Trends in Particle Formation of Bioactive Compounds Using Supercritical Fluids and Nanoemulsions

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Abstract This review discusses the recent developments in the application of supercritical fluid technologies for the production of composites or encapsulates of bioactive compounds. Various supercritical particle formation technologies are briefly described, including processes in which the supercritical fluid acts as a solute, solvent, and antisolvent. The main features and mechanisms of antisolvent techniques that contribute to the understanding of the fundamentals of the Supercritical Fluid Extraction of Emulsions (SFEE) process are described. The published literature on SFEE, including the results and perspectives of its application in various industrial fields, are discussed. This article is the first comprehensive review specifically focused on the formation of particles using the SFEE technique.

Keywords SAS, SFEE, Supercritical, Bioactive Compounds, Novel Processing Techniques

1. Introduction

Particle formation and encapsulation technologies are widely employed in the pharmaceutical, cosmetic, and food industries. Examples of classical micronization processes include spray drying, spray chilling and spray cooling; extrusion coating; fluidized bed coating; liposome entrapment; coacervation; inclusion complexation; centrifugal extrusion and rotational suspension separation[1]. However, all of these techniques have inherent limitations.

Supercritical fluids have been used as solvents, solutes, and antisolvents for micro- and nanoparticle formation in a variety of compounds and have overcome all of the limitations of the traditional techniques. These limitations include poor control of particle size and morphology, degradation of thermosensitive compounds and low encapsulation efficiency[2]. The possibility of obtaining solvent-free microparticulate particles with a narrow size distribution curve using supercritical fluids is very attractive[3].

Supercritical fluids, which were first discovered in 1879, have an exceptional solubility for solids and liquids compared with liquid or gaseous fluids. Variations in the operating conditions to increase the solvation power make this technology a solid option for the recovery of several types of substances. The properties of these fluids have been extensively explored in the extraction and/or separation steps to obtain valuable compounds, such as flavors, colorants, and other biomolecules[4]. A promising new field for supercritical fluids is the formation of particles containing these compounds.

The aim of this review is to discuss several of the recent developments in the application of supercritical fluid technologies for the production of composites or encapsulates of bioactive compounds. Bioactive compounds are extranutritional constituents that typically occur in small quantities in nature, are part of the food chain, and have an effect on human health. In this review, the various supercritical particle formation technologies are briefly described. The main features and mechanisms of the antisolvent techniques that primarily contribute to understanding the fundamentals of the Supercritical Fluid Extraction of Emulsions (SFEE) process and the results of and perspectives on applying these techniques in various industrial fields are discussed.

2. Principles of Particle Formation

Encapsulation has been defined as packaging solid, liquid or gaseous materials into microcapsules that release their contents at controlled rates over prolonged periods of time under specific conditions [5],[6]. The size of the particles formed through encapsulation may be classified as follows: macro (>5000 µm), micro (1.0–5000 µm), and nano (<1.0 µm) [7]. Different morphologies can be obtained depending on the physicochemical properties of the core and wall materials and the encapsulation techniques used during production. In general, the two main structures are mononuclear capsules, which contain one core material

enveloped by a carrier material, and aggregates, which consist of many core materials embedded in a matrix of coating material [8],[9].

Composites are frequently produced by the simultaneous precipitation of the core and coating materials, which leads to the dispersion of the core material particles into a matrix of coating material. Encapsulates are produced when the coating material is precipitated as a thin shell over a previously existing particle of the core material [10]. Both types are produced for multiple purposes, such as controlling the release of core material in a desired quantity and location, increasing the dissolution rate of slightly water-soluble materials and modifying the surface properties of particles used in pharmaceutics, catalysts, cosmetics, the printing industry and energetic materials [11]. Figure 1 shows the general structures of encapsulates and composites.
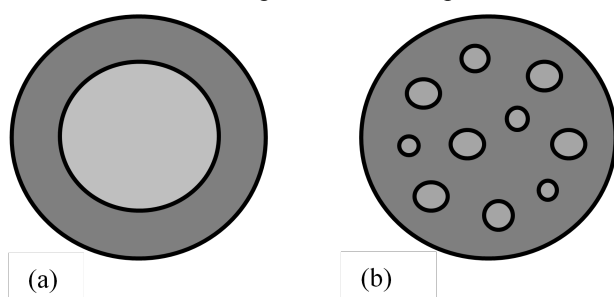

Figure 1. The structures of (a) an encapsulate and (b) a composite

In the food industry, the primary reasons that the encapsulation process is applied are summarized as follows[5], [12]: (i) to protect unstable materials from degradation; (ii) to decrease the evaporation or transfer rates of the core material to the outside environment; (iii) to modify the physical characteristics of the original material to be easier to handle; (iv) to control the release of the core material; (v) to mask the odor or taste of the core material; (vi) to dilute the core material when small amounts are required, yet still achieve a uniform dispersion in the host material; and (vii) to separate components within a mixture that would otherwise react with one another.

Nanoencapsulation offers numerous benefits. The application of this technique in the food industry has received attention from the scientific community due to its potential to protect and improve the efficiency of delivering bioactive compounds in functional foods to improve human health [13].

3. Particle Formation by Nano- and Microencapsulation

There are several studies that describe the nano- and microencapsulation technologies that are used to encapsulate bioactive compounds [6], [9], [14-16]. Microencapsulation techniques can be divided into chemical processes, such as molecular inclusion and interfacial polymerization, physicochemical techniques, such as coacervation and liposome entrapment, and physical processes, including

spray drying, spray chilling or spray cooling; extrusion; co-crystallization and fluidized bed coating. Additional information on conventional techniques is provided in [5] and [17].

There are specific features and characteristics that are disadvantages in each process, such as thermal denaturing, large residual solvent concentrations, and difficulties in controlling particle size and size distribution during processing. These limitations may affect particle stability, flow properties, and delivery efficiency [18].

The use of supercritical fluids as an alternative medium for nano- and microencapsulation can improve the results obtained using conventional techniques. Published reviews indicate that these methods have the potential to overcome the drawbacks previously described [19-23].

4. Nano- and Microencapsulation Using Supercritical Fluids

Carbon dioxide is the primary fluid applied to produce composite particles using supercritical fluid methods[11] because it enables the process to be performed at near ambient temperatures in an inert atmosphere, which avoids the degradation of the bioactive compounds. The supercritical region can be achieved at moderate pressures and temperatures (T_c = 304.2 K, P_c = 7.38 MPa). A number of modified processes that use supercritical fluids in particle formation have been described in the literature. These processes are classified according to the role of the supercritical fluid in the process, as follows: solvent[Rapid Expansion of Supercritical Solutions (RESS)]; solute[Particles from Gas-Saturated Solutions (PGSS)]; or antisolvent[Supercritical AntiSolvent (SAS)], including its numerous modifications [24]. These classifications are briefly described in the following sections, and additional details regarding these methods can be found in other reviews [10], [11], [19], [23-25].

4.1. Supercritical CO_2 as a Solvent

The first review article on applying the supercritical fluid method in particle design focused on the RESS method, which was the first method used to produce particles [26].

In the RESS process, the substance to be powdered is first dissolved in a supercritical fluid. This mixture is then depressurized through a nozzle, which leads to the rapid precipitation of the dissolved matter as the supercritical fluid vaporizes. The absence of liquid organic solvents, the mild processing temperatures, and the purity of the final product make this process particularly attractive for biomedical applications [27]. Many drugs, such as salicylic acid [28], naproxen [29], ibuprofen [30], griseofulvin and β-sitosterol [31], have been micronized using the RESS technique.

4.2. Supercritical CO_2 as a Solute

The solubility of compressed gases in liquids is generally quite high. Production of particles using the gas-saturated

solution (PGSS) process is based on the high solubility of supercritical CO_2 in many substances, including molten polymers, oils and fats [32]. The PGSS process consists of solubilizing supercritical CO_2 in melted or liquid-suspended substance(s), which leads to a gas-saturated solution that is expanded through a nozzle to form fine particles through precipitation after rapid expansion as a consequence of a drastic reduction in solubility [33]. The PGSS process has been applied in various fields to produce products ranging from inorganic powders to pharmaceutical compounds. Jung & Perrut [19] and [34] list several applications of this method for food and food-related products.

Another application of the PGSS process is drying liquid solutions to produce fine powders, or PGSS-drying [34]. Varona et al.[35] recently used PGSS-drying to encapsulate lavandin oil in starches by removing the water from an oil-in-water emulsion stabilized with N-octenyl succinic anhydride (OSA) starches as surfactants.

4.3. Supercritical CO_2 as an Antisolvent

Supercritical antisolvent precipitation is also known as GAS (gas antisolvent), PCA (precipitation by compressed antisolvent), ASES (aerosol solvent extraction system), SEDS (solution enhanced dispersion by supercritical fluids), and SAS (supercritical antisolvent) [36]. These processes are essentially the same, with differences in the feed mode of the solvent and antisolvent, which can be co-current or counter-current, depending on the type of injector used, and can use batch or semi-continuous modes [37].

Encapsulation using the SAS technique is based on the same simple principles of the RESS method in which a core material and a carrier are co-precipitated together [2]. This process is well known and has been applied to several types of compounds, including explosives [38], polymers [39], [40], pharmaceuticals[41], [42], and pigments [43]. The SAS method has been thoroughly reviewed by [36]. The advanced application of supercritical fluids in micro/nanoencapsulation technology, with the emulsion process referred to as Supercritical Fluid Extraction of Emulsions (SFEE), will be evaluated in this review.

4.4. Supercritical Fluid Extraction of Emulsions (SFEE)

SFEE combines the emulsion techniques and the SAS processes. Emulsion techniques generally require large quantities of organic solvents, and their removal involves additional separation techniques and the use of high temperatures. In addition, SAS is not able to produce particles within the nanometric scale, and the resulting products have an increased tendency for particle agglomeration [10]. To overcome these disadvantages, Chattopadhyay et al. [44] combined the two technologies and patented a new encapsulation method termed the Supercritical Fluid Extraction of Emulsions (SFEE). This method allows the removal of organic solvents during the process and enables the production of nanoscale particles that improve the solubility of bioactive compounds in

aqueous solutions, which increases their bioavailability.

The SAS method is based on combining the substance to be micronized or encapsulated dissolved in an organic solvent with a supercritical fluid, which acts as an antisolvent. Upon mixing, the supercritical fluid saturates and depletes the liquid solvent by decreasing its solvation power through extraction, and the solute precipitates as microparticles. If a wall material is also dissolved in the organic solvent, composites or encapsulates are formed by co-precipitation with the solute [10], [19]. The experimental setup and principles of the SFEE process are basically the same as those of SAS, but in SFEE, supercritical CO_2 is used as an antisolvent to eliminate the organic solvent from the droplets of an oil-in-water (O/W) emulsion [45]. An O/W emulsion containing the core materials to be precipitated dissolved in its dispersed phase (e.g., a conventional organic liquid solvent) is injected into the precipitation vessel with a CO_2 flow rate. The final product is a micro- or nanosuspension of the substance in water.

The differences in the SAS and SFEE processes are as follows: (a) in SFEE, an emulsion containing the substance to be precipitated dissolved in its dispersed phase is injected, whereas in SAS, a simple solution of the substances is injected; (b) SFEE requires additional steps to produce a powdery product because an aqueous product is formed; (c) the preparation of the initial materials is more complex in SFEE; and (d) emulsion droplet size distribution is a controlling parameter in addition to the other parameters involved in the SAS process (e.g., pressure, temperature, flow rate, and concentration)[10]. However, the SFEE technology is a promising method for producing nanometer particles of natural substances in water [46]. Narrower size distributions can be produced by SFEE because particle size is strictly related to the droplet size and distribution of the starting emulsion, and particle agglomeration can be prevented by the water/surfactant external phase [47]. Using the same pressure, temperature, and solution flow rate for both the SFEE and SAS methods, Shekunov et al. [45] observed a substantial difference in the resulting size and shape of the particles. SFEE produced prismatic crystals with a volume-weighted diameter typically between 0.5 and 1 μm, whereas SAS produced longer crystal dimensions of between 20 and 200 μm and a volume-weighted diameter above 10 μm. Thus, a 10-fold reduction in the particle size was achieved using SFEE compared with the particles produced using SAS.

Mattea et al. [48] described the phenomenon that occurs during the SFEE process by investigating a system composed of a β-carotene + dichloromethane-CO_2-water + starch-based surfactant. Each drop of the organic solvent behaved as a miniature gas antisolvent precipitator, and multiple particles formed inside the drop. Depending on the CO_2 pressure and temperature, the solubility of CO_2 in the aqueous and organic phases changed and caused swelling and shrinking of the drop due to the diffusion of supercritical CO_2 into the drop and dichloromethane out of the drop.

4.5. SFEE steps

4.5.1. Emulsion Preparation

Before initiating the SFEE process, an oil-in-water emulsion must be prepared. In general, these emulsions are prepared with the aid of surfactants.

Certain of the surfactant materials used to prepare the O/W emulsion have a double functionality in the SFEE process as both a surfactant to stabilize the emulsion and a coating material in the final dry product [46]. Surfactants also act as protective layers and reduce the agglomeration of the final particles[45],[49]. Mezzomo et al. [50] used a Pluronic F127 surfactant/coating material to encapsulate the extract from pink shrimp residue and observed that the emulsion was not stable due to incorrect Hydrophilic-Lipophilic Balance (HLB) values from the surfactant. The authors then used a modified starch (Hi-Cap 100) to achieve high encapsulation efficiency. Additional research is needed to optimize the effectiveness of SFEE for encapsulation.

When using a polymer without emulsification properties as a coating material, such as poly-lactic-co-glycolic acid (PLGA), surfactants are only used to stabilize the emulsion. Polyvinyl alcohol (PVA) is the most popular surfactant used in the production of PLGA-stable nanoparticles in the SFEE process.

From a food application perspective, the use of food-grade surfactants is important. Studies that have used food-grade surfactants for bioactive compound encapsulation via SFEE are extremely scarce. In the literature, all of the studies are related to the precipitation of carotenoids using a modified starch as the surfactant [46], [48], [50], [51]. Table 1 in section 4.8 lists all of the surfactants that have been tested using the SFEE process.

Silva et al. [52] provided an overview of the surfactants used in nanoemulsion production for food applications. The authors focused on nanoemulsion production methods, which are classified as either high-energy or low-energy.

There are a number of mechanisms available for the production of emulsions. High-speed stirring mixers [46],[47],[51], high-pressure homogenization [45], [53], [54], and ultrasonication [55-58] have been used to form fine emulsions for use in the SFEE process. Microfluidization is an additional alternative for preparing submicron emulsions. Jafari et al.[59] investigated the efficiency of sonication and microfluidization in the production of nanoemulsions and reported that the microfluidizer produced emulsions with narrower size distributions, whereas sonication was a better option in terms of operation and cleaning.

Emulsification is one of the important steps in the SFEE process. An advantage of this process is that growth of the particles is limited by the size of the emulsion droplets [49]. However, stable emulsions are required, and the droplets must be protected against flocculation followed by creaming or sedimentation. Coalescence via collisions and Ostwald ripening, which is a molecular diffusion degradation, are the

primary reasons for instability in nanoemulsions. Additional details regarding the principles of the formation and stabilization of nanoemulsions are provided in a review article by Tadros et al. [60].

Abismaïl et al. [61] reported that smaller average drops can be obtained using ultrasound. Ultrasound requires less surfactant, consumes less energy and produces emulsions that are less polydispersed and more stable compared with the emulsions produced by mechanical processes. Furlan et al. [57] studied the influence of sonication duration on the final particle size distribution. The authors concluded that the duration of sonication slightly influenced the average particle size but had a strong influence on the particle size distribution.

4.5.2. SFEE Processing

The SFEE process can be performed in a batch, semi-continuous or continuous mode using a similar apparatus. In the SFEE batch mode, an aliquot of the emulsion is placed into the precipitation vessel to be processed. In the semi-continuous mode, the aqueous suspension is removed from the bottom of the precipitation vessel when the extraction process is complete. In the continuous mode, the suspension is continuously removed through a needle valve [45], [53], [58]. Chattopadhyay et al. [53] observed that there were no differences in mean particle size and morphology between the batch and continuous modes.
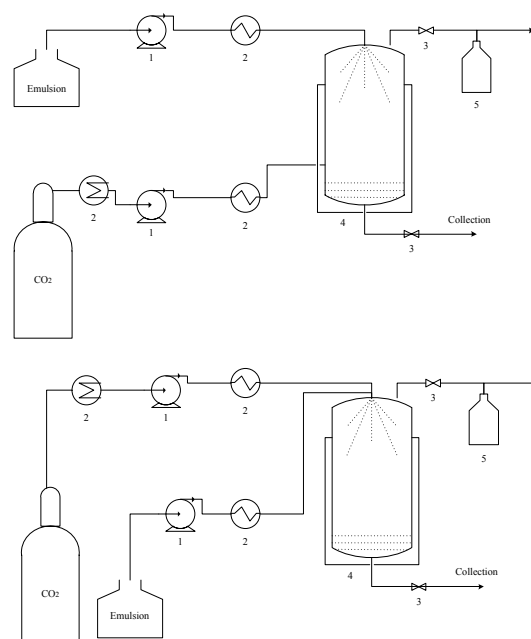

Figure 2. A schematic diagram of the SFEE apparatus. (A) Counter-current; (B) Co-current; 1, Pump; 2, Heat exchangers; 3, Valves; 4, Precipitation vessel; 5, Flash tank separator

A schematic representation of the different SFEE processes is presented in Figure 2. Briefly, the O/W emulsion and the antisolvent fluid are continuously injected

into the precipitation vessel, resulting in organic solvent extraction and particle precipitation due to contact between the supercritical fluid and the organic phase. The organic solvent diffuses into the water, followed by subsequent extraction of the solvent from the drop to achieve supersaturation and precipitation of the solute and surfactant [53].

The process can be co- or counter-current in that the antisolvent can be introduced in the top [49], [57] or the bottom [53],[58] of the precipitation vessel. The antisolvent is first injected into the precipitation vessel through a frit [54] or a nozzle[57] until the desired pressure, temperature, and flow rate are reached and maintained constant. According to Shekunov et al. [45], the use of a frit maximizes the mass-transfer efficiency during organic solvent extraction. The O/W emulsion is then injected at the desired flow rate through a capillary [58] or a nozzle [45], which breaks the emulsion into droplets to increase the surface area in contact with the antisolvent, until a selected amount is processed. The ratio between the antisolvent and emulsion flow rates, the temperature, and the pressure are maintained constant during the SFEE process. The effluent CO_2 and organic solvent exit from the top of the vessel into a flash tank separator to recycle both of the solvents. After the extraction process is complete, the antisolvent flow is maintained constant for a specific period of time to eliminate the remaining organic solvent from the suspension.

4.5.3. Elimination of Water

After SFEE processing, the final product is an aqueous micro- or nanosuspension. Water can subsequently be removed by conventional drying processes, such as spray drying, lyophilization, and microwaving. The high temperature used in most conventional dryers is unsuitable for drying suspensions of bioactive compounds because it accelerates the degradation process. This step can also promote destabilization of the nanoparticles dissolved in water, leading to an increase in the particle size. Santos et al.[51] and Mezzomo et al. [50] spray dried nanosuspensions to produce a dry powder, which increased the size of the particles due to the precipitation of the surfactant during the spray drying process. Most previous studies regarding SFEE did not remove the water, and there is a lack of research evaluating the influence of this step on particle destabilization.

4.6. The Effects of Various Parameters in the SFEE Process

4.6.1. Particle Size

The effects of various parameters in the SFEE process on precipitate particle size have been evaluated by several authors.

No significant changes in particle size have been observed by varying the operating parameters, such as pressure, temperature, processing time and solvent/antisolvent flow rates, in the SFEE process [46], [47], [53].

The literature shows that the primary parameters responsible for particle size control are the emulsion droplet size, solute/solution concentration and organic solvent content in the emulsion [45]. The literature confirms that the particle size is influenced more by the nature of the emulsions than by the mass transfer conditions [47], [53].

The literature reports that an increase in organic solvent and polymer concentration alters the particle size. Shekunov et al. [45] observed a reduction in particle size with a decrease in the organic solvent and solute concentrations. According to Chattopadhyay et al. [53] and [54], an increase in the organic solvent concentration in the emulsion can lead to the increased aggregation of the emulsion droplets, resulting in the precipitation of larger particles. Solute and polymer concentrations can be associated with specific functional groups in these compounds, which can change the interfacial tension of the emulsion droplets. The increase in particle size based on the solute concentration is likely due to an increase in the surface tension of the organic solution, resulting in emulsions with larger droplets.

In general, an increasing amount of surfactant leads to a decrease in particle size until a minimum value is reached. However, continuously increasing the amount of surfactant in water decreases the polydispersity index of the final product [57]. Kluge et al. [62] studied the effects of PLGA concentrations on the organic droplets at two different emulsion stirring rates and observed that increasing PLGA concentrations led to a higher viscosity of the dispersed organic phase, which favors the formation of larger droplets during emulsification. The authors also observed that the average particle size decreased with an increased emulsion stirring rate, whereas the particle size distributions generally became narrower [47], [53].

4.6.2. Stability of the Emulsion

The stability of the emulsion is related to the interfacial tension. If the interfacial tension increases as a result of a mass transfer of CO_2 to the drop, the emulsion becomes destabilized. Emulsion destabilization also occurs during the depressurization step due to the intense stirring caused by CO_2 release from the organic phase [46], [48].

Contact between the emulsion and CO_2 to achieve precipitation through the antisolvent effect must occur over a short period of time to minimize the possibility of emulsion destabilization prior to precipitation. However, the elimination of the remaining organic solvent may be slower because emulsion destabilization is no longer an issue after the particles have been produced [46].

Varona et al. [35] observed that the stability of the emulsion is drastically reduced when the pressure is increased. Although temperature has a minor effect, stability is related to the creaming effect. According to Chattopadhyay et al. [53], high temperatures and pressures can affect the stability of the emulsion by altering the surfactant-organic phase interactions. In general, a high

concentration of surfactant increases the stability of the emulsion [63].

4.6.3. Elimination of Solvent

The operating pressure and temperature conditions are selected to facilitate the maximum extraction of the organic phase of the emulsion with minimal loss of the solute and polymer due to dissolution in CO_2 and to avoid the loss of any emulsion that may wash out in the CO_2 stream [47],[53].

Mattea et al. [48] concluded that at pressures below the critical point pressure of CO_2-solvent mixtures, the swelling caused by CO_2 can be overcome by the diffusion of the solvent out of the drop due to the lower solubility of CO_2 in water. Thus, shrinking of the drop can be observed over time.

Chattopadhyay et al.[53] and Della Porta & Reverchon[47] observed an increased organic solvent extraction rate with an increased CO_2 flow. The efficiency of the solvent extraction increased with pressure, which was basically independent of the solution flow rate under the conditions investigated by Shekunov et al. [45].

4.6.4. Encapsulation Efficiency

The effectiveness of SFEE in encapsulation can be associated with several parameters, such as polymer type, solute concentration and emulsion formation[50]. Other factors that can influence the encapsulation efficiency include the nucleation rate of the compounds, the size of the formed particles, and the interactions between the carrier material and the solute[51]. Higher solute solubility in the antisolvent can result in a higher loss of the solute, which decreases the encapsulation efficiency due to dissolution in the antisolvent + organic solvent flow.

The CO_2 flow rate is directly related to the rate of solvent extraction from the emulsion droplet and solute/carrier material losses [47], which have a significant effect on the encapsulation efficiency [51]. Santos et al. [51] observed that a high emulsion flow rate resulted in high encapsulation efficiency, whereas an increased concentration of the surfactant/carrier material led to decreases in encapsulation efficiency. No significant changes were observed by varying the pressure.

4.7. Limitations to the SFEE Process

The most obvious drawback of SFEE is that the resulting suspension is an aqueous product instead of dry particles. Additional steps are required to produce a powdery product, which can lead to an increase in particle sizes due to agglomeration.

This technique has only been applied in the precipitation of solid solutes. Martín et al. [32] suggested the use of SFEE as a possible alternative for the production of compounds extracted from micelles loaded with essential oils, which can have a high viscosity.

Another limitation of this technique is that it is only suitable for the encapsulation of hydrophobic compounds. Kluge et al. [62] observed that solvent extraction from an emulsion, similar to the SFEE process, is not ideal for the encapsulation of hydrophilic compounds.

In the SFEE process, it is not possible to operate in a completely miscible zone. Due to the nature of the emulsion, there is additional resistance to the transport of CO_2 into the organic solvent droplet produced by the water from the emulsion. Different parameters, such as the droplet size when exiting the mixers and the design of the mixer, can affect the feasibility of the process [49].

Figure 3 represents a decision-making diagram for evaluating whether the SFEE process can be applied to encapsulate the solute of interest. This diagram must be considered based on certain conditions because temperature and pressure play an important role during the process. As shown in Table 1, the temperature and pressure for the SFEE process selected in most of the studies were 45°C and 8 MPa, respectively. These conditions were selected based on the solubility of the solute in the solvent and antisolvent. Consequently, the maximum extraction of the organic phase of the nanoemulsion with minimum solute and carrier material losses due to dissolution in CO_2 render the process viable [51].

It is important to note that when using a solute in solid form, the final product is a micelle system with powder inside at ambient temperature, but when using a solute with a viscous component that needs to be dissolved in an organic solvent to be pumped, the viscous compound is in the core of the micelle.
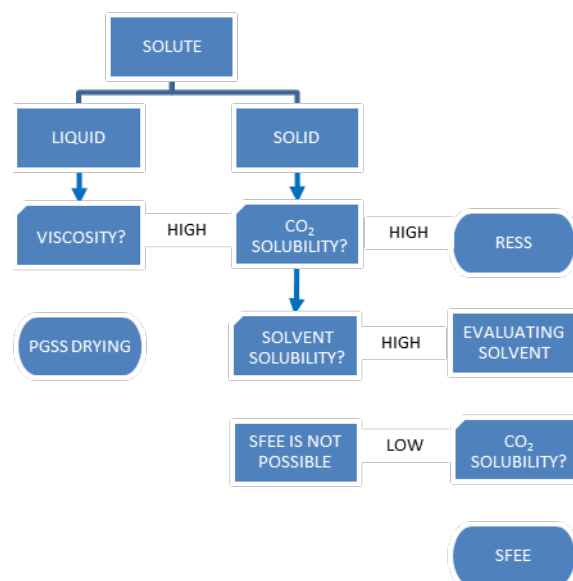

Figure 3. Diagram of a decision-making tree for the SFEE process

4.8. Applications

A variety of active pharmaceutical and food ingredients have been processed using the SFEE process. Reported applications of SFEE are presented in Table 1. Shekunov et al.[45] evaluated the SFEE method for the production of micro- and nanoparticles of cholesterol acetate, griseofulvin and megestrol acetate utilizing both batch and continuous processing for drug delivery applications. Chattopadhyay et

al.[53] successfully fabricated composite micro- and nanoparticles using a model system consisting of indomethacin, ketoprofen, and the biodegradable polymers poly(lactic/glycolic) acid and Eudragit RS to form composite particles ranging between 100 and 200 nm in size. SFEE has been used to produce nanoparticles of water-insoluble drugs combined with lipids for pulmonary delivery [54].

The compounds lysozyme (hydrophilic) and ketoprofen (hydrophobic) have been incorporated in poly-lactic-co-glycolic acid (PLGA) using the SFEE process [55],[62]. These authors investigated the phase equilibrium established between ketoprofen and PLGA to further underline the potential of SFEE to serve as a viable manufacturing technique and to highlight a novel application opportunity for this process [56]. In the SFEE processing of PLGA, variations in the PLGA concentration and stirring rates during the preparation of the emulsion have produced particles of pure PLGA with average sizes ranging between 100 nm and several μm with very narrow size distributions. PLGA has been used as a drug delivery system for magnetite nanocrystals stabilized by ricinoleic acid via SFEE[57].

Della Porta & Reverchon [47] evaluated the effectiveness of the supercritical extraction of CO_2 from the oil phase of oil-in-water emulsions to obtain spherical PLGA/piroxicam nanostructured microspheres. This process was described as occurring very rapidly due to the enhanced mass transfer of supercritical CO_2, resulting in the precipitation of microparticles with a narrower particle size distribution and preventing droplet coalescence or aggregation. Mayo et al.[58] demonstrated that the SFEE process allowed high actual loading of pDNA (19.7%, w/w), a high loading efficiency (>98%), and low residual solvents (<50 ppm) in preparing gene delivery nanoparticles.

Table 1. Reported Applications of SFEE

Core material	Surfactant/Polymer	Solvent	Operating parameters	Results and observations	References
Cholesterol acetate (CA)	Polyvinyl alcohol (PVA)	Ethyl acetate	Pressure: 8 MPa Temperature: 35 °C CO_2 flow: 12 kg·h^{-1} Emulsion flow: 1.200 ml·h^{-1}	Particle size: 100-1000 nm Emulsion size: 200-1000 nm Residual solvent (MA): <20 ppm Morphology (GF and MA): crystalline	[45]
Megestrol acetate (MA)	Tween 80	Toluene			
Griseofulvin (GF)	Lecithin	Dichloromethane			
	Polyvinyl pyrrolidone (PVP)	Dichloromethane; Ethyl acetate			
	PVA	Ethyl acetate			
Indomethacin (IN)	PVA/PLGA; PVA/Eudragit RS	Ethyl acetate	Pressure: 8 MPa Temperature: 45 °C CO_2 flow: 12 kg·h^{-1} Emulsion flow: 1.200 ml·h^{-1}	Particle size: 0.1-2 μm Residual solvent: <50 ppm	[53]
Ketoprofen (KP)					
Solid lipid formulations	Soy lecithin	Chloroform	Pressure: 8 MPa Temperature: 35 °C CO_2 flow: 2.4 kg·h^{-1} Emulsion flow: 120 ml·h^{-1}	Particle size: < 50 nm Residual solvent: <20 ppm	[54]
Lysozyme	PVA/PLGA	Ethyl acetate	Pressure: 8 MPa Temperature: 45 °C CO_2 flow: 4.8 kg·h^{-1} Emulsion flow: 120 ml·h^{-1}	Particle size: 100 nm-several μm with very narrow size distributions Encapsulation efficiency: >48.5%	[62]
Ketoprofen	PVA/PLGA	Ethyl acetate	Pressure: 8 MPa Temperature: 45 °C CO_2 flow: 4.8 kg·h^{-1}	Particle size: 100-200 nm	[55]

			Emulsion flow: 120 ml·h^{-1}		
Ketoprofen	PVA/PLGA	Ethyl acetate	Pressure: 8 MPa Temperature: 45 °C CO$_2$ flow: 4.8 kg·h^{-1} Emulsion flow: 120 ml·h^{-1}	Morphology: Spherical amorphous nanoparticles	[56]
Ricinoleic acid-stabilized magnetic nanocrystals	PVA/PLGA	Dichloromethane	Not available	Morphology: Janus type, with the magnetite accumulated on one hemisphere of the particle	[57]
Plasmid DNA (pDNA)	PVA/PLGA	Ethyl acetate	Pressure: 8 MPa Temperature: 45 °C CO$_2$ flow: ~240 ml·h^{-1} Emulsion flow: 24 ml·h^{-1}	Loading of pDNA: 19.7%, w/w Loading efficiency: >98% Residual solvent: <50 ppm	[58]
Piroxicam	PVA/PLGA	Ethyl acetate	Pressure: 8.5-15 MPa Temperature: 38 °C CO$_2$ flow: 0.1-0.5 kg·h^{-1}	Particle size: 1-3 μm Residual solvent: <40 ppm Encapsulation efficiency: 90-95%	[47]
β-carotene	OSA-modified starch Tween 20 + Span 20	Dichloromethane	Pressure: 8-13 MPa Temperature: 35-50 °C CO$_2$ flow: 2-4 kg·h^{-1} Emulsion flow: 0.3-1 kg·h^{-1}	Particle size: 400 nm in suspension in an aqueous medium	[46]
β-carotene	OSA-modified starch	Dichloromethane	Pressure: 5 and 10 MPa Temperature: 35 °C	Drop undergoes swelling and shrinking processes due to the diffusion of CO$_2$ into the drop and dichloromethane out of the drop	[48]
β-carotene	OSA-modified starch Lycopene	Dichloromethane	Pressure: 9-13 MPa Temperature: 50 °C CO$_2$ flow: 3 kg·h^{-1} Emulsion flow: 150-330 ml·h^{-1}	Particle size: 344 – 366 nm Residual solvent: <10 ppm Encapsulation efficiency: 34-89%	[51]
Shrimp residue extract enriched in astaxanthin	Hi-Cap 100 (modified starch)	Dichloromethane	Pressure: 10 MPa Temperature: 40 °C CO$_2$ flow: 4 kg·h^{-1} Emulsion flow: 240 ml·h^{-1}	Particle size: 0.7±0.1 μm Encapsulation efficiency: 93.1%	[50]

In the food industry, SFEE technology was used to form particles from carotenoids, which are an important class of bioactive compounds, to improve the stability and dissolution rates in water and to facilitate the dosing and handling of the product. Mattea et al. [49] published a review of the developments in carotenoid particle formation and co-precipitation with biodegradable polymers using supercritical fluids as an antisolvent, including the use of GAS, SAS, and SFEE. The same authors presented the feasibility of using antisolvent techniques to precipitate β-carotene from dichloromethane in a water emulsion, resulting in a suspension of sub-micron and nanoparticles

with final organic solvent concentrations as low as 1 ppm [46]. Santos et al. [51] recently produced sub-micrometer particles of β-carotene and lycopene in an aqueous medium using SFEE.

Mezzomo et al. [50] were the first researchers to apply SFEE in the co-precipitation of a complex mixture of bioactive compounds using a modified starch. The authors studied the encapsulation of extracts enriched in astaxanthins, the most representative carotenoid from crustaceans like shrimp, to obtain nanoemulsions with a high encapsulation efficiency and low particle size.

5.Conclusions and Perspectives

From a scientific point of view, particle design using the SFEE process is an attractive option due to the possibility of obtaining solvent-free particles with a narrow size distribution curve in addition to avoiding the degradation of thermosensitive compounds. The concept of using SFEE in an industrial context is currently under development. The primary factor limiting this process is that the final product is a suspension of the desired compound in water. The pharmaceutical industry represents a major focus for particles produced using SFEE technology.

Although SFEE has not been widely used for food applications, recent studies applied the technique to the formation of particles from carotenoids, which are an important class of bioactive compounds. Additional bioactive compounds and core materials must be explored in the near future. The results of these researches will have a positive impact in public health: (1) if the target compounds are drugs then, due to increased efficacy smaller amounts of drugs will be needed to treat illness or (2) if bioactive compounds are the target substances then we can expect that new foods can be formulated incorporating these particles.

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