Encapsulation and Co-Precipitation Processes with Supercritical Fluids: Applications with Essential Oils

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Abstract: Essential oils have important commercial applications as preservatives and flavours, and more recently as natural antimicrobial agents. These applications require a suitable formulation constituted by biodegradable compounds that protect the essential oil from degradation and evaporation at the same time that allows for a sustained release. Microcapsules of biopolymers loaded with essential oils meet these requirements. Such microcapsules can be prepared with different processes such as spray-drying, freeze-drying and coacervation, and supercritical fluids are an advantageous medium for this purpose. Some supercritical fluid-based precipitation processes have already been applied to produce these microcapsules. Amongst them, the results obtained with Particles from Gas Saturated Solutions (PGSS), PGSS-drying and Concentrated Powder Form (CPF) processes are particularly promising. Recent developments in the preparation of formulations with supercritical fluids include the preparation of liposomes and micelles, which can be suitable carriers for essential oils.

Keywords: Supercritical carbon dioxide, spray drying, emulsion, particles from gas saturated solutions, concentrated powder form, micelle, liposome.

1. INTRODUCTION

Essential oils are the ethereal fraction obtained by physical means from a plant. Nowadays, their main uses are in perfume and flavour industries although they are also used in food and pharmaceutical industries as preservatives (mainly antioxidants) and flavours, for applications like cosmetics and personal care products, pharmaceutical products, insecticides, preparations for paints or textile industries, additives for rubber and plastics or adhesives [1]. More recently, many applications of essential oils as natural antimicrobial agents have been described. Compounds in essential oils with phenolic structure have been identified to be active against microorganisms [2, 3]. Burt [4] has selected some plants that can be used in food industry owing to their antibacterial properties: cilantro, coriander, cinnamon, oregano, rosemary, sage, clove and thyme. Table **1** shows some reported applications of essential oils [5-26].

Essential oils are sensitive materials which can easily suffer degradation under the action of oxygen, light and moderate temperatures. Furthermore, they are insoluble in water, and for certain applications a controlled release is required. Therefore an adequate formulation of the essential oil which takes into account these aspects is required for commercial applications. Common goals in the development of essential oil formulations are to protect the essential oil from degradation or from losses by evaporation, to achieve a controlled release, and to facilitate handling. Possible formulations include liquid forms (emulsions, micelles, liquid solutions etc.), semi-liquid forms (gels, liposomes etc.), and solid forms (microcapsules or microcomposites).

Essentials oils exist in liquid form at room temperature. Therefore the simplest form of encapsulation consists in emulsifying or dispersing the components in an aqueous solution. The main drawback of this formulation is the handle difficulty. This problem can be overcome by producing a dry formulation by microencapsulation, entrapping the oil drops in a carrier material.

Encapsulation techniques can be divided into three classes: chemical processes like molecular inclusion or interfacial polymerization, physicochemical techniques like coacervation and liposome encapsulation and physical processes like spray drying, spray chilling/cooling, co-crystallization, extrusion or fluidized bed coating. The use of supercritical fluids as an alternative medium for formulating essential oils can improve the results obtained with other physical or physicochemical techniques, or even make possible innovative formulations, due to the peculiar properties of supercritical fluids in general and of supercritical carbon dioxide in particular (adjustable solvent power, favourable transport properties, no contamination of the product) which are significantly different from those of gas and liquid solvents [27]. The objective of this review is to provide an account of the principal technologies used for encapsulation of essentials oils, and to present innovative applications of supercritical fluids in this field.

2. COATING MATERIALS

The coating or carrier material plays an important role because it is responsible for the protection of the oil and for enabling a controlled release. This material must be selected

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Table 1. Reported Applications of Essential Oils

Essential Oil	Application	Ref.
Artemisia afra, Pteronia incana oils, Lavandula officinalis, and Rosmarinus officinalis	Preservatives in aqueous cream formulation	[5]
Australian tea tree oil	Recurrent herpes labialis	[6]
Basil	Tratment for acne	[7]
Blend of the spices Capsicum, Cinamon, anise and oregano oil	Feed complement for piglets	[8]
Chamomile	Treatment of inflammatory dermatoses	[9]
Chamomile	Insect bite, dry skin, sunburn, acne and psoriasis	[10]
Cinnamon, clove	Yoghurt conservation	[11]
Cinnamomum zeylanicum (cinnamon), Mentha piperita (peppermint), Ocimum basilicum (basil), Origanum vulgare (origanum), Teloxys ambrosioides (the flavoring herb epazote), Syrygium aromaticum (clove), and Thymus vulgaris (thyme)	Inhibition of fungal development in Maize	[12]
Cinnamon bark (Cinnamomum zeylanicum), lemongrass (Cymbopogon flexuosus), savory (Satureja montana), Roman chamomile (Chamaemelum nobile), rosewood (Aniba rosaeodora), spearmint (Mentha spicata) and tea tree (Melaleuca alternifolia)	Selected Bacteria, Fungi and Viruses	[13]
Cymbopogon citratos	Inhibitor of storage fungi	[14]
Eucalyptus citriodora, Eucalyptus hybrida and Ocimum basilicum	Nematicidal activity	[15]
Laurus nobilis, Eucalyptus globulus and Salvia officinalis	Preservatives in O/W skin cream, a hydrogel and a non-alcoholic hydrolyte	[16]
Lavender	Bites, stings, boils, burns, stretch marks, rashes, spots, cold sores, sunburns	[17]
Lavender	Allergies	[18]
Lemongrass	Storaged grain protection	[19]
Oregano	Fish food conservation	[20]
Oregano and Thyme	Staphylococcus aureus and Salmonella typhimurium	[14]
Pyrethrum and neem	Pesticides	[21]
Rosemary, sage	Comercial food preservative	[22]
Tea tree oil and eucalyptus oil	Herpes simplex virus type 1	[23]
Thyme	Nosema disease in honeybees	[24]
Thyme (Thymus vulgaris L.), Oregano (Origanum vulgare ssp. Hirtum), Clove (Syzygium aromaticum), nutmeg (Myristica fragrans), black pepper (Piper nigrum L.), geranium (Pelargonium graveolens)	Gram positive and Gram negative Bacteria	[25]
Vetiver essential oil	Protection of wood against Termites	[26]

depending on the specific oil to be coated and the desired characteristic of final microcapsules. Ideally, the coating material should be soluble in water, biodegradable, form low viscosity solutions, yield powders with specific properties (non-hygroscopic, non-porous, soluble, stable, etc.), have a low cost and be easy to dry and non reactive.

In applications with supercritical fluids the properties of the coating material and particularly the interactions of coating materials with supercritical fluids are especially important. It is well known that supercritical carbon dioxide interacts with many polymeric materials, causing several important variations in their physical properties such as swelling, reduction of cristallinity or reduction of melting and glass transition temperatures [28, 29]. These interactions may be important for enabling the incorporation of essential oils into carrier materials, for example by facilitating the diffusion of the essential oil due to the swelling and opening of the pores of carrier material particles.

Different coating materials have been used in encapsulation techniques, including gums, starches, gelatines and polymers. Most commonly used wall materials for essential oil encapsulations are octenyl succinic anhydride (OSA)

starches, β-cyclodextrines, acacia gums, maltodextrines and its mixtures. Recently chitosan and phospholipids have been used as coating materials as well.

Acacia gum is widely used because it presents favourable properties as emulsifier [30]. However, it is expensive, it suffers supply shortages and acacia gum capsules present a limited protective capacity against oxidation because they act as semi permeable membranes. The use of mixtures of gum and maltodextrines results in good relation between price and efficiency [31].

Hydrolysed and chemically modified starches (OSA-starches) are less expensive, very soluble in water, with adequate heat tolerance, and OSA-starch solutions have moderate viscosities [32]. OSA-starches can be effectively used to form an essential oil-in-water emulsion, which can be then dried to produce microcapsules [33].

β-cyclodextrines are enzymatically modified starches shaped like a hollow cone, which had been widely used for encapsulation of essential oils giving some of the highest encapsulation yields, due to the formation of inclusion complexes between the oil and the apolar cavity of cyclodextrines [34]. The inclusion complex protects the oil efficiently during storage but it has low thermal stability [31].

Chitosan is a natural linear biopolyaminosaccharide, biodegradable, biocompatible, mucoadhesive and its production is economically feasible (chitin is the second most abundant polymer in the nature after cellulose). For these reasons it had been widely used in medicine applications, and it is also suitable for essential oil formulations [35, 36].

Phospholipids (phosphatidylcholines, phosphatidylserines and phosphatidylethanolamines) are a class of amphiphilic lipids formed by a hydrophilic head (a phosphate group, a diglyceride and a simple organic molecule) and a hydrophobic tail (long fatty acid), which spontaneously form liposomes, bilayer sheets, micelles, or n-lamellar structures, depending on hydration and temperature. Liposomes can encapsulate hydrophilic compounds in the aqueous internal cavity, and hydrophobic substances can be dissolved into the membrane. Because of their ease of biodegradation and their similarity to cell biomebranes, they are less toxic than polymer or other wall materials and they are excellent carrier systems for a variety of applications, and in particular for essential oils [37, 38].

3. FORMULATION OF ESSENTIAL OILS BY TECHNIQUES NOT BASED ON SUPERCRITICAL FLUIDS

3.1. Spray-Drying

Microencapsulation by spray-drying is one of the oldest encapsulation methods, as well as one of the most common and cheapest techniques for producing microcapsules. In this method, the material for encapsulation is homogenized with the carrier material at different ratios. Then the mixture is fed into a spray dryer and atomized with a nozzle or spinning wheel. The contact between drop and hot air takes place during atomization. Rapid evaporation of solvent (usually water) maintains droplet temperature at low level and allows quasi-instantaneously entrapping the active compound. The microcapsules are then collected after they fall to the bottom of the drier [39]. The liquid feeding the sprayer can be a solution, an emulsion or a suspension. The main factors in spray-drying that must be optimized are feed temperature, air inlet temperature, and air outlet temperature [40].

Morphology of final product is related to drying conditions. Furthermore the key mass transport phenomenon in the spray drying process is the drying of individual atomized droplets in the chamber. Evaporation stages and heat and mass transfer in spray drying can be described through the evolution of drying rates, as it was suggested by Alamilla-Beltran [41]. Five stages of drying were proposed by Dolinsky [42], which are heating from its initial temperature to the equilibrium evaporation temperature, equilibrium evaporation, crust formation, boiling period and bound moisture removal period.

Spray drying is widely used for the drying of heat-sensitive food, pharmaceuticals and other substances such as essential oils. Applications of spray-drying for encapsulating essential oils are reviewed in Table **2** [43-60]. Typical encapsulation materials used with essential oils are acacia gum, maltodextrins, hidrophobically modified starches, proteins and mixtures of thereof.

3.2. Other Techniques

Different techniques have been successfully used for preparing essential oil-loaded microcapsules. Two of the most successful methods apart from spray drying are freeze-drying and coacervation. Other techniques such as annular-jet or spinning-jet drying can be used to produce particles loaded with liquids such as essential oils. Table **3** shows some applications of these techniques for essential oil formulation [34-38, 61-75].

In the freeze-drying process solvent is removed from a frozen solution by vacuum sublimation, maintaining the drying chamber pressure and temperature bellow the triple point of solvent. Freeze drying appears as one of the most suitable methods for dehydration of almost all heat-sensitive materials and aromas, due to the lower operating temperatures, slow drying rate and to the use of vacuum. This technique has also been used to encapsulate essentials oils [37, 61].

Coacervation consists in the separation of a polymeric solution into two liquid phases, a polymer-rich phase called coacervate and diluted phase called equilibrium solution, induced by media modifications (pH, ionic strength and polyion concentrations), followed by the coating of the coacervate phase around suspended core particles or suspended droplets. Complex coacervation is based on electrostatic interactions between two oppositely charge polyions in aqueous media, in which the core material in dispersed form is added to the polymer solution. This mixture is then suspended in an aqueous solution phase containing a surface-active agent. Finally, solidification of the coating is achieved by thermal, cross-linking or desolventization techniques. Another closely related technique is the organic phase separation, which can be considered as a reversed simple coacervation: a polymer phase separates and deposits on a core that is suspended in an organic solvent rather than in water Several essentials oils were encapsulated by coacervation employing gelatine [37, 62], proteins [63] and other polymers [64].

Table 2. Applications of Spray-Drying for Encapsulation of Essential Oils

Essential Oil	Coating Material	Operation Conditions			Encapsulation Efficiency %	Ref.
		Inlet air T (ºC)	Outlet air T (ºC)	Feed Rate		
Caraway fruit oil	Maltrodextrine and gum arabic	200	80 ± 2			[43]
	HI-CAP100					
	β-cyclodextrin				75%	
Peppertmint (*Mentha piperita*)	OSAN-starches	200 ± 10	120 ± 10		79%	[44]
	Acid and enzyme-hydrolyzed starches					
D-limonene	Gum Arabic	200	110 ± 10	45mL/min	99%	[45]
	HI-CAP100				94%	
	Maltodextrin				100%	
Caraway essential oil	Whey protein concentrate + Maltodex-trines	180 ± 5	90 ± 5		78-86%	[46]
	Maltodextrins, N-Lok, Encaps-855					
	Skimmed milkpowder+ Maltodextrines					
	Capsul-E, Nlok,Encaps 855+ Maltodextrines					
L-Menthol	Gum arabic (GA)	180	100 ± 5	45mL/min	72%	[47]
	CAPSUL				85%	
Cardamom oleoresin	Gum Arabic	178 ± 2	120 ± 5	300 g/h	23%	[48]
	Hi-CAP100				15%	
	Maltodrextrine				15%	
Lippia sidoides	Maltodextrin DE10 and gum arabic	160	140			[49]
Cinnamon oleoresin	Gum arabic, maltodextrin, and modi-fied starch					[50]
Ginger essential oil	Maltrodextrine and wey protein	120 ± 3	60 ± 3		100%	[51]
Citral and linalyl acetate	Arabic gum Arabic and maltrodextrine	300-400				[52]
Orange oil, ethyl acetate, ethyl propionate and ethyl butyrate	Acacia gum, maltodextrine and CAP-SUL	200	100			[53]
Elettaria cardamomum (Cardamom)	Mesquite gum	200 ± 5	110 ± 5			[54]
Terpenes(limonene, li-nalool, citral, α-myrcene,and α-pinene)	Arabic gum	150	93	15 ml/min	44 - 97%	[55]
Orange peel essential oil	Arabic gum				100%	[56]
	Mesquite gum				91%	
Elemi and peppermint oils	Maltodextrin					[57]
Oregano, citronella, majo-rana oil	Whey protein concentrate	200 ± 3	110 ± 5		84%	[58]
	Skimmed milk powder					
Orange oil	Whey protein isolate	120	80-84		73%	[59]
	Soy protein isolate				86%	
	Sodium caseinate				81%	
	Gum Arabic				75%	
Cardamon oleoresine	Binary and ternary mixtures of arabic gum, maltodextrin, and modified starch	178 ± 2	120 ± 5	300 g/h		[60]

Table 3. Applications of Different Techniques not Based on Supercritical Fluids for Encapsulation of Essential Oils

Essential Oil	Coating Material	Encapsulation	Encapsulation Efficiency %	Application	Ref.
Cinnamon leaf	β-cyclodextrin	Molecular-complex with ethanol. After filtration and drying	95%	Food preservation	[34]
Garlic			95%		
Citronella	Chitosan	Modified orifice method	95-98%		[35]
Mentha piperita	Chitosan	Gelled chitosan dispersions		Cosmetic formulation	[36]
Tumeric	Chitosan-sodium alginate	Emulsification, gelification and solvent removal	70%	Food additive and household medicine	[65]
Zanthoxylum limonella	Chitosan	Complex coacervation			[63]
Origanum dictamnus L.	Phosphatidyl choline and cholesterol	Liposome (thin film hydration method)	4%	Insect pest control	[37]
Artemisia arborescens L.	Hydrogenated (P90H) or non-hydrogenated phosphatidylcholine (P90)	Liposome (thin film hydration method)	60-74%	Therapeutic proposes (controlled release of an antivirial agent)	[38]
Oregano, Cassia, Red thyme	Corn Zein	Coacervation		Control release of the oils	[62]
Thymus vulgaris	Gelatine	Coacervation coupled with freeze-drying	99%	Insect pest control	[66]
Rosmarinus officinalis			99%		
Camphor	Gelatine blended and arabic gum	Coacervation	99%	Therapeutic proposes	[67]
Artemisia arborescens	Sodium alginate	Coacervation	86%-100%	Therapeutic proposes (controlled release of an antivirial agent)	[68]
Rosmari-nus officinalis and Thymus herba-barona	Gelatin 120 Bloom and glutaraldehyde	Coacervation coupled with freeze drying	98%	Insect pest control	[69]
Lemograss	Poly(vinyl alcohol) and glutaraldehyde	Coacervation		Insect pest control	[61]
Peppertmint and orange	Saccharomyces cerevisiae	Permeation of oil into the yeast cell. Cell wash and freeze dried	99%	Development of a yeast cell microcapsule	[70]
Orange peel	Sucrose syrups	Co-crystallization		Evaluation of the cocrystallization process for the encapsulation of orange peel oil with sucrose.	[71]
Lemon	β-cyclodextrin	Molecular-complex formation			[72]
Eugenol	β-cyclodextrin	Molecular-complex and freeze-dried	90%	Food flavoring, fragance, cosmetics and tobacco products	[73]
	Polycaprolactone	Emulsion-diffusion and freeze-dried	100%		
Lippia gracilis	β-cyclodextrin	Slurry complexation	64%	Insect pest control	[74]
		Paste method	100%		
Eucalyptus	Sodium alginate and calcium chloride as cross-linking	Interfacial insolubilization reaction	90%-92%	Antibacterial and animal pathogen microorganisms	[75]

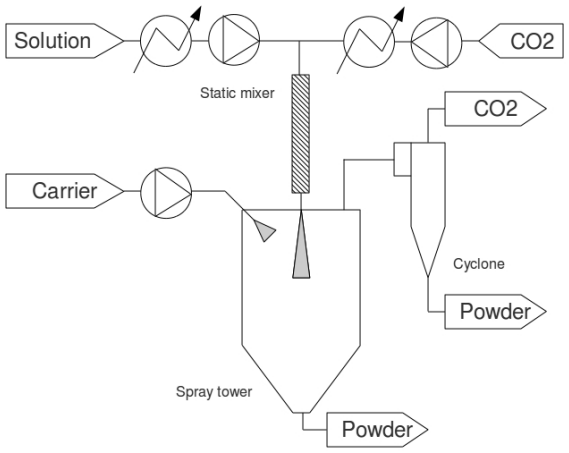

Fig. (1). Schematic diagram of a PGSS process.

4. APPLICATIONS OF SUPERCRITICAL FLUIDS FOR FORMULATIONS WITH ESSENTIAL OILS

4.1. Co-Precipitation and Encapsulation Processes

Particle formation has been one of the most active fields of research related to supercritical fluids in the last years. Many different precipitation processes based on the use of supercritical fluids have been proposed, in which the supercritical fluids performs different functions as solvent (Rapid Expansion of Supercritical Solutions, RESS), antisolvent (Supercritical Anti Solvent precipitation, SAS), co-solvent or solute (Particles from Gas Saturated Solutions PGSS), propellant (Carbon dioxide-Assisted Nebulisation with a Bubbler Dryer CAN-BD), etc. [28, 76]. Most of these processes were originally developed for producing solid composites, but with some modifications some of them can be used to obtain solid-liquid composites such as essential oil-loaded microcapsules.

In particular, the PGSS process has been adapted for producing solid-liquid composites with promising results. The PGSS process is based on the high solubility of supercritical carbon dioxide in many molten fats, lipids or polymers at moderate pressures (e.g. up to 30 wt% in poly ethylene glycol -PEG- with P < 30MPa [77]). PGSS is a two-step process, as presented in the flow diagram shown in Fig. (**1**). The first step consists in the saturation of the solute with carbon dioxide, which is accomplished in a static mixer operating at high pressure. The second step consists in expanding the gas saturated solution (typically down to atmospheric pressure) through a nozzle. Joule-Thomson effect induces a pronounced and fast reduction in temperature, which causes particle formation [78]. The PGSS process can be used to encapsulate liquids if the liquid to be encapsulated (e.g., an essential oil) is admixed together with the coating material and CO_2 into the static mixer. With this, an emulsion of the essential oil into the gas-saturated coating material is formed in the static mixer and the coating material becomes solid during the expansion, encapsulating the essential oil. The PGSS process has been successfully used to produce solid capsules filled with different liquids [79]. Varona *et al.* [80] used this technology to produce particles of PEG filled with lavandin essential oil. Encapsulation efficiencies of up to 66% were achieved and spherical microcapsules of sizes ranging between 80 and 130 μm could be obtained.

Another modification of the PGSS process which is suitable for producing liquid-loaded microcapsules is PGSS-drying [78]. The flow diagram of a PGSS-drying process is very similar to that of a conventional PGSS. The main difference between the two techniques is that in PGSS-drying the coating material is fed to the static mixer in an aqueous solution. A certain amount of the gas is dissolved into this solution in the static mixer (higher when the concentration of the coating material in the solution is higher [81]), and the release of this gas from the solution during depressurization considerably enhances solution atomization. Temperature and pressure conditions before the expansion as well as gas-to-solution flow ratios have to be chosen in order to ensure that a single fluid phase region is attained after the depressurization and thus dry particles are obtained. PGSS drying can be used to produce liquid-loaded particles if an emulsion is fed to the static mixer. Usually, in this emulsion the liquid to be encapsulated is the organic disperse phase, and the coating material is dissolved in the aqueous continuous phase. With this, the emulsion acts as a template for the particles, whose shape is preserved when the coating material precipitates in the spray. Varona *et al.* [80] also used this method to produce lavandin oil-loaded microcapsules, in this case using OSA-starches as coating materials. Fifty percent of the oil in the emulsion was encapsulated in the particles. Particle sizes varied between 15 μm and 200 μm, with a residual moisture content of about 5%.

A supercritical fluid-based process which was specifically developed to produce particles loaded with liquids is the Concentrated Powder Form (CPF) process. A schematic flow diagram of a CPF process is shown in Fig. (**2**): the liquid to be encapsulated is put into contact with a compressed gas, and the gas saturated solution is sprayed through a nozzle. Once again, the release of the gas from the solution dur-

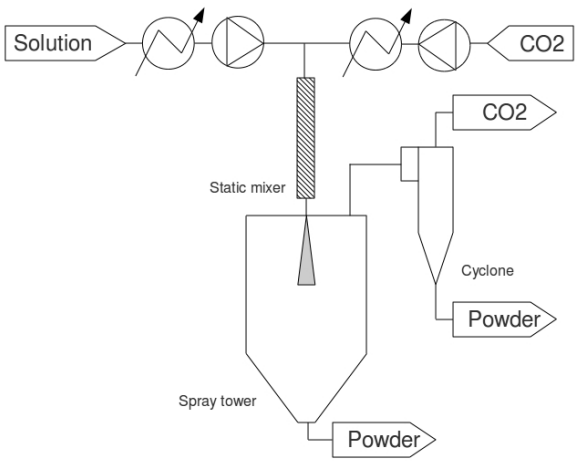

Fig. (2). Schematic diagram of a CPF process.

ing depressurization enhances atomization. After this, previously formed carrier material particles are blown into this spray by means of an inert gas. The two materials are then intensively mixed, and agglomerates of carrier material particles containing high amounts of liquid (up to 90 wt%) are obtained [78]. The maximum loading capacity mainly depends on the characteristics of the carrier material particles rather than on the properties of the liquid to be encapsulated [82]. Over 100 liquids have been encapsulated with CPF [78, 83]. This process was applied to encapsulate citrus oil [83], demonstrating that because the process is carried out in inert conditions, no citrus oil degradation products were formed.

Both PGSS and CPF processes are well established technologies with favourable economical conditions for commercial application [78]. They show significant advantages over other formulation processes, including the reduced use of organic solvents, the possibility of operating at moderate temperatures in an inert atmosphere, thus avoiding oxidation or thermal degradation of the essential oil as well as oil losses due to evaporation, and an enhanced control of particle size enabled by the fast precipitation kinetics. Although the reported applications of these technologies for encapsulation of essential oils are not too numerous, the knowledge obtained with the study of the encapsulation of other similar liquids is also relevant for applications with essential oils. Therefore, these technologies are already mature for industrial applications with essential oils.

4.2. Encapsulation in Micelles and Liposomes

Water-insoluble substances such as essential oils can be stabilized in an aqueous environment in the inner core of a micelle. Polymeric micelles have been recognised as a very promising carrier for water-insoluble drugs, due to their prolonged stability times *in vivo* [84]. Therefore they can be suitable formulations for applications of essential oils as antibiotics. The effectiveness of micelle formulations of essential oil active compounds for bacteria growth inhibition has indeed been proved [85]. Although micelles spontaneously form when the concentration of a surfactant or an amphiphilic block copolymer is higher than the critical micelle concentration, a more elaborate process is required if a high encapsulation efficiency of the oil in the micelle is to be achieved. Nowadays, the most popular micelle production method is to dissolve the active compound and the polymer in an organic solvent, which is then replaced with water by dialysis. This method is adequate for laboratory scale, but has important disadvantages for large scale implementation related to the small productivity of dialysis equipments, and the long processing times required (>1 day). An alternative is the formation of an organic solvent-in-water emulsion followed by the evaporation of the organic solvent, but this approach has his own disadvantages due to the presence of residual organic solvent in the final product and the necessity of exposing the product to high temperatures during prolonged times [86].

A possible alternative is the use of compressed gases or supercritical fluids to remove the organic solvent from the emulsion in order to produce essential oil-loaded micelles, according to the Supercritical Extraction from Emulsions (SFEE) process [87]. A schematic diagram of this process is presented in Fig. (**3**). It consists in putting the emulsion into contact with a compressed gas. Possible contacting devices include packed columns and sprays. Gas extracts the organic solvent and an aqueous suspension is obtained.

In a recent study performed with the objective of precipitating β-carotene from the organic phase of a dichloromethane-in-water emulsion [88], it was demonstrated that with this technique it is possible to eliminate the organic solvent from the emulsion down to concentrations below 1 ppm. With a study of the evolution of drop sizes with time, it was shown that final particle size matched the particle size of empty micelles in the initial emulsion. Therefore, it could be concluded that the produced β-carotene particles were encapsulated in surfactant micelles. The feasibility of carrying out the process at moderate pressures (5 MPa) was also demonstrated. Due to this, this process in principle is also feasible for obtaining micelles loaded with active com-

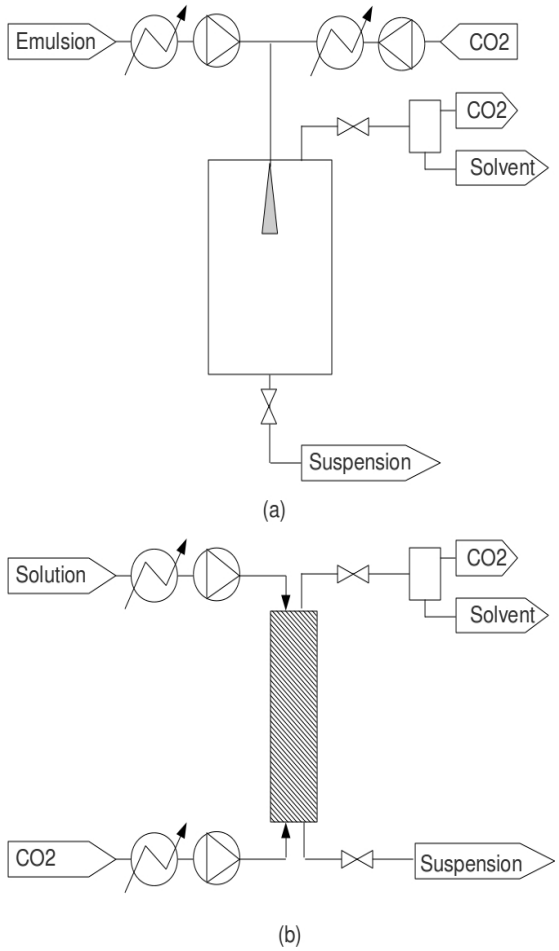

Fig. (3). Schematic diagram of the Supercritical Fluid Extraction from Emulsions process, (**a**) using a spray as contacting device and (**b**) using a packed column.

pounds extracted from essential oils: the solubility of many essential oil compounds in carbon dioxide at moderate pressures is much lower than that of organic solvents (e.g. at 7 MPa and 313 K, the solubility of linalool, a typical essential oil component, in supercritical carbon dioxide is approximately 1/25 of that of hexane [89, 90]), and in any case the fraction of essential oil compound extracted during micelle formation can be easily recovered and recycled by depressurization of the gas effluent. Still the experimental validation of this approach has not been carried out.

Liposomes also are promising carriers for essential oils. The antimicrobial and antiviral activity of liposomes loaded with some essential oils has already been proved [37, 38]. An extensive review of conventional and dense gas-based methods for producing liposomes was presented by Meure *et al.* [91].

Two of the oldest dense-gas techniques for the production of liposomes are the injection and the decompression methods presented by Castor and Chu [92], which are suitable for incorporating hydrophobic compounds such as essential oils into the liposomes. In the injection method, the lipid is dissolved into CO_2 with the aid of cosolvents and then decompressed into water, while in the decompression method a biphasic CO_2-cosolvent-lipid + water mixture is sprayed. Hydrophilic substances to be encapsulated are included in the water phase, and hydrophobic substances in the CO_2-cosolvent-lipid phase. Liposomes of less than 200 nm incorporating hydrophilic and hydrophobic drugs have been produced with this technique, and *in vivo* tests demonstrated the therapeutic activity of the formulation [93].

Otake *et al.* [94] developed the so-called "Improved Supercritical Reverse-Phase Evaporation Method" for the production of liposomes. In this method a dispersion of the lipid in water with the substances to be encapsulated was prepared in a batch stirred vessel and CO_2 was injected up to the operating pressure (typically 20 MPa). This mixture was stirred during a certain time to ensure homogenization (approximately for 40 min) and then pressure was slowly released to obtain the liposomes. The use of CO_2 enabled additional stability of the liposomes due to the formation of carbonic acid by dissolution of CO_2 in the water phase, which was incorporated in the liposomes membrane producing a static repulsion effect. Indeed, it was found that the liposomes produced by this technique were stable during more than 30 days, while liposomes produced by other techniques not based on supercritical carbon dioxide were only stable for a few hours. As in the case of micelles, essential oil-loaded liposomes have not yet been produced using supercritical fluids, but previous results obtained with other substances are promising and demonstrate that there are possibilities for applications with essential oils.

In addition to a direct one-step fabrication of the liposomes, some researches have suggested to produce particles of phospholipids loaded with the active compounds of interest as dry precursors of liposomes which can then be reconstituted by hydration. Several supercritical fluid technologies have been used for this purpose, as for example the Supercritical Anti Solvent process (SAS) or the Aerosol Solvent Extraction System (ASES) [95]. Thus the methods described in Section 4.1 are in principle suitable to produce microcapsules of phospholipids loaded with essential oils which can be used as liposome precursors.

5. CONCLUSIONS

The use of dense gases and supercritical fluids for the preparation of essential-oil microcapsules is advantageous for several reasons: product degradation or contamination with toxic organic solvents is minimized, in some occasions supercritical fluid processes allow for a enhanced control of product characteristics, a closed and inert system is used for the precipitation which can be easily made compliant with Good Manufacturing Practices, and moreover successful implementations in pilot scale indicate that supercritical precipitation processes present promising technical and economical conditions for large scale production. Several supercritical precipitation processes such as PGSS, PGSS-drying and CPF have already been successfully used to produce essential-oil loaded microparticles. Even though the number of applications with essential oils studied with these processes is relatively small, the satisfactory results with essential oils as well as with many other similar liquids demon-

strate good perspectives for the commercial application of these techniques. More recent developments include the preparation of micelles and liposomes with supercritical fluids. Although promising results have been obtained with other hydrophobic substances, the application of these techniques for preparation of formulations with essential oils is still a pending task.

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