Supercritical Fluids applications in the Pharmaceutical Industry

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Abstract

A detailed literature and patent review on recent development of supercritical fluids applications in the pharmaceutical industry is presented: Extraction from solids, mainly from natural raw materials, fractionation of liquids and preparative chromatography for highly-selective separations and active substances purification, reactions in supercritical media for selectivity and kinetics improvement, particle design leading to innovative formulations in order to increase the drug bio-activity and bio-availability, to design controlled-release systems and to replace parenteral drug delivery by less invasive routes (pulmonary, transdermal, oral). Basic guidelines are proposed in order to help the scientists in choosing the optimal process and technology, especially for drug formulation where numerous processes are presently proposed.

Scope

The pharmaceutical industry is facing many challenges: Invention of new drugs and improvement of the therapeutic drug efficacy against numerous pathologies, meanwhile supporting a continuous effort to move to environment-friendly processes and reducing the use of potentially harmful solvents. As Nature is an almost unlimited source of active substances, a great interest is paid to concentrate them or to remove undesired compounds, using mainly extraction with organic solvents or water or ethanol/water mixtures, depending on the polarity of the targeted molecules. As *carbon dioxide*, used pure or added with ethanol, presents the definitive advantages to be a « green », abundant and cheap solvent perfectly adequate to process food or pharmaceutical products at a temperature near to ambient, Supercritical Fluid Extraction (SFE) - referring to *fluid-solid extraction* - and Supercritical Fluid Fractionation (SFF) - referring to *fluid-liquid fractionation* – are widely investigated for extraction and purification of natural or synthetic active products (for instance, elimination of toxic residues).

Recently, a great interest has been paid to use Supercritical Fluids as *reaction media*, especially for very selective synthesis (hydrogenation mainly) that may find attractive applications in pharmaceutical active preparation. Enzymatic reactions and supercritical decontamination/sterilization are also subjected to extended R&D work.

Moreover, Supercritical Fluid (SCF) technology is very attractive for *drug formulation* and manufacturing *innovative* therapeutic particles, either of pure active compounds or composites of excipient and active compounds. In fact, it is important to notice that optimized drug formulation and delivery improve therapeutic efficacy of the drug, reduce adverse effects and bring better comfort to the patient. Several issues can be addressed through innovative processes using Supercritical Fluid technology:

- Very low solubility of active molecules in biological fluids,

- Alteration along the digestive track,
- Delivery of very unstable bio-molecules,
- Substitution of injection delivery by less invasive methods, like pulmonary delivery (inhalation),
- Need for controlled release due to high toxicity or long-term delivery.

Present status of SCF industrial applications:

During the last two decades, industrial applications of Supercritical Fluids have been mostly developed for natural products extraction/fractionation, both for food and pharmaceutical products, as detailed in many books and symposium proceedings [1-8]. At present time, these applications are still continuing to spread worldwide as requirements for high quality products and concerns on environment/health are growing [9].

- **Extraction** (SFE) from solid materials is the most developed application, mainly for natural products processing: Food products (coffee, tea, low-fat cholesterol-free egg yolk powder, etc.), food ingredients and supplements (hops and aromas, colorants, carotenoids and vitamin-rich extracts, specific lipids, etc.), natural insecticides (*Neem, Pyrethrum)* and nutra-/phytopharma-ceuticals. I estimate at about 100 the number of industrial-scale SFE units now under operation with a growth of about 10% per year (figures 1 and 3a).

  Among the drugs presently registered in Europe, the saw palmetto (*Serenoa repens*) extract is obtained by large scale SFE; *Pygeum africanum* can also be extracted without chlorinated solvents, among many other active principles of natural origin (*Kava-kava, Tanacetum parthelium, bee pollen, etc.*).

  Residual organic solvents and pesticides are also removed from final active compounds (natural, like *ginseng*, or synthetic) at large scale. Delipidation is also operated, especially for protein extracts.

Moreover, some "niches" applications concern high-added value bio-medical products, like bone delipidation for allografts [10,11], or specialty polymer stripping (bio-medical implants).

- **Fractionation** (SFF) of liquid mixtures are designed to take profit of the very high selectivity of supercritical fluids with attractive costs related to continuous operation; nevertheless, few industrial units are now used, mainly for aroma production from fermented and distilled beverages (figures 2 and 3b).

  The more promising pharmaceutical applications seem to be:

  - *Fractionation of lipids*: Mono-, di- and tri-glycerides [12], polyunsaturated fatty acid esters [13] like EPA/DHA, tocopherols concentration [14], polar lipids as for separation of ceramides, glyco-lipids (mono- and di-galactosyl-diglycerides) and phospholipids from wheat gluten oil [15], etc.
  - *Fractionation of specialty polymers*: It is possible to obtain very "narrow" fractions as the SFF process is extremely selective if operated in adequate conditions: For example, clinical lots of a pharmaceutical poloxamer were successfully processed on a large-scale SFF unit in order to eliminate the shortest chains that present some toxicity;
  - *Recovery of active compounds from fermentation broths*.

- **Preparative-Scale Supercritical Fluid Chromatography** (PSFC) is operated for ultimate fractionation of very similar compounds [16], especially for lipids like polyunsaturated fatty acids in a few large-scale units. Recent development of simulated-moving bed chromatography with a SCF eluent (SF-SMB) proved that the variability of the elution power of a SCF is a key-advantage over liquid solvents, leading to a significant increase in fractionation performance in comparison with

classical SMB. This opens an attractive route for enantiomer fractionation that constitutes one of the main issues in pharmaceutical synthesis [17,18].

- **Reactions** (SFR) are operated in Supercritical media [5,19,20], and very promising processes are being developed for fine highly selective synthesis, especially hydrogenation with the recent commissioning of a toll-processing SCF hydrogenation plant for fine chemical synthesis in the UK. Enzymatic reactions operated in carbon dioxide also received a great attention, although no major development happened yet.
- **Pollution abatement:** SCF, and especially carbon dioxide, lead to environment-friendly processes through organic solvent substitution. Moreover, water streams polluted with organic compounds can be treated with CO_2 for pollutants elimination. On the other hand, supercritical water appears as a unique medium for safe destruction of dangerous wastes by total oxidation due to its special physicochemical properties, especially for highly hazardous wastes, as proven on a few demonstration plants, including one for recycling precious metals from spent catalysts [21]. Moreover, pollutant destruction in subcritical water is also used in pharmaceutical companies, even if the oxidation rate is lower than in supercritical water.

Particle design and drug formulation by SCF processes:

Particle formation processes using supercritical fluids [22-24] are now subjected to an increasing interest, especially in the pharmaceutical industry with three aims: Increasing bio-availability of poorly-soluble molecules, designing sustained-release formulations and preparing drug delivery less invasive than parenteral (oral, pulmonary, transdermal). The most complex challenge is related to therapeutic proteins as it is extremely difficult to process and deliver bio-molecules due to their instability and very short half-life *in vivo*. In fact, SCF technology comprises several processes that offer various possibilities to address the different issues to solve. Moreover, although most previous works dealt with water-insoluble (or poorly

soluble) molecules, recent development permits to also process very hydrophilic molecules, including fragile bio-molecules.

- **Rapid Expansion of Supercritical Solutions (RESS)** consists in atomizing a solution of the product in a supercritical fluid into a low-pressure vessel [25]. This process could find valuable applications at commercial scale only when the product solubility in the supercritical fluid is not too small ($\geq 10^{-3}$ kg/kg), limiting the process application to non-polar or low-polarity compounds when CO_2 is used as solvent. However, recent works demonstrated that a much wider range of molecules can be processed by RESS when using polar SCF like dimethyl ether. In fact, the particle morphology (shape, size, crystalline pattern) can be tuned by playing on the process and equipment parameters, as shown on two examples:
  - Micronization of *Lovastatin*, an anti-cholesterol drug, by RESS with CO_2 (figure 4): From large and irregular particles (4a), we obtained either highly-porous agglomerates of nano-particles (4b), or micro-particles in form of rod crystals (4c) or spheres (4d) depending on the type of nozzle.
  - Micronization of *Celecoxib*, a COX-2 inhibitor registered for arthritis cure [26,27]: Rapid depressurization of Celecoxib solution in SCF CO_2 (50°C, 29 MPa) led to fluffy agglomerates of elementary nano-particles. The XRD patterns of these particles are compared on figure 5 with the starting material one (upper curve), the two latter curves being presented with an offset of 2,000 and 3,000 counts per second respectively for ease of interpretation. It clearly appears that the starting material is highly crystalline and the generated particles are completely amorphous when the temperature in the atomization vessel is kept low (sample b: lower curve), and mostly amorphous (sample a: intermediate curve) when this temperature is near ambient. We consider that the very short RESS nucleation

leads to amorphous material that immediately tends to re-crystallize during the particle residence time in the atomization vessel and on the collection filter, if the temperature is not kept lower than a "re-crystallization temperature" that is much below (at least 30°C according to some estimations) the solid glass transition temperature.

- **Supercritical Anti-Solvent (SAS)** applies to most molecules that can be dissolved in a very wide range of organic solvents. Recent development opens a bright future for "engineering" new types of particles of different morphologies (figure 6), leading to nano-particles (50-500 nm) or micro-particles (0.5-5 µm) or empty "balloons" (5-50 µm) made of nano-particles, permitting a very significant increase in bio-availability of poorly water-soluble drugs, or preparation of drug with a narrow particle size distribution dedicated to pulmonary delivery. It has been shown on numerous examples that the particle morphology can be tuned, including the generation of one or the other crystal polymorph in case of polymorphism [24,27]. Moreover, micro-spheres of drug embedded in an excipient for sustained-release delivery can be prepared by this process (see below).
- **Supercritical Fluid Drying** permits to prepare dry powders from aqueous solutions. The main target is to obtain stabilized dry powder of proteins or other bio-molecules that may be denatured by the classical drying processes like spray-drying. At the difference with lyophilization, it is also possible to control the particle size and particle size distribution. Several processes can be used to "extract" water:
  - *Supercritical Anti-Solvent:* This process is used for obtaining particles from aqueous solutions using a CO_2 – Ethanol mixture as fluid, the alcohol serving as entrainer of water into the fluid; in fact, this process requires huge amounts of fluid (the fluid/solid mass ratio is in the range of 10,000) as water is very slightly

soluble in the fluid mixture and the solvent residue adsorbed on the particles must be eliminated by a final stripping with pure carbon dioxide. Moreover, it was found that the protein bio-activity may be significantly altered as shown on trypsin, or not for lysozyme [24], depending on pH and temperature stability of the molecule.

- *Emulsion Extraction:* The solution of active in an aqueous medium is emulsified into a polar organic solvent, often in presence of a surfactant; this emulsion (possibly a micro-emulsion) is then pulverized into a supercritical fluid stream that extracts the solvent and water, leading to a dry powder of particles consisting in the active mixed with other compounds dissolved in the aqueous medium (salts, sugars, etc.) [28]. According to our recent experience, it is possible to prepare dry particles (moisture less than 5% wt) of controlled size from aqueous solutions of very hydrophilic compounds emulsified in n-pentanol: Sugars (sorbitol), amino-acids (valine), and proteins (BSA, insulin, various enzymes), as presented on figure 7. It is noteworthy to notice that the particle size distribution can be tuned in order to fit the specifications for inhalation (figure 8). Stabilized formulation of proteins incorporating buffer salts, sugars and possibly surfactants can be obtained and bio-activity is preserved as shown on several enzymes (catalase, trypsin, lactase).
- *Polar SCF Extraction:* Instead of using carbon dioxide that requires a polar co-solvent for extracting water, it is possible to use a polar fluid that does dissolve water, like dimethyl ether as described in a recent patent application [29] that claims isolation of a water-borne bio-molecule to form solid particles without activity alteration of the molecule structure, as exemplified on BSA, insulin, antibodies and DNA.

- **Fluid-Assisted Micro-encapsulation** uses the concept known as Particle Generation from Supercritical Solutions or Suspensions (PGSS [30]), consisting in atomizing a solution of compressed gas or supercritical fluid inside the coating agent in which the particles of active are dispersed in form of a slurry, by decompression towards a low-pressure vessel; the rapid fluid demixion induces solidification of the coating agent, leading to very small core-shell micro-capsules of active inside the excipient. According to this process developed and patented by Perrut [31], micro-capsules of proteins can be easily prepared in "mild" conditions that do not lead to protein denaturation and loss of bio-activity as demonstrated on lactase; however, this should be confirmed on therapeutic proteins and other fragile bio-molecules through on-going experimental work. It is to be noticed that this process is very easy to scale-up and to be operated in compliance with GMP rules, possibly in a sterile environment when required.

  As shown on figure 10, various release curves can be obtained depending on the coating agent; it is to be noted that, for most excipients, the "burst" effect is very limited, proving the quality of the active particle coverage by the coating. Extended works are now on going with several therapeutic peptides and proteins, including *in vivo* tests in animals.

- **Other coating/encapsulation processes:**
  - A first class of processes can apply when the coating is soluble in the supercritical fluid, such as waxes, glycerides, alcohols, fatty acids and esters, and some rare polymers. The RESS process can be used as firstly demonstrated by Debenedetti et al. [32]. Benoit et al. [33,34] are developing a deposition process consisting in dissolving the coating agent into supercritical carbon dioxide and, by changing the pressure and the temperature, precipitating the coating agent onto the active

substance particles dispersed into the supercritical solution inside a stirred vessel, leading to microcapsules that are collected after depressurization.

- But in most cases, the coating is not soluble in the supercritical fluid. A significant number of works are based on the anti-solvent process after the pioneering patent of Fischer and Muller [35]. Among other works [23,24,35-41], Subramaniam et al. [41] patented a process where the coating solution is sprayed into the supercritical fluid, in a way to generate high frequency sonic waves inside the precipitation vessel where particles are fluidized. Benoit et al. [42] proposed a process where the active substance particles are in suspension in a solution of a slightly polar polymer in an organic solvent; this suspension is contacted with supercritical carbon dioxide causing coacervation of the coating polymer onto the particles by anti-solvent effect. Perrut [43] described a method to collect nano-/micro-particles suspended in a stream of supercritical fluid by scrubbing this fluid with a liquid consisting in a saturated solution of coating agent in an organic solvent: Extraction of part of this solvent by the fluid causes super-saturation and nucleation of the coating agent preferably onto the particles.
- A process derived from the PGSS concept was recently patented [44] for tablet coating: It consists in pulverizing the coating agent(s) suspension into a supercritical fluid on the tablets processed in a classical coating equipment.
- Wakayama [45] and Filardo et al. [46,47] proposed to polymerize (or copolymerize) monomers onto particles of substrate suspended in supercritical carbon dioxide, in the presence of a surfactant and a polymerization initiator, in order to obtain micro-capsules.

- **Impregnation:** High diffusivity and tunable solvent power of SCF are the basis of supercritical impregnation. Supercritical fluid-soluble substrates can be easily

impregnated inside porous media as demonstrated by many investigators using various matrixes like polymers, wood, paper. This can be used to prepare controlled drug delivery systems [48], food-grade carrier micro-particles impregnated with flavors or colorants [49], etc.

Majewski and Perrut [50] recently patented a process that leads to a homogeneous distribution of substrate into the excipient and is illustrated by *kava-kava* extraction with on-line impregnation of the kavalactone-rich extract into maltodextrine. This process is of special interest to combine on-line supercritical fluid extraction and impregnation, especially for nutraceuticals production. However, these impregnation processes are only feasible when the active compound is soluble in the supercritical fluid.

That is not the case for the so-called Concentrated Powder Form (CPF) process [51] through which powdery agglomerates with unusual high liquid concentrations of up to 90 wt.% can be obtained by spraying gas-saturated solutions and admixing a solid carrier material with the spray: The gas, which must be at least partially soluble in the liquid, generates small droplets that infiltrate the porous carrier particles or agglomerate the non-porous ones.

- **Process choice:** Table 1 summarizes the different cases in order to guide the reader in his choice through these various formulation processes.

Biological applications:

As biotechnological synthesis of therapeutic products are in progress, *cell lysis* by SCF is the more interesting because this process does not lead to very small membrane fragments at the difference with classical homogenization, preserving fragile molecules and easing downward-processing [52].

Regarding *sterilization*, it is known for long that CO_2 has a biocide effect on most bacteria [53]. Recent works showed that combination with ethanol addition or pressure cycling [54] greatly boosts the micro-organisms alteration. It was also proven that *virus inactivation* can be obtained on plasma fractions [55-57] with N_2O or CO_2 in "mild" conditions to avoid denaturation of the very fragile proteins, and during CO_2-delipidation of bone implants [11]. For pharmaceutical and bio-medical applications, this biocide effect is of key-importance as SCF processing does not at all increase the bio-burden but contributes to maintain or reach sterility.

Conclusion:

Even if Supercritical Fluid technology is not yet widespread in the pharmaceutical industry, except for extraction of active compounds from vegetal sources (phytopharma-/nutraceuticals), many promising applications are now under development, especially for new drug formulations for which SCF processes propose innovative routes adapted to each case. Ironically, the intense R&D work is leading to many attractive results, but also to many patents, and is rendering the Intellectual Property situation rather complex, that may refrain pharmaceutical companies to enter this technology in their formulation "tool-box" on the short term. However, even if no application has yet reached commercial stage, there is no doubt that the pipe-line is now rich in several formulations to be shortly introduced for registration, especially for manufacturing inhalable particles. Scale-up and compliance to GMP seem accessible at present, as we showed by building three semi-industrial particle-design plants under strict quality assurance and documentation according to GMP rules. Moreover, the SCF technology has the great advantage of intrinsic sterility.

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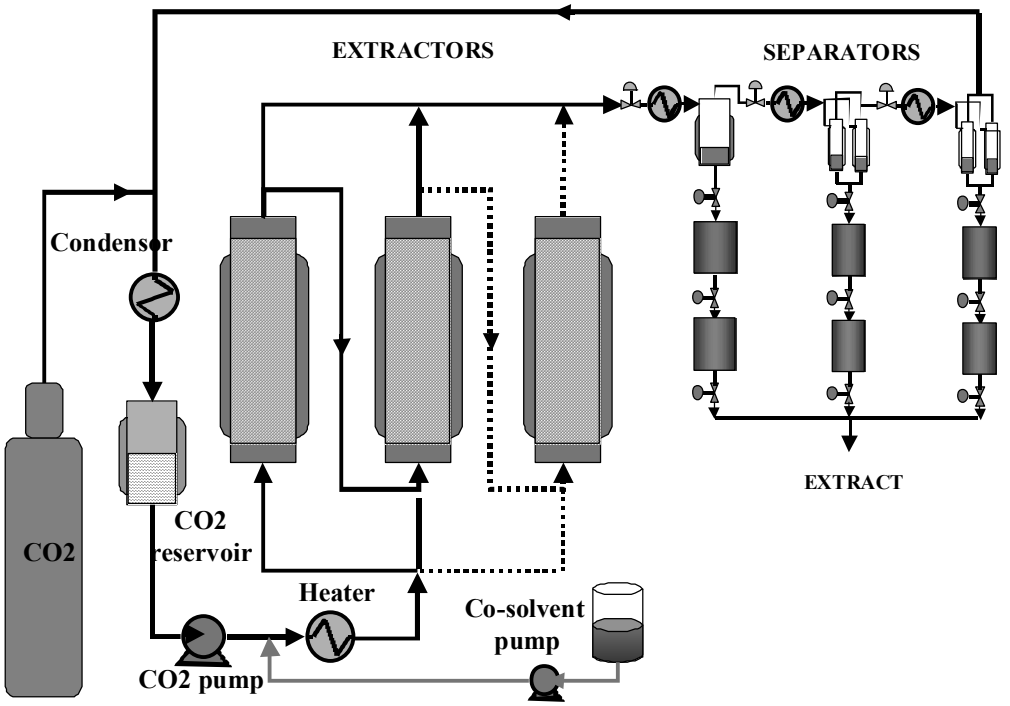
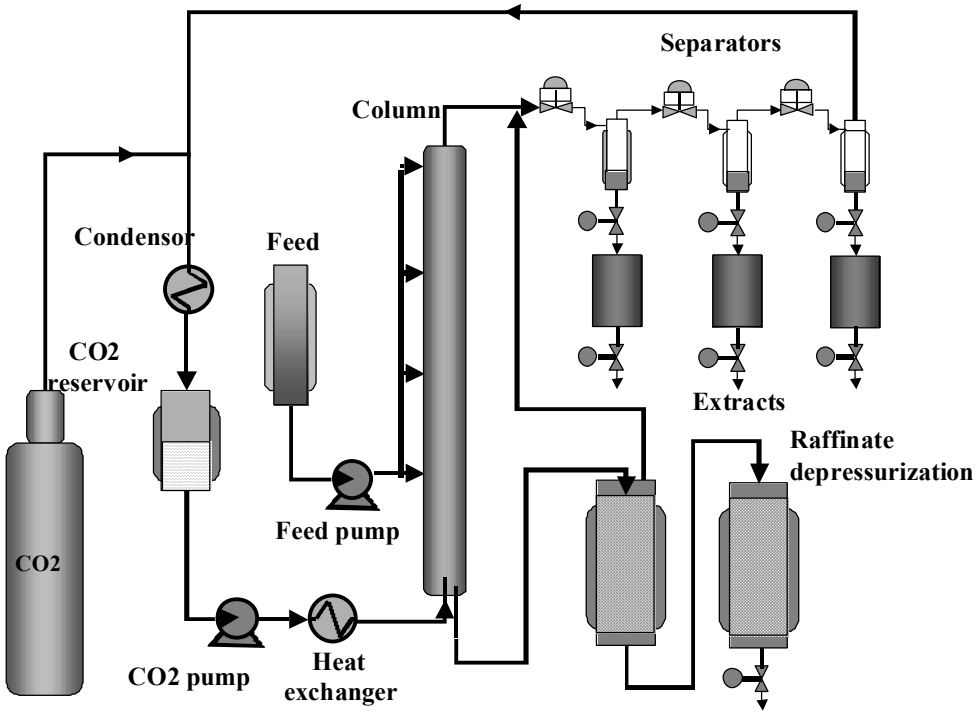
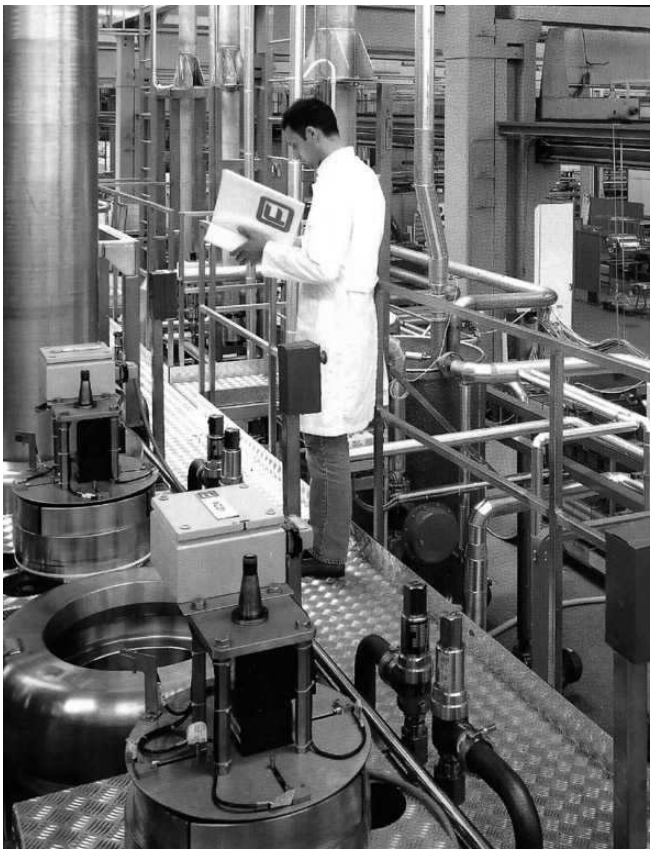
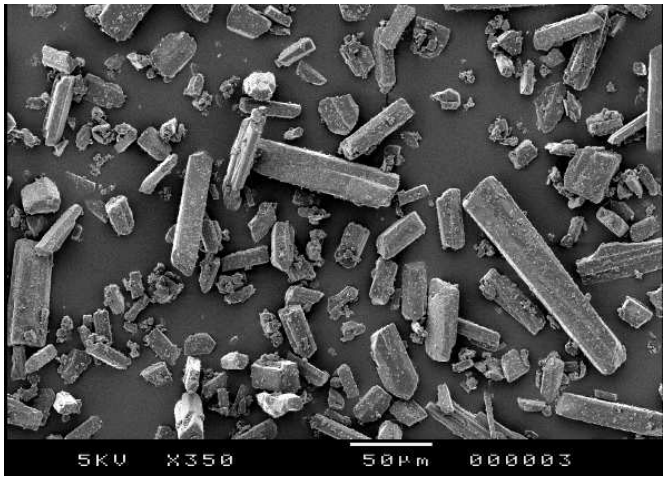
- ***List of tables :***

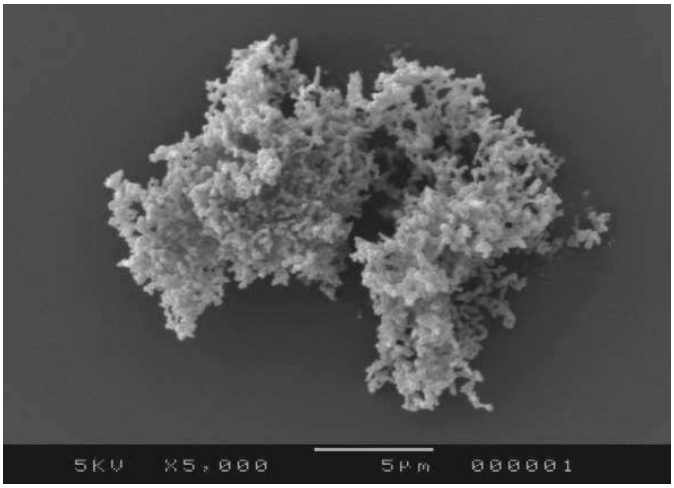
- ***List of captions :***

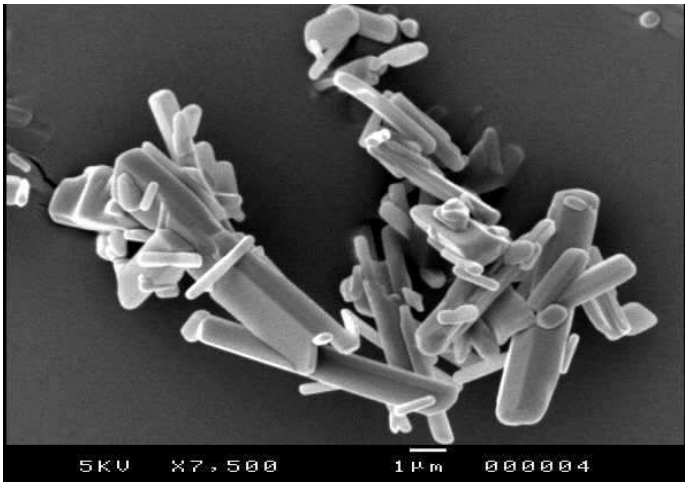

EXTRACTORS
SEPARATORS
Condensor
CO2
CO2
reservoir
Heater
Co-solvent
pump
CO2 pump
EXTRACT


Separators
Column
Condensor
Feed
CO2
reservoir
Extracts
Raffinate
depressurization
Feed pump
CO2
CO2 pump
Heat
exchanger




5KV X350 50µm 000003


5KV X5,000 5µm 000001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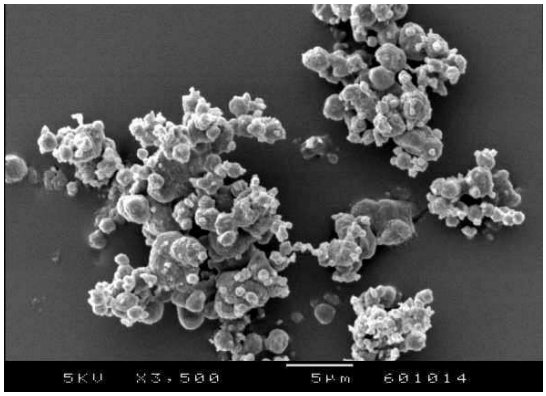
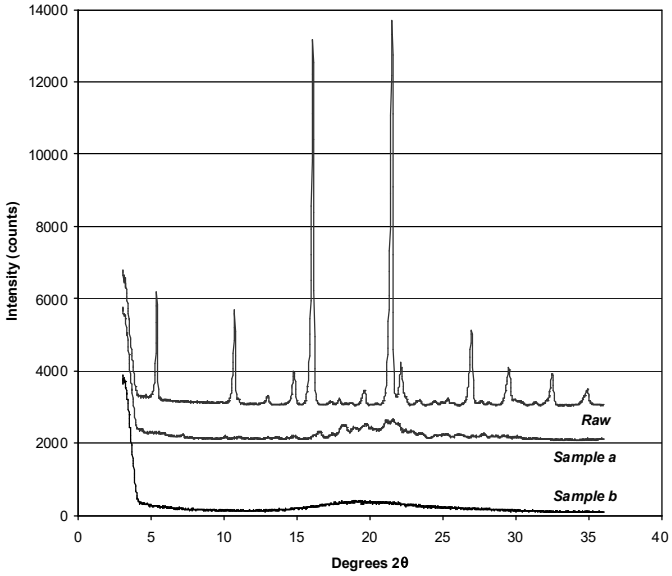
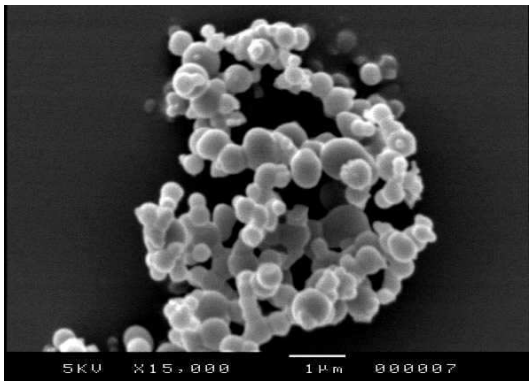

5KV X3,500 5µm 601014


14000
12000
10000
8000
6000
4000
2000
0
Intensity (counts)
0
5
10
15
20
25
30
35
40
Degrees 2θ
Raw
Sample a
Sample b


10KV X1,500 10µm 000032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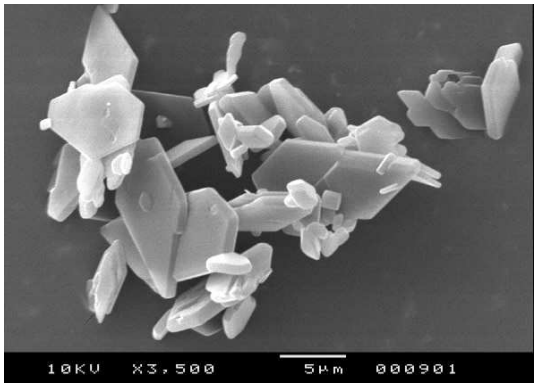
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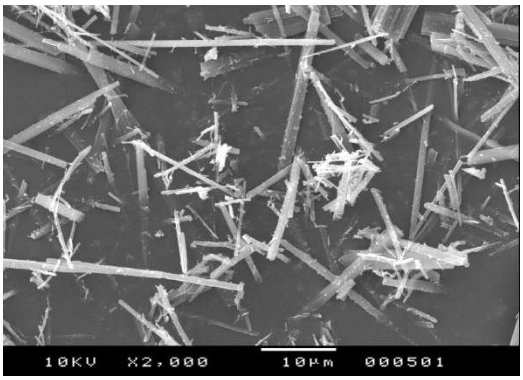
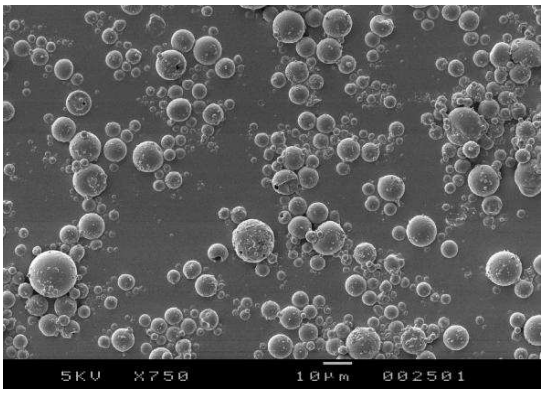
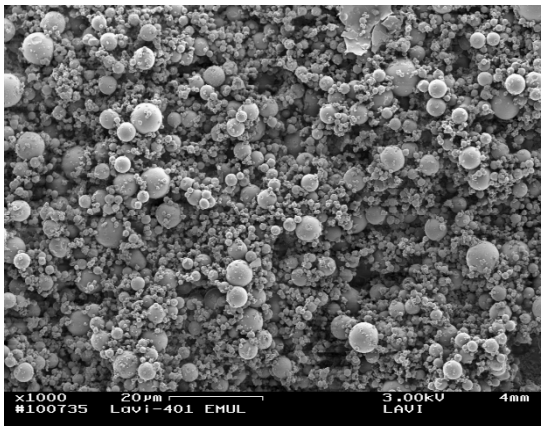
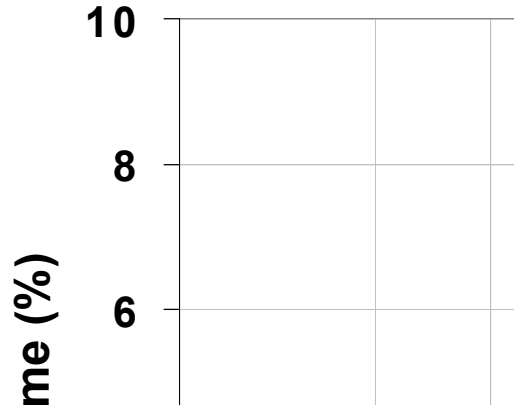
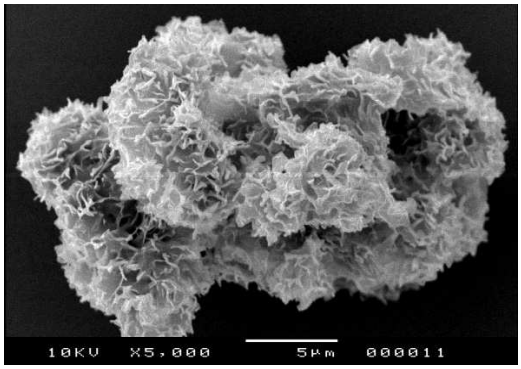
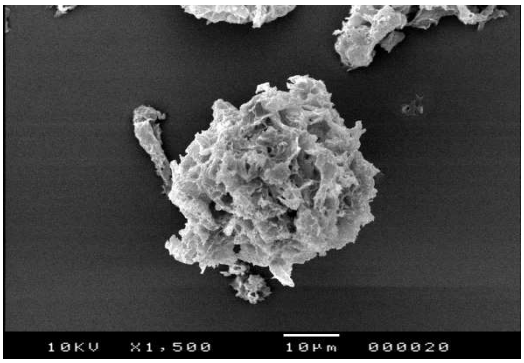

10KV X2,000 10μm 000501


5KV X750 10µm 002501


x1000 20µm 3.00kV 4mm
#100735 Lavi-401 EMUL LAVI


10
8
6
me (%)


10KV X5,000 5µm 000011


10KV X1,500 10µm 000020

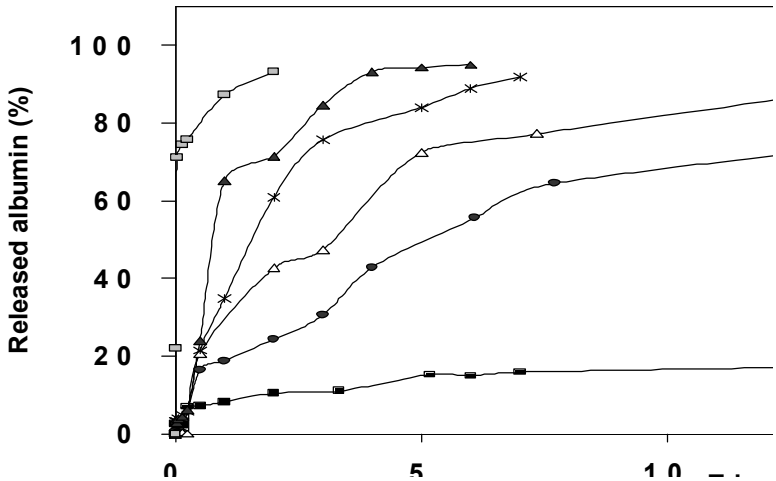

Released albumin (%)
100
80
60
40
20
0
0
5

Table 1: Formation of neat or composite microparticles

Substrate solubility in SCF	Matrix solubility in SCF	Available process	Type of particles produced	Remarks
Yes	**--**	RESS	Nano/Micro-particles	Few substrates soluble SCF CO_2 Use of polar SCFs
Yes	**Yes**		Micro-spheres	Few substrates / coatin both soluble in SCF C Use of polar SCFs
Yes	**No**	Impregnation	Micro-spheres	Carrier impregnation
No	**Yes**	Liposome-RESS process	Liposomes	To be demonstrated at commercial scale
		RESS Fluidized-bed coating	Micro-capsules	Few coatings are solub in SCF CO_2
		Coating deposition	Micro-capsules	Use of polar SCFs
		Anti-solvent processes	Nano/Micro-particles Micro-spheres/capsules	Huge fluid ratio Difficult solvent/ fluid separation and scale-up
No	**No**	Coating coacervation	Micro-capsules	To be demonstrated at large scale
		Fluid-Assisted Micro-encapsulation	Micro-capsules	Very low CO_2 consumption Easy scale-up
		CPF process	Micro-spheres	Continuous process Easy scale-up
No	**--**	Emulsion drying	Nano/Micro-particles	For water-soluble or biological molecules
No	**--** **Yes**	Polar SCF drying	Nano-Micro-particles Micro-spheres/capsules	For water-soluble or biological molecules