O8

Molecular Weight: 957,52 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: clear, viscous

Colour: dark brown

Safety data

Flash point 113 °C - closed cup

Density 0,952 g/cm3

4. HAZARDS IDENTIFICATION

Symbol	 GHS07
Signal word	Warning
Hazard statements	H315-H319
Precautionary statements	P305 + P351 + P338
Personal Protective Equipment	Eyeshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	Xi
Risk Statements	38
Safety Statements	26
WGK Germany	1
RTECS	WG2934550
Flash Point(F)	235.4 °F
Flash Point(C)	113 °C

Appendix E – Washing Solvents Safety Data Sheets

E1 – Pluronic®/F68

1. IDENTIFICATION OF THE SUBSTANCE

Product name: phase Synperonic PE/F68

Product Number: 81112

Brand: Fluka

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol); Pluronic® F68; Poly(propylene glycol)-block-poly(ethylene glycol)-block-poly(propylene glycol)

Formula: C₅H₁₄O₄

Molecular Weight: 138,2 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: solid

Safety data

Melting point: 58 °C

Relative density: 1,050 g/cm³

4. HAZARDS IDENTIFICATION

Symbol	 GHS07
Signal word	Warning
Hazard statements	H319
Precautionary statements	P305 + P351 + P338
Personal Protective Equipment	Eyeshields, Gloves, type N95 (US), type P1 (EN143) respirator filter
Safety Statements	23-24/25
WGK Germany	2
RTECS	MD0911050

E2 – Ethanol

1. IDENTIFICATION OF THE SUBSTANCE

Product name: Ethanol absolute PA

Product Number: 121086.1212

Brand: PANREAC

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: Ethyl Alcohol

Formula: C₂H₆O

Molecular Weight: 46,07 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: liquid clear

Colour: colourless

Safety data

Boiling Point (°C): 78,5 °C

Melting Point (°C): -114,1 °C

Flash Point: 14 °C

Ignition Temperature: 425 °C

Vapour pressure: (20 °C) 59 hPa

Viscosity: 25 °C 1,2 mPa.s

Miscible with water and most of the solvents

4. HAZARDS IDENTIFICATION

Symbol	 GHS02
Signal word	Danger
Hazard statements	H225
Precautionary statements	P210
Personal Protective Equipment	Eyeshields, Faceshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	F
Risk Statements	11
Safety Statements	7-16
RIDADR	UN 1170 3/PG 2
WGK Germany	1
RTECS	KQ6300000
Flash Point(F)	57.2 °F
Flash Point(C)	14 °C

E3 - n-Hexane

1. IDENTIFICATION OF THE SUBSTANCE

Product name: n-Hexano p.a. ACS, Reag. Ph Eur

Product Number: 104374

Brand: Merck Schuchardt OHG

2. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms: 1-methyl pentane, Hexanes, Isohexane

Formula: C₆H₁₄

Molecular Weight: 86.18 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form: liquid

Colour: colourless

Safety data

Boiling Point (°C): 69 °C (1013 hPa)

Melting Point (°C): -94.3 °C

Autoignition temperature: 240 °C

4. HAZARDS IDENTIFICATION

Symbol	 GHS02, GHS07, GHS08, GHS09
Signal word	Danger
Hazard statements	H225-H302-H304-H315-H319-H335-H336-H361-H373-H411
Precautionary statements	P210-P261-P273-P281-P301 + P310-P305 + P351 + P338
Personal Protective Equipment	Eyeshields, Faceshields, full-face respirator (US), Gloves, multi-purpose combination respirator cartridge (US), type ABEK (EN14387) respirator filter
Hazard Codes	F, Xn, N
Risk Statements	11-38-48/20-51/53-62-65-67
Safety Statements	36/37-61-62
RIDADR	UN 1208 3/PG 2
WGK Germany	3
Flash Point(F)	-9.4 °F
Flash Point(C)	-23 °C

Appendix F –List of Scientific Publications**F1- Full papers in peer-reviewed international journals**

- (1) Rodrigues, S. N.; Fernandes, I.; Martins, I. M.; Mata, V. G.; Barreiro, F.; Rodrigues, A. E. (2008). Microencapsulation of limonene for textile application. *Industrial & Engineering Chemistry Research* 47(12): 4142-4147.
- (2) Martins, I. M.; Rodrigues, S. N.; Barreiro, F.; Rodrigues, A. E. (2009). Microencapsulation of thyme oil by coacervation. *Journal of Microencapsulation* 26(8): 667-675.
- (3) Rodrigues, S. N.; Martins, I. M.; Fernandes, I. P.; Gomes, P. B.; Mata, V. G.; Barreiro, M. F.; Rodrigues, A. E. (2009). Scentfashion (r): Microencapsulated perfumes for textile application. *Chemical Engineering Journal* 149(1-3): 463-472.
- (4) Martins, I. M.; Rodrigues, S. N.; Barreiro, M. F.; Rodrigues, A. E. (2010). Polylactide-based thyme oil microcapsules production: Evaluation of surfactants. *Industrial & Engineering Chemistry Research* 50(2): 898-904.
- (5) Rodrigues, S. N.; Martins, I. M.; Mata, V. ; Barreiro, M. F.; Rodrigues, A. E. (2011). Characterization and evaluation of commercial fragrance microcapsules for textile application. *Journal of the Textile Institute* 103(3): 269-282.
- (6) Teixeira, M. A.; Rodríguez, O.; Rodrigues, S.; Martins, I.M.; Rodrigues, A. E. (2011). A case study of product engineering: Performance of microencapsulated perfumes on textile applications. *Aiche Journal*: n/a-n/a. (In press) DOI: 10.1002/aic.12715.
- (7) Martins, I. M. ;Rodrigues, S. N.; Barreiro, M. F.; Rodrigues, A. E. (2011). Release of thyme oil from polylactide microcapsules. *Industrial & Engineering Chemistry Research* 50(24): 13752-13761.

F2- Conference Proceedings

- (1) Rodrigues, S.; Fernandes, I.; Martins, I.; Barreiro, F.; Mata, V.; Rodrigues, A. Microencapsulação de limoneno para aplicação têxtil. XVII COBEQ - Engenharia Química: Energia e novos desafios, Recife, Brasil, 14 a 17 Setembro de 2008. Paper ID:667
- (2) Martins, I. M.; Barreiro, F.; Rodrigues, A. E. Microencapsulation of Thyme oil by coacervation. CHEMPOR 2008 "10th International Chemical and Biological Engineering Conference", Braga, Portugal, págs. 431-432, Setembro 2008.
- (3) Martins, I. M.; Rodrigues, S. N.; Barreiro, F.; Rodrigues, A. E. Microencapsulation by coacervation of biodegradable polymer with thyme oil. "Particles 2009: Micro and Nano Encapsulation", Berlim, Alemanha, págs. 49-50, 11-14 Julho 2009.
- (4) Rodrigues, S. N.; Martins, I. M.; Barreiro, F.; Rodrigues, A. E. Synthesis of polyurethane-urea microcapsules with perfume for textile application. "Particles 2009: Micro and Nano Encapsulation", Berlim, Alemanha, págs. 64, 11-14 Julho 2009.
- (5) Rodrigues, S. N.; Martins, I. M.; Barreiro, F.; Rodrigues, A. E. TECHNOLOGIES FOR PRODUCING MICROCAPSULES WITH ADDED VALUE. "WCCE8 - 8th WORLD CONGRESS OF CHEMICAL ENGINEERING". Montréal, Canadá, 23-27 Agosto 2009.
- (6) Martins, I. M.; Rodrigues, S. N.; Barreiro, F.; Rodrigues, A. E. Evaluation of surfactants of thyme oil microcapsules prepared by coacervation. "XVIII International Conference on Bioencapsulation - Porto, Portugal - October 1-2, 2010", Paper ID:P010
- (7) Martins, I. M.; Barreiro, F.; Rodrigues, A. E. Release studies of essential oil of *Thymus vulgaris* L. from PLA microcapsules. CHEMPOR 2011 "11th International Chemical and Biological Engineering Conference", Lisboa, Portugal, 5 a 7 de Setembro de 2011. Paper ID:432