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REVIEW ON ANALYTICAL TOOLS OF SUPERCRITICAL FLUID EXTRACTION AND ITS APPLICATION IN PHARMACEUTICAL INDUSTRY

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Abstract

Supercritical fluid extraction is the process of separating one component from another using super critical fluid as the extracting solvent. We have done critical review on supercritical fluid extraction and its application in pharmaceutical industry. This review will be useful for pharmacy students as well as industry.

Keyword : Supercritical fluid, extraction, pharmaceutical, industry

Introduction

Supercritical fluid extraction is the process of separating one component from another using super critical fluid as the extracting solvent. Extraction is usually from a solid matrix, but can also be from liquids. SFE can be used as a sample preparation step for analytical purpose or on a large scale either strip unwanted material from a product (decaffeination) or collect a desired product CO2 is the most used super critical fluid, sometimes modified by co solvents such as ethanol or methanol. Extraction conditions for super critical carbon dioxide are above the critical temperature of 31 degree Celsius and critical pressure of 74 bars. Addition of modifiers may slightly alter this.[2,3]

Supercritical fluid is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases' do not exists. It cans effuse through solids like a gas, and dissolves materials like liquids. In addition, close to the critical point, small changes in pressure or temperature results in large changes in density, allowing many properties of super critical fluids to be fine-tuned. SCF are suitable as a substitute for organic solvents in a range of industrial and laboratory process.

PRINCIPLE OF SUPER CRITICAL FLUID EXTRACTION

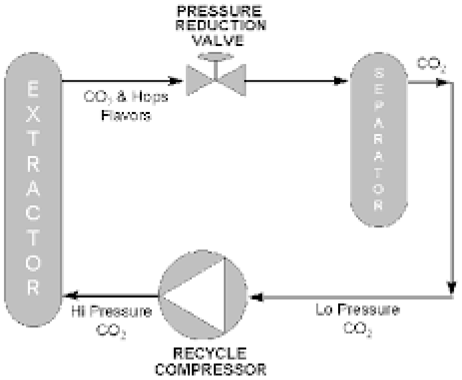

Figure 1 super critical fluid extraction

SFE is based on the fact that, near the critical point of the solvent, its property change rapidly with only slight variations of pressure.

The critical point marks the end of the vapour liquid coexistance curve. A fluid is termed supercritical when the temperature and pressure are higher than the thereisnophase transiton in that the fluid cannot undergo a transission to a corresponding the critical values. Above the critical temperature liquid phase, regardless of applied pressure.

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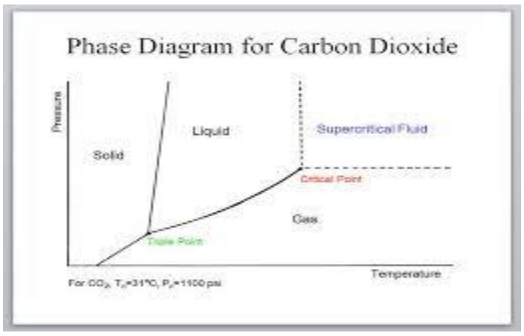


Figure. 2 phase diagram of carbon dioxide

Super critical fluid is characterised by physical and thermal properties that are between those of the pure liquid and gas. The fluid density is a strong function of temperature and pressure. The diffusivity of SCF is much higher than for a liquid and super critical fluid readily penetrates porous and fibrous solids. Consequently, SCF can offer good catalytic activity. SFE can be used to extract analytes from dsamples. The main Advantage of using super ciritical fluids for extraction is that they are inexpensive, extract the analyte faster and more environment friendly than organic solvents. For this reason super critical fluid CO_2 is the reagent widely used as supercritical solvent.[9]

Supercritical Fluids

The critical temperature of a substance is the temperature above which that substance can no longer exist as a liquid, no matter how much the pressure is increased. In the same way, the critical pressure is the pressure above which the substance can no longer exist as a gas, no matter how high the temperature is. In a phase diagram, these pressure and temperature values define the critical point. (Figure 3)

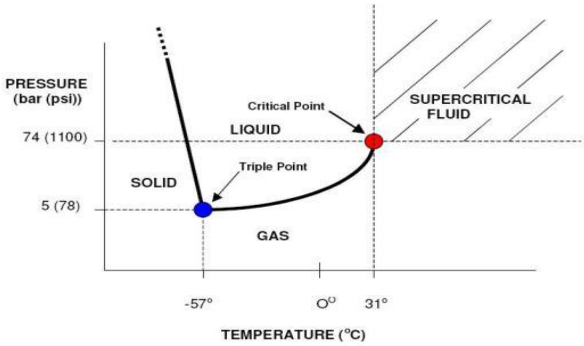
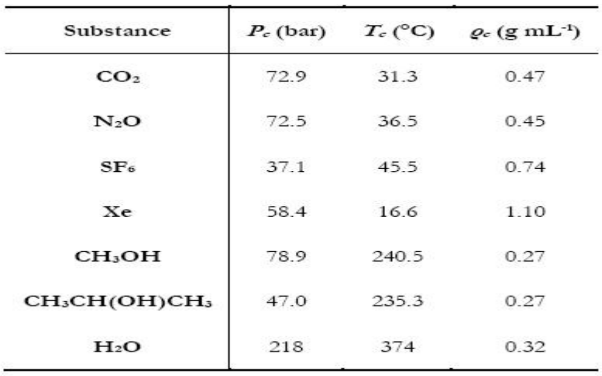


Figure 3. Phase diagram of a pure substance. Shown temperature and pressure values are those of pure carbon dioxide.

Supercritical fluids are obtained either by heating a gas above its critical temperature or by compressing a liquid at a higher pressure than its critical pressure. It is impossible to draw a clear line between supercritical fluids and liquids or gases, since the transition from liquid to supercritical fluid by raising the temperature at constant pressure or from gas to supercritical fluid by increasing the pressure at constant temperature is continuous. Critical temperatures, pressures and densities for a number of pure substances are shown in Table 1.

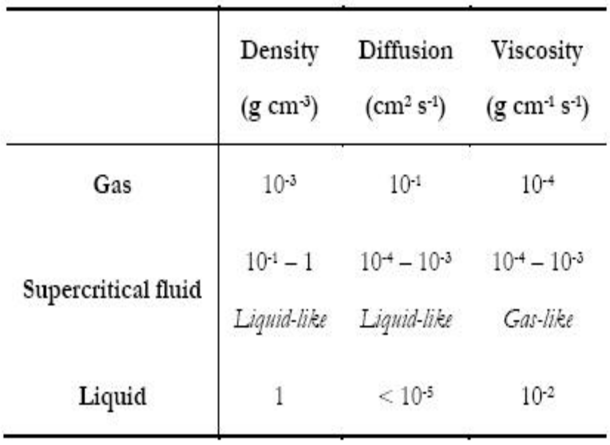
Table1. pressure(P_c),temperaturere (T_c) and density f pure substances.



Substance	P_c (bar)	T_c (°C)	ϱ_c (g mL^{-1})
CO_2	72.9	31.3	0.47
N_2O	72.5	36.5	0.45
SF_6	37.1	45.5	0.74
Xe	58.4	16.6	1.10
CH_3OH	78.9	240.5	0.27
$CH_3CH(OH)CH_3$	47.0	235.3	0.27
H_2O	218	374	0.32

Under supercritical conditions, the properties of the substance (e.g. density, viscosity, diffusion coefficient…) are intermediate between those of liquid and gas. The density is typically of the order of magnitude of liquid density (from 0.1 to 0.8 g cm-3). The solvating power of supercritical fluids is also very similar to the one of many conventional organic solvents and much higher than in gases. Conversely, the diffusion coefficient and viscosity of supercritical fluids are about 5 to 50 times higher than in liquids. This is illustrated in Table 2.

Table 2. Order of magnitude of physical properties density, diffusion and viscosity for gaseous supercritical and liquid states.



	Density (g cm^{-3})	Diffusion (cm^2 s^{-1})	Viscosity (g cm^{-1} s^{-1})
Gas	10^{-3}	10^{-1}	10^{-4}
Supercritical fluid	$10^{-1} – 1$ *Liquid-like*	$10^{-4} – 10^{-3}$ *Liquid-like*	$10^{-4} – 10^{-3}$ *Gas-like*
Liquid	1	$< 10^{-5}$	10^{-2}

The properties of supercritical fluids, intermediate between the properties of gas and liquids, make them interesting for use as chromatographic mobile phases. Gas chromatography (GC) allows high resolution separation of complex mixtures. However, GC is limited to thermally stable compounds that are volatile and of low molecular mass. Reversed phase high performance liquid chromatography (RP-HPLC) is recognized to be the most convenient separation technique for a wide range of compounds, including substances of high molecular mass and with thermal lability. Nevertheless, long analysis times and low column efficiency due to the low solute diffusion in the mobile phase are often observed. According to the intermediate properties of supercritical fluids between those of a gas and a liquid, supercritical fluids appear to be a good solution to avoid problems of both HPLC and GC.

PROCEDURE

The system must contain a pump for the CO_2, a pressure cell to contain the sample, a means of maintaining pressure in the system and a collecting vessel. The liquid is pumped to a heating zone, where it is heated to supercritical conditions. It then passes into the extraction vessel, where it rapidly diffuses into the solid matrix and dissolves the material to be extracted. The dissolved material is swept from the extraction cell into a separator at lower pressure, and the extracted material settles out. The CO_2can then be cooled, re-compressed and recycled, or discharged to atmosphere.

Pumps

Carbon dioxide (CO_2) is usually pumped as a liquid, usually below 5°C and a pressure of about 50 bar. The solvent is pumped as a liquid as it is then almost incompressible; if it was pumped as a supercritical fluid, much of the pump stroke would be "used up" in compressing the fluid, rather than pumping it. For small scale extractions (up to a few grams / minute), reciprocating CO_2 pumps or syringe Kpumps are often used. For larger scale extractions, diaphragm pumps are most common. The pump heads will usually require cooling, and the CO_2 will also be cooled before entering the pump.

Pressure vessels

Pressure vessels can range from simple tubing to more sophisticated purpose built vessels with quick release fittings. The pressure requirement is at least 74 bar, and most extractions are conducted at under 350 bar. However, sometimes higher pressures will be needed, such as extraction of vegetable oils, where pressures of 800 bar are sometimes required for complete miscibility of the two phases.

The vessel must be equipped with a means of heating. It can be placed inside an oven for small vessels, or an oil or electrically heated jacket for larger vessels. Care must be taken if rubber seals are used on the vessel, as the Supercritical carbon dioxide may dissolve in the rubber, causing swelling, and the rubber will rupture on depressurization.

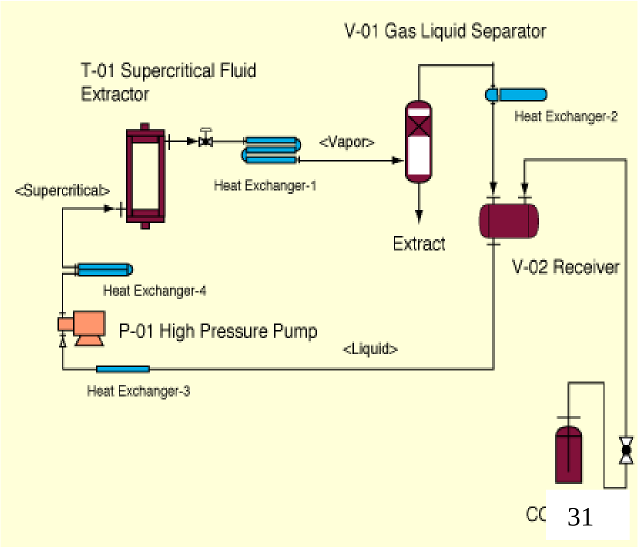


Figure 4. Schematic diagram of SFE apparatus.....

Pressure maintenance

The pressure in the system must be maintained from the pump right through the pressure vessel. In smaller systems (up to about 10 mL / min) a simple restrictor can be used. This can be either a capillary tube cut to length, or a needle valve which can be adjusted to maintain pressure at different flow rates. In larger systems a back pressure regulator will be used, which maintains pressure upstream of the regulator by means of a spring, compressed air, or electronically driven valve. Whichever is used, heating must be supplied, as the adiabatic expansion of the CO_2 results in significant cooling. This is problematic if water or other extracted material is present in the sample, as this may freeze in the restrictor or valve and cause blockages.

Collection

The supercritical solvent is passed into a vessel at lower pressure than the extraction vessel. The density, and hence dissolving power, of supercritical fluids varies sharply with pressure, and hence the solubility in the lower density CO_2 is much lower, and the material precipitates for collection. It is possible to fractionate the dissolved material using a series of vessels at reducing pressure. The CO_2 can be recycled or depressurized to atmospheric pressure and vented. For analytical SFE, the pressure is usually dropped to atmospheric, and the now gaseous carbon dioxide bubbled through a solvent to trap the precipitated components.

Heating and cooling

This is an important aspect. The fluid is cooled before pumping to maintain liquid conditions, then heated after pressurization. As the fluid is expanded into the separator, heat must be provided to prevent excessive cooling. For small scale extractions, such as for analytical purposes, it is usually sufficient to pre-heat the fluid in a length of tubing inside the oven containing the extraction cell. The restrictor can be electrically heated, or even heated with a hairdryer. For larger systems, the energy required during each stage of the process can be calculated using the thermodynamic properties of the supercritical fluid.

1. Detection

Theoretically, SFC displays the widest possible choice of detection techniques, being compatible with most of LC and GC detectors. However, most of them are not commercially available in combination with a SFC system. The most common detector used in SFC is the UV absorbance detector, because of its sensitivity, its wide dynamic range and also because SFC mobile phases are generally UV-transparent ($CO2$ is UV-transparent below the cut-off wavelength of most UV detectors). Other non-informative detection techniques can be used such as evaporative light scattering detection (ELSD), chemiluminescent nitrogen detection (CLND) or flame ionization detection (FID). Recently, Brunelli and co-workers have described the hyphenation of pSFC with corona-charged aerosol detection. However, it is more and more desirable for high-throughput analysis methods to use informative detection techniques, *i.e.*techniques that provide structural information about the eluted compounds.

As with LC, mass spectrometry (MS) is an obvious choice of informative technique. pSFC-MS has been known in the literature for more than twenty years.and a substantial amount of research has been carried out demonstrating the efficiency of the technique using different ionization conditions such as positive or negative ion atmospheric pressure chemical ionization (APCI) or electrospray ionization (ESI). SFC-NMR and SFC-IR have also been studied. In this project, a hyphenated pSFC-UV-MS using a quadrupole mass spectrometer with an electrospray ionisation source was used.

Using a supercritical fluid as a mobile phase to perform chromatographic separations was suggested more than forty years ago..The first packed column SFC system was marketed in 1982 by Hewlett Packard (HP), followed by a capillary SFC instrument in 1986 by Less Scientific SFC. Analytical chemists had been long awaiting the possibilities offered by SFC. The fact that, in SFC, one can develop chromatographic methods not only by varying mobile phase composition but also temperature and pressure was very much anticipated. At the time, many people even thought that SFC would replace HPLC altogether. This, obviously, did not happen. The poor reproducibility obtained with capillary SFC in the early 1990s, combined with the lack of user-friendliness and the high cost of early SFC systems resulted in the technique being disregarded as inefficient and too expensive. SFC is still in many analysts' minds, at best, a substitute for normal phase LC. Another factor that might have influenced the poor acceptance of SFC is its market situation. The SFC market is composed of small companies that cannot compete with the main HPLC manufacturers in terms of marketing and sales staff. Several companies gave SFC a try and then decided to give up. In 1995, HP sold its SFC section to Terry Berger who founded Berger Instrument, which was subsequently bought by Mettler-Toledo in 2000, before being ceded again, to Thar, in 2007.

Despite all this, there has been recently a resurgence of interest in SFC, noticeably in the pharmaceutical industry. The many advantages of SFC (in terms of speed of analysis, resolution, solvent consumption, etc…) make it a technique of choice for high-throughput pharmaceutical analysis and SFC, at both analytical and preparative scales is used routinely in an increasing number of pharmaceutical companies. Interest is also growing in the petroleum industry with SFC being very suitable for the analysis of biofuels and in environmental analysis for the detection of pesticides, for instance.

SUPERCRITICAL FLUID CHROMATOGRAPHY (SFC)

To achieve the quality and safety requirements expected for new drug compounds, analytical chemists are faced with the challenge of developing new analytical methods capable of quick, highly efficient separations for the characterization of all compounds and impurities. Until recently, HPLC-MS has been preferentially used for this purpose. However, with the aim of maximising the information gathered in a given time, packed column supercritical fluid chromatography coupled to mass spectrometry (pSFC-MS) appears more and more as a complementary technique for high-throughput analysis (HTA). Interestingly, pSFC is suitable for most of the tasks undertaken by LC-MS, whether it be structure analysis (pSFC-MS-MS), quantification (pSFC-CLND/ELSD-MS), purification (preparative scale pSFC), chiral separations (pSFC-MS) or purity assessment (pSFC-UV-MS). For all these applications, high chromatographic resolution is required. This means that the need is for a technique providing chromatographic efficiency (i.e. a high number of theoretical plates), speed of analysis, sensitivity and selectivity. The "green" potential of SFC, noticeably on a preparative scale, has been demonstrated. In terms of covered chemical space, any analyte soluble in methanol or a less polar solvent is suitable for SFC analysis. The technique is therefore suitable for non-polar analytes. The addition of an organic modifier in the mobile phase (possibly with the addition of a third component at low concentration, *vide infra*) even affords elution of polar compounds such as organic acids and bases and their salts. The elution of peptides using SFC has also been reported.[2] The feasibility of pharmaceutical analysis using SFC has also been demonstrated. Pinkston and co-workers, for instance, analysed a large and diverse library of pharmaceutical compounds and found that SFC was suitable for the analysis of 75 % of the analytes (as compared with 79 % for rpHPLC). Zhao and co-workers came to the conclusion that pSFC can be implemented in all steps of drug discovery and is a valuable complement to LC. However, they also concluded that dedicated academic research and hardware development are still needed to reach that stage, especially because in-depth knowledge of retention mechanism in SFC is still missing.

SOME INSTITUTIONS IN INDIA PERFORMING SFE

1. National botanical reserch institute ;Lucknow.
2. Patki herbs and species institute; Guwahiti.
3. Sami lab ltd; Banglore.
4. Tea research association; Toklai Assam.
5. Regional research laboratory; Jammu.
6. Ace agro ltd; Lonavala; maharashtra.

APPLICATIONS AND COMMERCIAL PROCESSES OF SUPERCRITICAL FLUIDS [7,9,11]

Any commercialization of a process that uses supercritical fluids must involve a cost analysis that should indicate that the advantages in the new process offsets the penalty of high pressure operations. A variety of supercritical fluid processes have been commercialized. Details of a few such processes are given below. Many other processes have been investigated on a lab or pilot plant scale and have the potential to be scaled up in the near future.

1. **Supercritical Fluid Chromatography.**

Supercritical fluid chromatography is now often used as an analytical tool. The density is used as the controlling feature. Separations are based on a user programmed density profile with the supercritical fluid as the mobile phase. This analytical technique has been successfully used to separate oligomers and high molecular weight polymers. Supercritical fluids are used as the extracting solvents for the removal of polyaromatic hydrocarbons from soil. It is now a standard method for gas chromatography sample preparation because the extraction is considerably faster than Soxhlet extraction.

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.

2. Fractionation

Supercritical fluids an be used to fractionate low vapor pressure oils and polymers. This fractionation is difficult to achieve in distillation because the impurities have about the same volatility as the primary components reducing the overall selectivity. Kerr-McGee Inc. has developed a commercial process for the separation of heavy components of crude oil. Fractionation with respect to chemical composition is possible and has been investigated to produce polymer fractions of low polydispersity starting from a parent material of high polydispersity. 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.

3. Reactions

Supercritical fluids are attractive media for several chemical reactions. The properties of supercritical fluids mentioned earlier can be used to advantage. By small adjustments in pressure, the reaction rate constants can be altered by two orders of magnitude. Equilibrium constants for reversible reactions can also be changed 2-6 fold by small changes in pressure. This dramatic control over the reaction rates has led to the design of several reactions in different areas of biochemistry, polymer chemistry and environmental science. In bioreactions, increased solubilities of hydrophobic material and the potential to integrate the separation and reaction steps has led to research in this area. The use of lipase and synthesis of mondisperse biopolymers holds commercial promise. Carbon dioxide has also been extensively studied for homogeneous polymerization of a few polymers such as fluoroacrylates. The feasibility of free radical polymerization of polystyrene and the polymerization of polyethylene has also been investigated. Carbon dioxide is also often used as a swelling agent for a polymer substrate. Though highly corrosive and a high critical temperature and pressure, supercritical water has [illegible]e of the most studied medium for chemical reactions. Supercritical water has the ability to dissolve many nonpolar organic compounds such as alkanes and chlorinated biphenyls and can dissolve in several gases. It is thus an attractive media for oxidative reactions and has been used to treat a wide variety of waste water streams from chemical, petroleum, textile industries. Huntsman Corporation has commercialized a hydrothermal oxidation unit to treat alcohol and amine contaminated water.

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.

4. Applications in the material and polymer industry

Supercritical fluids are used extensively in the material and polymer industry. Rapid expansion from supercritical solutions across an orifice or nozzle is used commercially to precipitate solids. In this technique, a solute dissolved in supercritical fluid is depressurized rapidly. By controlling the operating variables carefully, the desired precipitated morphology can be attained. In an another process, called gas anti-solvent, a supercritical fluid is rapidly added to a solution of a crystalline solid dissolved in an organic solvent. Since the solute has limited solubility in the fluid, the supercritical fluid acts as an anti-solvent to precipitate solid crystals. By varying the density of the fluid, the particle size distribution of final crystals can be finely controlled. Another process is the precipitation using a compressed fluid anti-solvent. In this process, the solution is sprayed through a nozzle into a compressed fluid and the solvent diffuses rapidly into the supercritical fluid while the fluid swells the solution to precipitate the solute. This process has been used commercially to form nanometric monodisperse microspheres of polymers. Another process that has been commercialized is the usage of supercritical fluid carbon dioxide to produce foamed parts. Since supercritical fluids depress the glass transition temperature of the polymer, polymer foams can be formed at room temperature by directly adding the supercritical fluid into the extruder.

5. Food applications

Carbon dioxide is the most common supercritical fluid in the food industry. Due to the non-toxicity and low critical temperature, it can be used to extract thermally labile food components and the product is not contaminated with residual solvent. Further, the extracts color, composition, odour, texture are controllable and extraction by supercritical fluid carbon dioxide retains the aroma of the product. Supercritical carbon dioxide extraction is used as a replacement for hexane in extracting soybean-oil and has been tested for extraction from corn, sunflower and peanuts. Supercritical fluid extraction provides a distinct advantage not only in the replacement but also extracts oils that are lower in iron and free fatty acid. To satisfy the consumer's need for 'lighter' foods, developmental work on supercritical extraction of oils from potato chips and other snack foods are been carried out. In addition, supercritical carbon dioxide has also been used to extract lilac, essential oils, black pepper, nutmeg, vanilla, basil, ginger, chamomile, and cholesterol.

A large amount of research has been concentrated on the decaffeination of coffee by supercritical carbon dioxide. Thus, it is not surprising to note that this was the first process to be commercialized (in 1978), whose primary step is supercritical extraction. Dry carbon dioxide cannot extract caffeine from dry coffee effectively and that the beans should be pre-wetted by water. Soaking of about 2 hours is necessary for efficient extraction of caffeine from coffee beans by supercritical carbon dioxide. Commercial processes for decaffeination of coffee include the Kraft General Foods in USA and several processes in Germany.

6. Environmental applications

Due to strict environmental regulations, supercritical fluids are used as replacements for conventional hazardous chemicals such as hexane. Supercritical fluid extraction has been proposed as an alternative technique for soil remediation and activated carbon regeneration. Over 99% of a majority of organics can be removed from contaminated soil. Organics that have been successfully extracted include PAHs, PCBs, DDT and toxophene. Carbon dioxide has been used with entrainers for the extraction of highly polar compounds. A commercial process to separate oils from refinery sludge and contaminated soil has been developed by CF Systems Corporation, USA. Chelating moieties that dissolve into carbon dioxide have been developed for the extraction of heavy metals from soil.

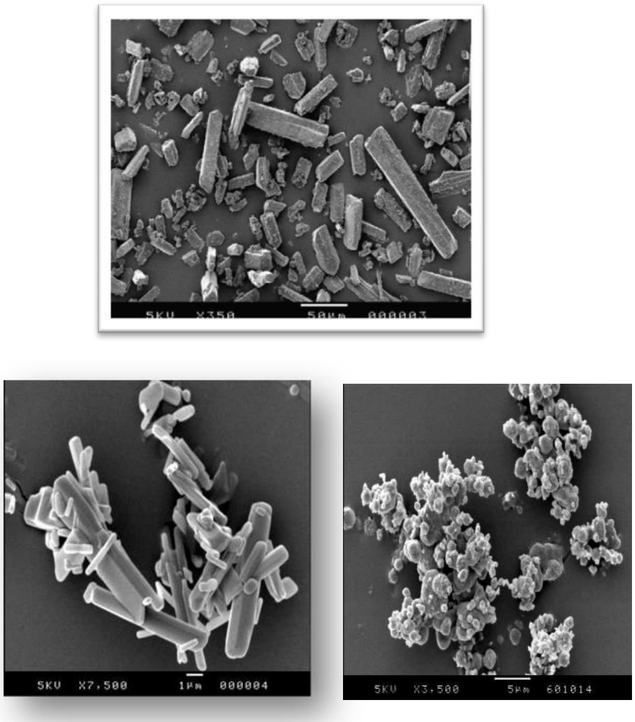
7. Pharmaceutical applications

Since the residual solvent present in the extracted material is of critical importance in the pharmaceutical industry, supercritical fluid carbon dioxide has found several applications. The extraction of vitamin E from soybean oil and a purification method for vitamin E has been well studied. The latter process avoids the step of vacuum distillation, which usually results in the thermal degradation of the product. Solubilities and recrystallization of various drugs has been demonstrated in supercritical fluids.

In Particle design and drug formulation **:**[11] Particle formation processes using supercritical fluids 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 tha[illegible]st cases, these solid morphology change does not co[illegible]e 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.



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

Figure 6: Lovastatin atomization by RESS

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), 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.

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 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 ; 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.

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, leading to a free-flowing powder

8. In 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

9. 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 .

Regarding sterilization, it is known for long that CO_2 has a biocide effect on most bacteria and fungi. A recent article 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 with N_2O or CO_2 in "mild" conditions to avoid denaturation of the very fragile proteins, and during CO_2-dilapidation of bone implants.

OTHER APPLICATIONS INCLUDE[12]

- Decaffeination (coffee & tea)
- Hop extracts (bitter)
- Spice extracts (oil & oleoresin)
- Flavours & fragrances
- Food colours
- Food preservatives
- Herbal medicines
- Pesticides (neem)
- Deoiling of fast foods
- Cholesterol free food products
- Nicotine / tar free tobacco

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. 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-phytopharmaceuticals. 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.

ADVANTAGES [10,12]

Cost Efficient

Several factors combine to reduce the cost of SFC separations without sacrificing purity, including:

- Faster run times
- Flexible solvent conditions
- Lower solvent use as SFC typically utilizes carbon dioxide as the mobile phase
- Reduced waste for disposal
- Less ancillary equipment required Selectivity

The properties of a supercritical fluid can be altered by varying the pressure and temperature, allowing selective extraction. For example, volatile oils can be extracted from a plant with low pressures (100 bar), whereas liquid extraction would also remove lipids. Lipids can be removed using pure CO_2at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent.

Speed

Extraction is a diffusion-based process, with the solvent required to diffuse into the matrix, and the extracted material to diffuse out of the matrix into the solvent. Diffusivities are much faster in supercritical fluids than in liquids, and therefore extraction can occur faster. Also, there is no surface tension and viscosities are much lower than in liquids, so the solvent can penetrate into small pores within the matrix inaccessible to liquids. Both the higher diffusivity and lower viscosity significantly increase the speed of the extraction: An extraction using an organic liquid may take several hours, whereas supercritical fluid extraction can be completed in 10 to 60 minutes.

- It can be easily recycled
- Chemically inert. (However Lorn (1988), notes the presence of some acetates on the raspberry extract, obtained with supercritical CO_2)
- Non flammable
- Has a good solvent characteristic for non-polar and slightly polar solutes.
- It is a “natural” substance, present in mineral waters and part of the life cycle
- It is easily removed from the product
- The dissolving power and selectively can be controlled by selection of suitable pressure/temperature combination
- It has a convenient critical temperature (31.04ºC). This enables extractions to be carried out at comparatively low temperature (often as low as 40 or 50ºC), decreasing the risk of damage of thermolabile compounds.
- Most of the volatile components, which tend to be lost in hydrodistillation, are present in the supercritical extracts [Martinez de La Ossa *et al.* (1991) and Vardag and Korner (1995)]. Partly because of this, extracts obtained in this way tend to have flavour and taste, which are well liked by tasty panels.
- Extraction of natural raw material with supercritical CO_2, allows the obtaining of extracts which fl d taste are perfectly respected and reproducible
- The supercritical fluid ability to vaporise non-volatile components (at moderate temperatures) reduces the r energy spent, when comparing to distillation.
- Once the pressure excess in the equipment prevents oxygen entry while extraction occurs, oxidation reactions don’t happen
- The number of solvents possible to be used on supercritical extraction is superior of classic organic solvents
- Supercritical fluids have a superior selectivity although they have an inferior solvent power than classic organic solvents
- Supercritical extraction can be connected directly to a chromatograph, allowing analysis immediately after extraction.

LIMITATION [12,14]

- The requirement for high pressures increases the cost compared to conventional liquid extraction, so SFE will only be used where there are significant advantages.
- Carbon dioxide itself is non-polar, and has somewhat limited dissolving power, so cannot always be used as a solvent on its own, particularly for polar solutes.
- The use of modifiers increases the range of materials which can be extracted. Food grade modifiers such as ethanol can often be used, and can also help in the collection of the extracted material, but reduces some of the benefits of using a solvent which is gaseous at room temperature.

Following rules depent on solvent power

It dissolves non-polar or slightly polar compounds.

• The solvent power for low molecular weight compounds is high and decreases with increasing molecular weight.

• SC-CO2 has high affinity with oxygenated organic compounds of medium molecular weight.

• Free fatty acids and their glycerides exhibit low solubilities.

• Pigments are even less soluble.

• Water has a low solubility (<0.5% w/w) at temperatures below 100oC.

• Proteins, polysaccharides, sugars and mineral salts are insoluble and;

• SC-CO2 is capable of separating compounds that are less volatile, have a higher molecular weightand/or are more polar as pressure increases

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