Invited lecture at the 8th Meeting on Supercritical Fluids (Bordeaux-2002)

Pharmaceutical applications of Supercritical Fluids

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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 almost an 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. However, for the past three decades, important R&D works about supercritical fluid technology have been dedicated to natural products processing, leading to some very well-known applications to food products like coffee and tea decaffeination or hops resins extraction, but also to manufacturing **phytopharmaceuticals/nutraceuticals** ; in fact, **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 from natural feedstocks at a temperature near to ambient, although it does require a sophisticated equipment designed for high pressure operation, generally up to 300 or 350 bar. But, **natural product needs a natural solvent !** And for non-polar compounds extraction, there is no alternative to supercritical carbon dioxide. Supercritical Fluid Extraction (SFE) - referring to *fluid-solid extraction* - and Supercritical Fluid Fractionation (SFF) - referring to *fluid-liquid fractionation* – are also widely investigated for purification of natural or synthetic active products (for instance, elimination of toxic residues).

Moreover, Supercritical Fluid technology is very attractive for manufacturing **innovative** therapeutic particles, either of pure active compounds or mixtures 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. In this domain, several issues are to be addressed :

- 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 delayed delivery due to high toxicity or long-term distribution.

As detailed in a recent literature and patent review [8], particle design using Supercritical Fluids can be operated by several different processes, the choice between them depending on the aimed particle structure, morphology and size distribution, opening new ways for solving drug delivery problems.

Present status of 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 [1-7]. At present time, these applications are still continuing to spread worldwide as requirements for high quality products and concerns on environment/health are growing.

- **Extraction** (SFE) from solid materials is the most developed application, mainly for food products (coffee, tea,…), food ingredients (hops and aromas, colorants, vitamin-rich extracts, specific lipids, …) and nutra-/phytopharma-ceuticals. Residual organic solvent or other impurities, like pesticides, are also removed from final active compounds (like ginseng) at large scale or residual solvents from synthetic drugs. I estimate the number of industrial-scale SFE units now under operation about 100 with a growth of about 10% per year. Some "niches" applications concern high-added value products, like bone delipidation for allografts, or specialty polymer stripping for medical applications.

- **Fractionation** (SFF) of liquid mixtures is 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 for aromas production from fermented and distilled beverages, fractionation of polyunsaturated fatty acids (EPA, DHA), polar lipids (ceramides, sphingolipids, glyco-lipids), specialty lipids (phytosterols) and vitamins (tocopherols), specialty polymers (hard-disk lubricants) ; the recovery of active compounds from fermentation broths may also appear as a fruitful application in the near future.

- **Preparative Scale Supercritical Fluid Chromatography** (PSFC) is operated for ultimate fractionation of very similar compounds, especially for lipids like polyunsaturated fatty acids in a few large-scale units ; moreover, enantiomers resolution is paid great interest, at lab-scale until now prior to further development.

- **Reactions** (SFR) are operated in Supercritical media [4-6], and very promising processes are being developed for fine highly selective synthesis, especially hydrogenation. As reaction rate and selectivity are drastically improved, a very high throughput can be obtained from rather small units, with the example of a recent start-up of a plant dedicated to hydrogenation of specialty chemicals in the UK.

Particle design and drug formulation :

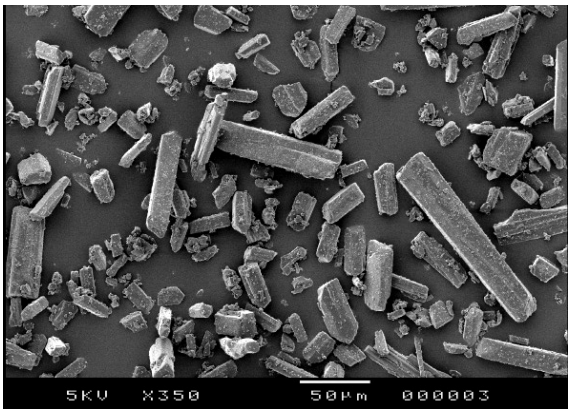
Particle formation processes using supercritical fluids [8] are now subjected to an increasing interest, especially in the pharmaceutical industry with three aims : Increasing bio-availability of poorly-soluble molecules, designing formulations for sustained-release and for less invasive than parenteral drug delivery (oral, pulmonary, transdermal). The most complex challenge is related to therapeutic proteins as it is extremely difficult to deliver bio-molecules due to instability and very short half-life *in vivo*.

Generally, a dramatic change of the solid morphology is observed after processing by SCF : Average particle size and size distribution, particle shape and porosity, and consequently,

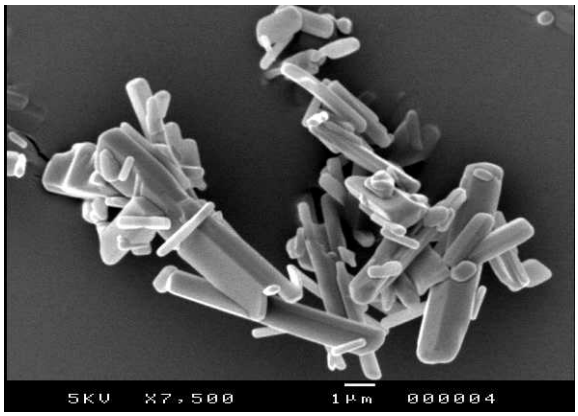
specific area and dissolution rate are often completely different form those of the starting material. However, it seems that, in most cases, these solid morphology change does not concern the microscopic crystal pattern, although relatively few results attested by XRD and DSC have yet been published in spite of its extreme importance in terms of dissolution kinetics and stability during further processing and storage.

- **Rapid Expansion of Supercritical Solutions (RESS)** consists in atomizing a solution of the product in a supercritical fluid into a low-pressure vessel. This process could find valuable applications at commercial scale only when product solubility in the supercritical fluid (preferably CO_2) is not too small ($\geq 10^{-3}$ kg/kg), limiting the process application to non-polar or low-polarity compounds. Below is presented the example of RESS micronization of lovastatin, an anti-cholesterol drug that is slightly soluble in CO_2 ; the nozzle geometry orients the particle size morphology from long rods generated by a capillary nozzle to spherical particles generated by a very short laser-drilled orifice.

Figure 1 : Lovastatin atomization by RESS

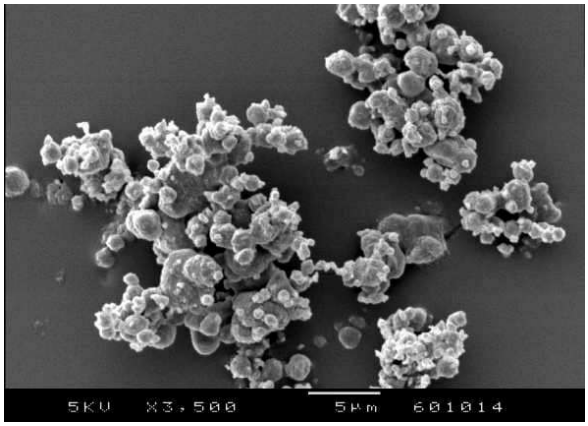


Raw material



a)

Micronized lovastatin *generated with a capillary nozzle (a) and with a laser-drilled short orifice (b)*

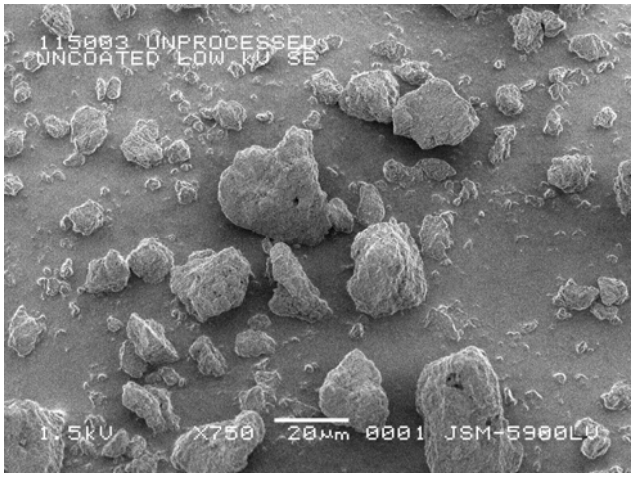


b)

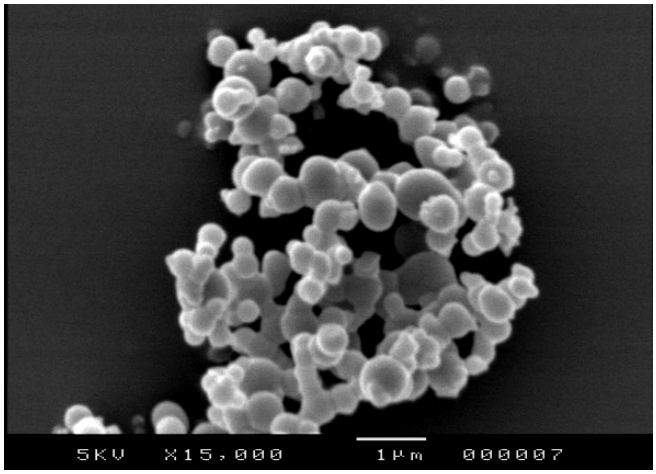
- **Supercritical Anti-Solvent (SAS)** applies to most molecules that can be dissolved in a very wide range of "strong" organic solvents. The supercritical fluid is used as an anti-solvent that causes precipitation of the substrate(s) dissolved initially in a liquid solvent. In the most commonly used implementation of this concept called **Aerosol Solvent Extraction System** (ASES) [8], the solution is sprayed through an atomization nozzle as fine droplets into compressed carbon dioxide. The

dissolution of the supercritical fluid into the liquid droplets is accompanied by a large volume expansion and, consequently, a reduction in the liquid solvent power, causing super-saturation within the liquid mixture and the formation of small and uniform particles. Many variants are proposed in order to control the particles morphology and size by using special nozzles. Recent development, especially for preparation of very fine drugs dedicated to pulmonary delivery, opens a bright future for "engineering" new types of materials, leading to nano-particles (50-500 nm) or micro-particles (500-5000 nm) or empty “balloons” (5-50 µm) made of nano-particles, permitting a very significant increase in bio-availability of poorly water-soluble drugs or micro-spheres of drug embedded in an excipient for sustained-release delievery. Shown on figure 2, two pictures of particles of a poorly water-soluble anticholesterol drug with the parameters of the particle size distribution before and after ASES micronization.

Figure 2 : Micronization of LAVI-115 by anti-solvent



Starting material
x50 = 41.9 µm
x10 = 2.9 µm
x90 = 150.8 µm



Micronized material
x50 = 1.1 µm
x10 = 0.5 µm
x90 = 2.8 µm

- **Micro-encapsulation** can be performed by several processes using SCF according to the precedent concepts (RESS, SAS), or some other innovative variants for liposome generation or carrier deposition by variation of pressure/temperature conditions. But the most attractive seems the PGSS (Particles from Gas-Saturated Solutions) process [8] where supercritical carbon dioxide is used as a viscosity reducing agent, similarly to many spraying applications with different products (application of paints and adhesives, dry paint/coating production). Typically, this process allows to form particles from substances that can absorb a large concentration of fluid, and consequently swell and melt at a temperature much below (~10 to 50°C) their melting temperature. Composite micro-capsules can also be prepared by atomizing a slurry of drug powder dispersed in a liquefied excipient in which is dissolved a compressed gas below its critical pressure, the rapid fluid demixion generating very small micro-capsules of drug entrapped inside the solidified excipient (figures 3 and 4) ; this process is very promising for protein encapsulation since it can be operated in “mild” conditions that do not lead

to protein denaturation and loss of bio-activity ; however, this should be confirmed on therapeutic proteins or other fragile bio-molecules. The simplicity of this concept, leading to low processing costs, and the very wide range of active and of carriers (lipids, polymers) that can be treated open wide avenues for application.

Figure 3 : Drug encapsulated in PEG

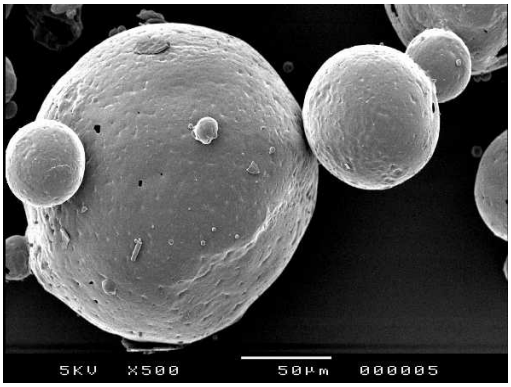
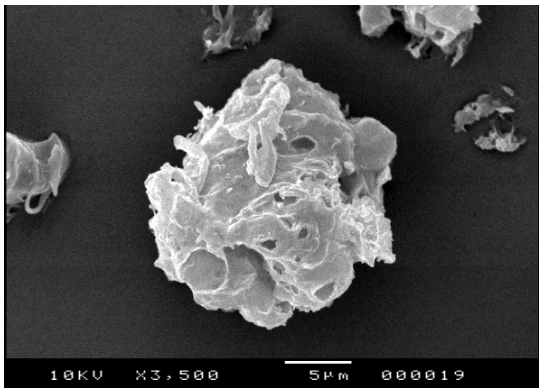


Figure 4 : Lactase particle encapsulated in a lipid



- **Impregnation** of active compounds into excipients is easily obtained using SCF as vectors, due to their high diffusivity and tunable solvent power, either through porous matrix or inside non-porous polymeric matrixes swollen by the fluid, like pharmaceutical patches, sponges, and catheters. We recently disclosed a new process for on-line impregnation after extraction, especially for natural products impregnation into a porous excipient [10], leading to a free-flowing powder.

Pollution abatement :

SCF, and especially **carbon dioxide**, leads to environment-friendly processes through organic solvent substitution. Water streams polluted with organic compounds can be treated with CO_2 for pollutants recovery. 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. Moreover, pollutant destruction in **subcritical water** is also receiving a keen interest in pharmaceutical companies, even if the oxidation rate is lower than in supercritical water.

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 [11].
Regarding **sterilization**, it is known for long that CO_2 has a biocide effect on most bacteria and fungi. A recent article [12] reviews the literature and reports attractive results showing that bacteria can be easily inactivated by exposition during a few minutes to carbon dioxide at relatively low pressure (74 bar) at 38°C ; moreover, spores can be inactivated only when using high temperatures (75°C) or through a more interesting process operating at low temperature (38°C) and rapidly cycling the pressure (50 - 150 bar) during one hour (30 cycles). The authors consider that this inactivation is related to dissolution of CO_2 in the cell

causing a rapid decrese of the intracellular pH and a deep modification of the membrane permeability due to the interaction with the membrane lipids.
It was also proven that a significant **virus inactivation** can be obtained on plasma fractions [13,14] 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 [14].

Conclusion :

Even if Supercritical Fluid technology is not yet widespread in the pharmaceutical industry, except for extraction of active compounds from vegetal sources (phytopharma-/nutra-ceuticals), many promising applications are now under development, especially for new drug formulations through innovative particle design [16].

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