Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications

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Abstract

The formulation of natural substances together with a biocompatible or biodegradable carrier material to form composites or encapsulates has a great relevance for pharmaceutical, cosmetic and food industries. Several precipitation methods with supercritical fluids can be successfully adapted to produce these materials. This article presents a review of the main aspects of supercritical encapsulation and co-precipitation processes, focused on a process mechanisms description as well as of the types of materials that can be formulated with them.

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1. Introduction

Many formulations of pharmaceutical, cosmetic and natural substances consist in composites or encapsulates comprising a core material (the active component) surrounded by a coating material or carrier (typically a bio polymer or a fat). Composites are frequently produced by the simultaneous precipitation of the core and coating materials, leading to a dispersion of particles of the core material into a matrix of coating material, while encapsulates are produced when the coating material is precipitated as a thin shell over a previously existing core material particle. It is also possible to produce composites by first forming particles of pure coating material that are then impregnated with the active components. Such formulations present several advantages. First of all, they can allow achieving a controlled delivery of the active ingredients into its targeted media (the organism of a patient, the soil of a cultivation, etc.) [1]. In addition to oral administration, these particulate carriers can also be injected intramuscularly or intravenously as long as their particle size is within physiologically acceptable range to achieve a controlled dissolution of the active substance. Moreover, when the
active substance is susceptible of degradation, the coating mate-
rial can act as a protective layer against aggressive agents. Finally,
composites and encapsulates are easier to handle and dose than
pure particles of the active substance, and particularly the forma-
tion of relatively large composites or agglomerates encapsulating
micronometric or nanometric particles of the active substance, can
be a convenient method for improving the collection of the parti-
cles after a precipitation, if the collection of particles of pure active
material is difficult due to their small size.
Composites and encapsulates can be prepared by several
micronencapsulation techniques. These techniques can be classified
in physical process like: spray-drying, spray-cooling, spray-chilling,
air suspension coating, extrusion, centrifugal extrusion, freeze-
drying, annular jet and rotational suspension separation; and
chemical process like coacervation, co-crystallization, liposome
entrapment, interfacial polymerization, molecular inclusion, etc.
[2]. Of these processes, spray-drying is one of the oldest encap-
sulation methods, and perhaps the most common and cheapest
technique to produce composites [3]. In this method, the material
for encapsulation is homogenized with the carrier (most often in
a water solution), and then this mixture is fed into a spray dryer
and atomized with a nozzle or spinning wheel. The evaporation of
solvent is rapid causing the entrapment of the active compound
into the carrier. The initial liquid feeding the sprayer can be a solu-
tion, an emulsion or a suspension [4]. Spray drying is widely used
for the drying of heat-sensitive food [5], pharmaceuticals [6] and
essential oils [7]. Typical shell materials are gum acacia, maltodex-
trins, hydrophilically modified starch, proteins and mixtures of
thereof. In spray-cooling methods, the coating material is a molten
fat in which the core material is dispersed. The coating material is
solidified by atomizing it into a cool air stream. The main advan-
tage of these processes is that particles solidify into almost perfect
spheres to give free-flowing powders [8]. Microcapsules produced
by spray-chilling and spray-cooling are insoluble in water due to
the lipid coating and thus these techniques are utilized for encap-
sulating water-soluble core materials such minerals [9], vitamins
[10], enzymes, acidulates and some flavours [11]. In fluidized-bed
coating processes, solid particles are suspended in a chamber where
coating material is atomized [12]. Gas–solid fluidization offers high
contacting efficiency due to its high rapid mixing which also serves
to create a convective flywheel that maintains near-isothermal
conditions. Typical coating systems are hot-melt coatings such as
hydrogenated vegetable oil, stearines, fatty acids, emulsifiers and
waxes, or solvent-based coatings such as starches, gums or mal-
todextrins [2]. Finally, freeze drying is another intensively used
process for the production of composites, particularly of proteins
[13]. In this process water is removed from a frozen solution by vac-
uum sublimination. This process 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.
The application of supercritical fluids as an alternative to these
conventional precipitation processes has been an active field of
research and innovation during the past two decades [14–16]. The
main motivation for this is the possibility of exploiting the peculiar
properties of supercritical fluids, and in particular of supercritical
carbon dioxide (sc-CO₂), the most used supercritical fluid for
precipitation processes. The properties of supercritical fluids are
often described as intermediate between those of a liquid and a
gas, moreover these properties can be easily changed with changes
in pressure and temperature [17]. In the case of carbon dioxide,
the supercritical region can be achieved at moderate pressures and
temperatures (Tc = 304.2 K, Pc = 7.38 MPa); therefore, working with
sc-CO₂ it is possible to carry out the process at near-ambient tem-
peratures, avoiding the degradation of thermolabile substances.

sc-CO₂ also provides an inert medium suitable for processing easily
oxidizable substances. Additionally, the use of the supercritical fluid
eliminates or reduces the use of toxic or contaminant organic
solvents in the process, the separation of the supercritical fluid
from the product can be easily accomplished by depressurization,
and the high solubility of most organic solvents in supercritical
fluids allows obtaining solvent-free products [18]. For this reason,
several precipitation processes based on supercritical fluids have
been developed. These processes can be classified according to the
role of the supercritical fluid in the process [14,19]: solvent, anti
solvent, co-solvent or solute, or even propellent gas.
The aim of this article is to discuss some of the recent develop-
ments in the application of these supercritical fluid technologies for
the production of composites or encapsulates of active materials.
The article is focused in the description of the main features and
mechanisms of some of the most relevant supercritical fluid tech-
niques used for this purpose, rather than on an extensive review of
the materials produced with these techniques.

2. Coating materials

The coating material must fulfil several requisites: its bio-
compatibility and lack of toxicity are of course important
considerations. It should also provide a suitable medium for pre-
serving the properties and activity of the active substance (e.g. the
activity of pharmaceutical proteins [20]). Additionally, it should be
easy to process with the selected precipitation technique. Most
frequently, natural or synthetic biopolymers are used as coating
materials, although other materials as fats [17] or sugars [21] can
also be used. Yeo and Kiran [1] and Tomasko et al. [24] presented
extensive reviews of the supercritical processing of polymers.
The key property that differentiates biopolymers from conven-
tional is their biodegradability, because they degrade in vivo or in
the natural environment to produce biocompatible or non-toxic
by-products. Table 1 presents several frequently used biopoly-
mers. Several of the most frequently used biopolymers are either
biopolymers or starch-based polymers. Starch-based polymers
often are a blend of starch and other plastics, which allows for
enhanced biological and environmental properties. On the other
hand, some biopolymers as polyactic acid (PLA) and polyglycolic
acid (PGA) were the first polymeric materials successfully used as
sutures, and their degradation pathways are well known and do
not produce toxic products. The chemical structures of PLA and
PGA are similar except that PLA is more hydrophobic. There are
also differences in their degradation kinetics, and therefore it is
possible to control the degradation rate of poly (ε-lactide)-co-
glycolide (PLGA) co-polymers by controlling the molar ratio of
lactide and glycolide in the polymer. These materials were later
successfully used for controlled release applications for oral and
parenteral administration. This success led to the development of
other aliphatic polyesters and particularly of polycaprolactones
(PCL). PCLs degrade slower than PLA and PGA and therefore they
are more suitable for long-term delivery systems. Some non-
biodegradable but biocompatible polymers can also be used in drug
delivery systems, including polyethylene co-vinyl acetate (EVA),
polyethylene glycol (PEG), and some acrylic polymers.

3. Physical properties of polymers under CO₂ pressure

The complex phase behaviour of mixtures of polymers, phar-
maceuticals and supercritical fluids has a crucial influence on
the performance of the precipitation processes. Frequently,
polymers + SCF systems exhibit type III phase behaviour [25].
In particular, it has been shown that in systems comprising a
### Table 1
Several frequently used coating materials [1,22,23].

<table>
<thead>
<tr>
<th>Biopolymer</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polyesters</strong></td>
<td>PET structure modified by incorporating aliphatic monomers that can be hydrolyzed by soil moisture. Smaller fragments of polymer are then degraded by naturally occurring microorganisms within a few days</td>
</tr>
<tr>
<td>Aliphatic-aromatic copolymers</td>
<td>Properties similar to those of PE and PP</td>
</tr>
<tr>
<td>Aliphatic polyesters</td>
<td>Made from polycondensation reaction of glycol and aliphatic dicarboxylic acids. Biodegradable in soil and water</td>
</tr>
<tr>
<td>Polylactide aliphatic copolymer (CPLA)</td>
<td>Made by copolymerising lactide with aliphatic polyester</td>
</tr>
<tr>
<td>Polycaprolactones (PCL)</td>
<td>In natural environments it decomposes within 6–12 months</td>
</tr>
<tr>
<td>Polylactic acid or polylactide (PLA)–PGLA (poly (n-lactide))-co-glycolyde)–PLA copolymer-PPLA (poly (l-lactide))–PDLA (poly (d-lactide))</td>
<td>Biodegradable biopolymer derived from lactic acid, can be obtained by fermentation of starch or by chemical synthesis</td>
</tr>
<tr>
<td>Polyhydroxyalkanoates (PHA)</td>
<td>Linear polymers produced by fermentation of sugar or lipids</td>
</tr>
<tr>
<td>Polyhydroyxylic acid (PHB)</td>
<td>Biopolymer present in living microorganisms as storing material (equivalent to starch)</td>
</tr>
<tr>
<td>Poly-lactic acid (HYAFF)</td>
<td>Biodegradable and biocompatible. Used in tissue engineering and for bone reconstruction</td>
</tr>
<tr>
<td>Polysaccharides and starch-based polymers</td>
<td>The main component (10–90 wt%) is starch, the rest are additives and other biodegradable polymers</td>
</tr>
<tr>
<td>Starch-polymer blends</td>
<td>Starch makes the blend more easily attacked and degraded by micro organisms, as they first attack the starch, creating pores in the material that weaken it</td>
</tr>
<tr>
<td>Dextran</td>
<td>Polysaccharide made of glucose molecules, molecular weights ranging from 10,000 to 150,000 Da</td>
</tr>
<tr>
<td>Inulin</td>
<td>Polysaccharide made of fructose molecules</td>
</tr>
<tr>
<td>Cyclodextrins</td>
<td>Oligosaccharides with annular structure made of 6 sugar molecules (α-cyclodextrin), 7 sugar molecules (β-cyclodextrin) or 8 sugar molecules (γ-cyclodextrin)</td>
</tr>
<tr>
<td>Others</td>
<td>Plastic of natural protein origin (milk, horn, wheat…)</td>
</tr>
<tr>
<td>Casein formaldehyde</td>
<td>Non-flammable and insoluble in water</td>
</tr>
<tr>
<td>Polyeletylene glycol (PEG)</td>
<td>Water soluble</td>
</tr>
<tr>
<td>Poly (amino acids) and pseudopoly (amino acids)</td>
<td>Used in suture materials, artificial skin substitutes and drug delivery</td>
</tr>
</tbody>
</table>

Note: Several forms exist due to the chiral nature of lactic acid. Biodegradation of PLLA is faster than that of other isomers due to the lower crystallinity of this form.
polymer, a supercritical fluid and a low molecular weight solvent or pharmaceutical, a liquid–liquid phase split may be observed when CO\textsubscript{2} pressure is increased [26]. One of the two liquid phases in equilibrium is polymer-rich, while the other is CO\textsubscript{2}- and solvent-rich [27]. Based on this phase behaviour, Pérez de Diego et al. proposed an improved precipitation with a compressed anti-solvent (PCA) process based on separating the liquid–liquid phase split step and the spraying and solidification step [28]. This was done by means of a static mixer used to induce the mixing of CO\textsubscript{2} with the polymer and the subsequent phase split prior to the injection into the precipitation vessel through a nozzle (Fig. 1). The separation of the two steps of the process allowed for a better control and reproducibility of powder characteristics.

Although the solubility of polymers in sc-CO\textsubscript{2} could be reasonably expected to be extremely low considering the high molecular weight of most polymers, it is remarkable that in certain cases it is possible to achieve certain solubility by an adequate choice of the structure of the polymers. In general, as SCF are very weak solvents, the solubility can be determined by the interactions of end groups of the polymer, or by polymer branching [25]. It has been observed that the solubility of some fluorine-substituted polymers as perfluoroalkyl ethers or acrylates is unusually high. This effect is perhaps related to the flexibility of the chain of the polymer, that can aid dissolution, or to the presence of carbonyl or ether groups that can interact with CO\textsubscript{2} [24]. The solubility of sc-CO\textsubscript{2} into polymers, on the other hand, can be rather large (e.g. up to 30 wt% in PEG 4000 [29]).

Regarding the variation of the solubility with pressure, normally two distinct regions are observed: at low pressures, the solubility of sc-CO\textsubscript{2} increases almost linearly with pressure. At a certain pressure, the polymer appears to be totally “saturated” with the CO\textsubscript{2} and for increasing CO\textsubscript{2} solubility in a small extent over this saturation point, it is necessary to apply a large increase in pressure. This behaviour may be related to the total occupation of the free intermolecular volume of the polymer by CO\textsubscript{2} at the saturation pressure. Generally, the solubility of CO\textsubscript{2} increases when the polymer has polar groups [24]. Different equation of state models can be used to model this phase behaviour, including the Perturbed Hard-Sphere-Chain Equation of State (PHSC EoS) [26], the Sanchez-Lacombe EoS and the Statistical Associating Fluid Theory (SAFT) equation [25]. Cubic equations of state are not suitable for this purpose because critical properties of polymers are not available.

Besides giving rise to this complex phase behaviour, it is well known that the presence of supercritical fluids significantly alter many physical properties of the polymer [24]. Of particular importance for polymer processing with supercritical processes is the glass transition and the melting point temperature depressions induced by the supercritical fluid. In particular, the dissolution of sc-CO\textsubscript{2} into the polymer can reduce the glass transition temperature of amorphous polymers by as much as 4–30 °C/MPa [24], an effect that is caused by intermolecular interactions between the dissolved CO\textsubscript{2} and the polymer. The melting point depression caused by the dissolution of CO\textsubscript{2} is less noticeable in magnitude. Measuring the melting temperature of PEGs of different molecular weights under CO\textsubscript{2} pressure, Weidner et al. [29] observed that the variation of the melting point temperature with CO\textsubscript{2} pressure follows a similar trend as CO\textsubscript{2} solubility: at low pressures, melting temperature decreases almost linearly with pressure. Upon reaching the maximum CO\textsubscript{2} solubility, the melting temperature reaches its minimum value, and then increases as CO\textsubscript{2} pressure increases due to the effect of hydrostatic pressure. These authors observed a reduction of melting temperature of about 15 °C for all the PEG molecular weights investigated; therefore, all their experimental data could be represented in a single plot of melting temperature vs. reduced temperature (defined as melting temperature under pressure divided by the melting temperature at low pressure). The dissolution of CO\textsubscript{2} also causes certain modifications in the structure of the polymer, as a decrease in the crystallinity [30] as well as a significant swelling (of up to 34% in PEG1500–CO\textsubscript{2} systems [31]). It has to be taken into account that if after the precipitation the particles stay in contact with pressurized CO\textsubscript{2} for a prolonged time, the decrease of glass transition and melting temperatures can promote agglomeration and cause other variations of the properties of the particles.

Although the properties of polymers in a supercritical fluid environment are crucial parameters for supercritical encapsulation and co-precipitation processes, there is a certain lack of experimental data. The solubility of CO\textsubscript{2} in some bio polymers (PEG, EVA, PLA, ...) has already been determined (see Tomasko et al. [24] for a detailed review of solubilities of CO\textsubscript{2} into polymers as well as of other thermodynamic and transport properties of

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**Table 1 (Continued)**

<table>
<thead>
<tr>
<th>Biopolymer</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-(2-hydroxypropyl)methacrylamide (HPMA) copolymers</td>
<td>Used to coat proteins, enzymes and cancer chemotherapy agents By controlling the molecular weight below the threshold of renal excretion the elimination of the polymers after the release of bioactive materials can be achieved</td>
</tr>
<tr>
<td>Polyethylene-co-vinyl acetate (EVA)</td>
<td>Produced by copolymerization of ethylene and vinyl acetate Stiffness and crystallinity depends on the proportion of vinyl acetate Biocompatible copolymer that has been used as the matrix for controlled drug release devices</td>
</tr>
</tbody>
</table>

*Fig. 1. Schematic flowsheet of the modified PCA process proposed by Pérez de Diego et al. [28]. A static mixer is incorporated at the top of the precipitation chamber to separate the liquid–liquid phase separation process and the spraying and solidification process, thus allowing for an improved control of each of these processes.*
CO$_2$-polymer systems), but experimental data regarding the variation of other properties caused by CO$_2$ dissolution (reduction of glass transition and melting temperatures, swelling, . . . ) are more scarce. More importantly, very little is known about the physical properties and phase behaviour of ternary CO$_2$-polymer-solute mixtures (e.g. about the adsorption equilibrium of drugs into CO$_2$-swollen polymers) that are even more important for developing impregnation and co-precipitation processes with supercritical fluids.

4. Characterization of composites and encapsulated materials

In general, the release of the active substance from the biopolymer matrix is controlled by diffusion of the substance and by the erosion and degradation of the polymer. Diffusion occurs during the release of small molecules from polymeric systems when the carrier retains its structural integrity even after the substance is depleted, while erosion refers to the dissolution and degradation of the polymer causing a progressive weight loss of the matrix. Both mechanisms depend on a number of parameters. Some of them are external (concentration of the active substance and carrier in the surrounding medium, pH, enzymatic action), and others are properties of the particles: particle size and morphology, drug loading, chemical interactions between drug and polymer, and polymer characteristics such as glass transition temperature and molecular weight [22]. Although the nature of the encapsulated substance determines the characterization methods to be used, several properties are commonly characterized for almost every particulate system as they affect the drug release: particle size (PS), particle size distribution (PSD), loading of the active compound, stability of the particles, release rate of the encapsulated material and degradation of the carrier material.

4.1. Particle size and particle size distribution measurements

The particle sizes to be considered in applications related to drug release are in the nanometric or micrometric scale. For this scale two different kinds of measurements are widely used: microscopic image analysis and light scattering techniques. Microscopic techniques allow to obtain information about the morphology of the particles besides PSD data, while light scattering techniques such as Dynamic Light Scattering (DLS) and Laser Diffraction (LD) [32] offer a more reliable alternative for determining PSDs.

The two main Electron Microscopy (EM) techniques most frequently used in particle characterization in this range of particle sizes are Transmitted Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM). Several modifications to these electron microscopic techniques have been applied to extend or enhance their application. Scanning Transmission Electron Microscopy (STEM) is like TEM but it uses a small probe to scan across the specimen, the rastering of this beam across the sample makes it suitable for several analysis techniques such as energy Dispersive X-ray Spectroscopy (EDX), Electron Energy Loss Spectroscopy (EELS) and Annular Dark-Field imaging (ADF) which allows direct correlation of the image and quantitative data. For example STEM combined with these analysis techniques has a very high atomic scale resolution and can be used to study the chemical composition at a surface or interface. Another new technique called Environmental SEM (ESEM) differs from a conventional SEM in that the sample is not viewed under high vacuum, and thus it allows studying samples without any previous preparation procedure, in wet conditions [33] and also dynamic imaging [34] in a non-destructive way. Also some scanning probe microscopes such as Scanning Tunneling Microscope (STM) and Atomic Force Microscope (AFM) are used to characterize particle size and surface structure [35].

4.2. Drug loading and drug release analyses

Some of the most important factors in the production of encapsulated materials are the relative amount of the active substance in the co-precipitate as well as the yield of the incorporation (defined as the ratio between the amount incorporated into the polymeric matrix and the initial amount). This parameter can be determined with several methods. The most usual methods involve the dissolution of the carrier and active substances into adequate solvents, followed by a quantitative analysis of the amounts of each substance by HPLC, GC, etc. It is also possible to determine the loading indirectly by measuring the amount of drug that was not incorporated into the carrier. In any case special care has to be taken to ensure that the active substance that has simply been deposited on the surface of the particles and has not been permanently incorporated into the carrier matrix is removed and properly accounted for. Some microscopy techniques allow the direct observation of the distribution of the encapsulated material, particularly when the encapsulated material exhibit a pronounced difference with the carrier material, such as fluorescence for which confocal laser scanning microscopy (CLSM) can be used, or a different atomic composition where EDX can be used to obtain the mapping of the characteristic atom in the particles. However this technique may be less accurate since it is based on a small sample analysis which may not be representative of the product.

Drug release analyses are performed into a solvent medium that emulates the target media (e.g. a phosphate buffered saline solution, PBS, with the same pH as the human organism for drugs intended for human consumption). Certified equipments are used that allow an accurate and reproducible control of parameters such as temperature, stirring and sampling method, which can affect the dissolution rate. Release analyses can also be used to measure the drug loading, but in this case it may be necessary to carry out the analysis for a very prolonged period in order to ensure the full release of the encapsulated materials [36].

4.3. Solid-state characterization

Most carrier materials are polymers or polymer-based materials, which can suffer modifications during the encapsulation or co-precipitation process. The crystallinity of polymers can be modified during the precipitation process [37] and also the crystallinity of the substances to be encapsulated [36]. Several authors also have reported the hindering effect of the polymer on active substance particle growth when both components are precipitated together [36,38,39].

There are different techniques to study the crystallinity of materials, such as X-ray diffraction (XRD) [40], differential scanning calorimetry (DSC) [41,42] and Raman spectroscopy (RS) [43]. These techniques give information about the interaction between the carrier and the encapsulated material, changes in the crystallinity of both substances and changes in the molecular weight of the carriers. For instance, XRD and DSC analysis can confirm if in a co-precipitation the active substance has been effectively incorporated into the carrier matrix, or if the product simply is a mixture of segregated particles of active substance and carrier: in the first case, the characteristic crystalline peaks and melting peaks of the active substance will not be observed in XRD and DSC analyses, while in the latter the XRD and DSC graphs will be a superposition of the diagrams of each substance, equivalent to what would be obtained with a physical mixture of particles of the two materials.
5. Particle formation processes with supercritical fluids

5.1. Supercritical fluid as solvent: (I) Rapid Expansion of Supercritical Solutions (RESS)

The Rapid Expansion of Supercritical Solutions process is based on exploiting the large variations in the solvent power of supercritical fluids with changes in pressure. A solute dissolved in a supercritical fluid in an extraction at a high pressure precipitates when the supercritical fluid is expanded and pressure is reduced. The RESS process can also be used to produce composites. The most direct implementation is to dissolve both the active substance and the coating material in the supercritical fluid, and then co-precipitate both substances. However, it is well known that the main limitation of RESS techniques is the low solubility of many substances in sc-CO2. This limitation is of course even more severe for co-precipitation applications in which both the carrier and active substance have to be soluble in sc-CO2. However, there are some applications related to the encapsulation of drugs with biopolymers [44,45]. It is also possible to overcome the limitation of low solubility in CO2 by employing alternative organic supercritical solvents such as trifluoromethane or chlorodifluoromethane [46]. Another modification of the RESS process intended for eliminating the solubility limitation is the RESS-non-solvent process (RESS-N). In this process, a liquid antisolvent for the polymer is used as a cosolvent for improving the solubility in the supercritical fluid. Mishima et al. [47] used this technique to encapsulate several pharmaceuticals and proteins in different polymers such as PEG, PLA or PMMA. Finally, although most biopolymers are insoluble in sc-CO2 and therefore cannot be processed by RESS techniques, other carrier materials which have high solubility in sc-CO2 such as paraffins or lipids can be considered for RESS co-precipitations.

Besides the solubility limitations, another major problem of RESS co-precipitation techniques is that since the precipitation by RESS is extremely fast, it is very difficult to control the morphology and loading of the composites, because these parameters are extremely sensible to the sequence of supersaturation, which in turn depends on most process parameters [1]. An alternative technique that eliminates this problem is the precipitation of the carrier over previously formed microparticles of the active substance. With this procedure, it is easier to control the loading of the particles or the polymer coating thickness simply by manipulating the feed compositions, as long as a homogeneous dispersion of the particles to be coated and the coating material is achieved in the precipitation chamber. With the objective of improving the coating homogeneity, Mishima [48] presented a modification of this procedure consisting in incorporating a high shear mixer into the precipitation chamber. This facilitates the circulation of particles and improves the control and homogeneity of the process. Another possibility that also improves product homogeneity is to perform the RESS precipitation over a fluidized bed of the active particles [49]. By precipitating the coating material in the upper section of the fluidized bed, the homogeneity of the coating is facilitated by the intense mixing of the particles inside the fluidized bed. Also the stratification of the particles in the bed caused by gravity can enhance the homogeneity, as particles with a coating layer thicker than average will tend to concentrate in the bottom section of the fluidized bed, away from the upper section in which the coating material is being precipitated. In general, the encapsulation processes using carrier materials that exhibit a good solubility in CO2 have very promising applications due to the simplicity and cleanliness of these processes.

5.2. Supercritical fluid as solvent: (II) Supercritical Solvent Impregnation (SSI)

Polymers can be impregnated with drugs by dissolving the drug in a supercritical fluid and then contacting the resulting fluid mixture with the polymer particles to be impregnated [50]. A schematic diagram of this process, called Supercritical Solvent Impregnation (SSI) is presented in Fig. 2. The two main items of the setup are the drug column, in which sc-CO2 is saturated with the drug, and the carrier column, in which this solution is brought into contact with the polymer. It is also possible to dissolve another substance in the CO2 to enhance the solubility of the drug (co-solvent) or to improve the dispersion of the drug in the polymer (surfactant).

Several product properties such as drug loading or drug depth penetration can be modified by adjusting parameters such as the rate of depressurization step, the time of impregnation or by changing the solvent density by changes in pressure and temperature [51]. The incorporation of the drug into the polymer can occur by precipitation of the drug during the depressurization by RESS process. However this mechanism of incorporation can result in poor product properties and therefore an unsuccessful
impregnation, because since a bound between carrier and active substance has not been created, the active substance can easily be segregated from the carrier and lost, especially if it is liquid at ambient conditions. An alternative mechanism which can result in much better product characteristics is the impregnation of the drug into the polymer during the contact step at high pressure due to the affinity of the solute for the polymer matrix. For this second mechanism to be possible, the partition coefficient of the drug between the supercritical fluid and the polymer must be favorable enough. The impregnation of the drug by this mechanism is aided by the plasticization and swelling effect caused by the dissolution of CO₂ into the polymer.

It has to be mentioned that as the RESS process, the SSI process can be hampered by the low solubility of drugs in the fluid. Alessi et al. [52] proposed to use certain solid co-solvents such as polydimethylsiloxanes (PDMS) to increase the solubility of the drug. PDMS are biocompatible and therefore their presence in the final product would not be a problem.

It appears that for improving the applicability and performance of this technology it would be necessary to gain a more detailed understanding of the interactions between polymer, CO₂ and active substance, as well as of the ways in which the active substance is attached or adsorbed into the polymer. This would allow a systematic selection of adequate polymer-active substance pairs. It may be also of interest to study the possibility of avoiding the deposition of active substance by precipitation during the expansion stage, because it can cause the formation of particles or droplets of the active substance that are not attached to the carrier matrix and therefore can be detrimental for the quality of the product.

5.3. Supercritical fluid as solute: Particles from Gas Saturated Solutions (PGSS)

PGSS is a process designed for making particles of materials that absorb supercritical fluids at high concentrations. SCF is dissolved in a (or substrate), or a solution of the substrate(s) in a solvent, or a suspension of the substrate(s) in a solvent, followed by a rapid expansion, at moderated pressures, of the saturated solution through a nozzle causing the formation of solid particles or liquid particles according to the system due to the intense cooling effect caused by the release of CO₂. A schematic diagram of a PGSS process is presented in Fig. 3. It consists in a precipitator into which the gas saturated solution is injected. The saturation of the solution with the gas is achieved by means of a static mixer.

The PGSS process is especially suitable for producing particles of polymers and also for incorporating active substances into these particles [53]. In this application the plasticizing and swelling effect caused by CO₂ dissolution can again enhance the incorporation of the active substance. The PGSS process can also be used for generating fluid-filled particles. In this process the carrier material is used in molten form and admixed with the liquid to be encapsulated. Both components are pressurized with pumps and dosed into a mixing system. Some examples are the encapsulation of theophylline in hydrogenated palm oil to obtain a controlled drug delivery system developed by Rodrigues et al. [54], the encapsulation of liquid paraffin in polyester and the encapsulation of liquid polyethylene glycol in polyethylene wax developed by Wendt and co-workers and encapsulation of aqueous solutions in fats [55]. As in the SSI process, the active substance can be incorporated into the carrier either in a segregated way (forming distinct particles or liquid vacuoles), or by adsorption into the carrier. In the encapsulation of liquids to form vacuoles inside the carrier, the yield of incorporation will depend on the relationships between the kinetics of solidification of the carrier, which forms a solid shell around the liquid preventing its evaporation, and the kinetics of diffusion and evaporation of the liquid to be encapsulated.

5.4. Supercritical fluid as antisolvent: (I) Supercritical Anti Solvent (SAS) precipitation

SAS processes are based on bringing into contact a solution of the solutes of interest in a conventional liquid solvent with a supercritical fluid. Upon mixing, the supercritical fluid saturates the liquid solvent and depletes it by extraction. The saturation of the liquid solvent by the supercritical fluid causes the precipitation of the solute by an anti solvent effect.

As with the RESS process, it is possible to produce either composites by simultaneously co-precipitating the core material and the carrier, or to encapsulate previously formed particles of the active substance by suspending it in a solution of the carrier and
then precipitating the carrier by SAS [56]. In the precipitation over a suspension of particles, the particles behave as nuclei for the precipitation of the polymer, and a polymer matrix of encapsulated particles is produced by agglomeration [57]. This procedure is especially suitable for organic-insoluble materials as proteins [58]. For co-precipitation applications, if the carrier and the core material are not soluble in the same solvent, it is possible to prepare two different solutions with each of the substances, and then subject them simultaneously to SAS precipitation. The Supercritical Enhanced Dispersion of Solutions (SEDS) process offers a nozzle design particularly suitable for simultaneously injecting several liquid solutions into the supercritical fluid [59]. As with other co-precipitation processes, one of the key parameters sought with a SAS co-precipitation is a high efficiency of loading. This depends on a number of parameters, and particularly on the initial concentrations of core and coating materials, and the affinity between these two materials [1]. As in the SSI process, if there is a high affinity between these materials it is possible to incorporate the core material in the carrier in an amorphous state. If this affinity does not exist, segregated precipitation of these materials may occur. It is possible to modify this affinity and improve the gas loading by including surfactants into the precipitating mixture [60].

The initial concentrations of active substance and carrier are also key parameters with respect to the morphology of the particles. In the co-precipitation of ß-carotene and PEG, it was possible to obtain different morphologies as hollow spheres, carotenoid particles partially covered with relatively small polyethylene glycol spheres, or smooth surface spherical particles, only by changes in the concentration ratio between the polymer and the carotenoid [61]. If the active substance is successfully incorporated in an amorphous state into the carrier material, the particle size of the composites can be smaller than the size of pure core material precipitated under the same conditions [61,62]. Temperature is another key parameter for a successful co-precipitation with the SAS process, because in some cases the addition of the organic solvent to polymer–scCO₂ mixtures can lead to the apparition of a liquid–liquid phase split at moderate temperatures [26].

5.5. Supercritical fluid as antisolvent: (II) Supercritical Fluid Extraction of Emulsions (SFEE)

The application of supercritical fluids in the particle technology with emulsions appears as a natural decision to avoid the main problems of each separated technologies. Emulsion techniques usually involve large quantities of organic solvents, and the removal of them involves additional separation techniques and the use of high temperatures. On the other hand supercritical fluids usually are not able to produce particles below the micrometer range or the products obtained present agglomeration problems ending in large particles of agglomerated nanoparticles. The combination of these two technologies was first presented by Perrut et al. [63,64] who proposed a process in which a aqueous soluble compound was dissolved in the dispersed phase of a W/O emulsion and supercritical carbon dioxide together with the organic solvent present in the continuous medium was used as a drying agent to form dried particles and especially for the production of proteins. Chattopadhyay et al. [65] patented a process to produce microparticles and nanoparticles from an O/W emulsion via the extraction of the solvent, having the solute dissolved in this solvent. During the extraction it can be expected that first the droplets of the disperse phase become saturated by CO₂, and then the solvent is extracted by CO₂ from them. Therefore during the saturation with CO₂ each droplet behaves as a miniature Gas Anti Solvent precipitator. The final product obtained by this method usually consisted in a micro or nano-suspension of the particles in water. The authors patented the process as Super-critical fluid extraction of emulsions (SFEE), and it was proposed as particularly suitable to produce nano-suspensions of pharmaceutical substances that are slightly soluble or insoluble in water instead of dry particles, which requires additional steps like air drying, vacuum drying or freeze drying to produce powder like particles. The same authors [66] presented the feasibility of the process to produce enhanced dissolution rate particulates, investigated the mechanism of particle formation and scale up of the process applying the SFEE to a model system consisting in cholesteryl acetate, megastrol acetate and griseofulvin from DCM, toluene or ethyl acetate in water emulsions. Furthermore, the encapsulation of particles using the same process was presented [67] to produce drug-polymer composites for sustained-release drug delivery formulations in both continuous and batch manners. An advantage of using emulsions for encapsulation is that if the active solution is dissolved in the disperse phase and the carrier in the continuous phase, the emulsion provides a template for the final morphology of the microcapsules. Recently the application of the SFEE technology was used to form particles from different substances, for example the PLGA/piroxam system was precipitated from an ethyl acetate in water emulsion for drug delivery purposes in a batch mode [68], and ß-carotene was also processed forming a nanosuspension of the carotenoid in water from DCM in water emulsions [69].

The experimental setup of these processes can be performed in a batch or continuous form, and the equipments involved in the precipitation process are practically the same of the batch GAS and semicontinuous SAS process, respectively. Figs. 4 and 5 present schematic diagrams of the batch and continuous process, respectively. The differences from GAS and SAS processes and the SFEE are (a) an emulsion containing the desired substance to be precipitated dissolved in its dispersed phase is injected instead of injecting a simple solution of the substances, (b) a liquid product is formed, allowing the continuous extraction of the product but requiring additional steps to produce a powdery product, (c) the preparation of the initial materials is more complex involving the use of surfactants, high energy dispersion techniques, (d) there is an additional controlling parameter besides the usual parameters of GAS and SAS processes (pressure, temperature, flow rates, and concentrations) that is the emulsion droplet size distribution. Almost all of the presented articles about SFEE observed a direct relationship between the particle size and the initial droplet size. So the effect and dependency of the droplet size distribution with the pressure, temperature and the remaining processing parameters is an important
factor in the SFEE processes. To optimize this technology it is also important to understand the variation of the properties of the emulsion during the saturation with the supercritical fluid, considering parameters as the changes in the droplet size of the dispersed phase due to the dissolution of sc-CO$_2$, or the possible reduction of stability of the emulsion caused by CO$_2$ and the relationship between the kinetics of de-emulsification and crystallization processes.

6. Concluding remarks

It is well known that the processing of natural and pharmaceutical substances with supercritical fluids in general and with supercritical carbon dioxide in particular has several inherent advantages: non-toxicity and easy removal of the solvent, operation at moderate temperatures and in an inert atmosphere that allows avoiding degradation of the product, etc. Besides, the possibility of tuning the properties of the supercritical fluid with changes in pressure and temperature or by the addition of co-solvents has given rise to several different precipitation techniques that are able to process a large variety of materials producing controlled particle sizes and morphologies. Those advantages are also applicable for encapsulation and co-precipitation processes with supercritical fluids, but these applications can also take advantage of the capability of the supercritical fluid for modifying the properties of the polymeric carrier material (plasticizing, swelling, reduction of melting temperature, etc.) as well as the interactions between the carrier and the active substance, a capability that can allow to modify and control the way in which the active substance is incorporated into the carrier. Nowadays, these possibilities are limited by the fact that the production of carrier-active substance formulations is largely a matter of empirical knowledge and not so much is known about the way in which the supercritical fluid interacts with the carrier and the active substance. A more detailed understanding of the fundamental aspects of these interactions may give rise to new opportunities of controlling the properties of co-precipitates and encapsulates produced by supercritical fluid techniques.

References


