



**- A REVIEW**

## **SUPERCRITICAL FLUID EXTRACTION**

**G. N. SAPKALE<sup>\*</sup>, S. M. PATIL, U. S. SURWASE and P. K. BHATBHAGE**

Department of Pharmacognosy, ASPM'S K. T. Patil College of Pharmacy, Siddharth Nagar,  
Barshi Road, OSMANABAD – 413501 (M.S.) INDIA

### **ABSTRACT**

Supercritical fluid extraction is the most effective and efficient way to extract valuable constituent botanicals. Supercritical Fluid Extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids that is CO<sub>2</sub> as the extracting solvent. CO<sub>2</sub> is the king of extraction solvents for botanicals. Extraction conditions for supercritical CO<sub>2</sub> are above the critical temperature of 31°C and critical pressure of 74 bar. Supercritical fluids are highly compressed gases, which have combined properties of gases and liquids in an intriguing manner. Supercritical fluids can lead to reactions, which are difficult or even impossible to achieve in conventional solvents. It is a fast process completed in 10 to 60 minutes. A supercritical fluid can be separated from analyte by simply releasing pressure, leaving almost no trace and yields a pure residue.

**Key words:** Supercritical fluid extraction, Applications.

### **INTRODUCTION**

Extraction can be defined as the removal of soluble material from an insoluble residue, either liquid or solid, by treatment with a liquid solvent. It is therefore, a solution process and depends on the mass transfer phenomena. The controlling factor in the rate of extraction is normally the rate of diffusion of the solute through the liquid boundary layer at the interface.

Extraction involves the separation of medicinally active portions of plant or animal tissue from the inactive or inert components using selective solvents in standard extraction procedures. The products so obtained from plants are relatively impure liquids, semisolids or powders intended only for oral or external use. So extraction continues to be of considerable interest in order to obtain improved yields of drug derived from plant and animal sources.

---

<sup>\*</sup> Author for correspondence; E-mail: geeta\_sapkale@rediffmail.com, shivapatilg@rediffmail.com, ulhas\_pharma@rediffmail.com, bpranita@ymail.com

Techniques of extraction methods continue to be investigated and applied to obtain higher yields of the active substances from natural sources. The principle methods of extraction are -

- Maceration
- Percolation
- Digestion
- Infusion
- Decoction

### **Solvent extraction**

Solvent extraction also known as liquid-liquid extraction and partitioning, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. It is a basic technique in chemical laboratories, where it is performed using a separatory funnel. In other words, this is the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. Solvent extraction may be made use analytically for concentrating or rejecting a particular substance, or for the separation of mixtures. This process usually separates a soluble compound from an insoluble compound. Solvent extraction is used in nuclear processing, ore processing, production of fine organic compounds, processing of perfumes and other industries. Thus, although the method can be used for extraction and preconcentration of a wide range of non volatile or semi volatile species from water using only routine laboratory equipments, its use is decreasing in most situations because solvents of the required purity tend to be expensive, and can also cause problems with proper disposal after use.

### **Supercritical fluid extraction**

Often the analysis of complex materials requires, as a preliminary step that is, separation of the analyte or analytes from a sample matrix. Ideally, an analytical separation method should be rapid, simple and inexpensive; should give quantitative recovery of analytes without loss or degradation; should yield a solution of the analyte that is sufficiently concentrated to permit the final measurement to be made without the need for concentration; should generate little or no laboratory wastes that have to be disposed off.

For many years, one of the most common methods for performing analytical

separations on complex environmental, pharmaceutical, food and petroleum samples was based upon extraction of bulk samples with hydrocarbon or chlorinated organic solvents using a Soxhlet extractor. Unfortunately, liquid extraction frequently fails to meet several of the ideal criteria. They usually require several hours or more to achieve satisfactory recoveries of analytes and even sometimes fails to do. The solvent costs are often high. The solutions of the recovered analytes are often so dilute so that a concentration step must follow the extraction. Analyte degradation or loss as well as atmospheric pollution may accompany this concentration step.

Beginning in the mid-1980, chemists began to explore the use of supercritical fluids for the separation of analytes from the matrix of many samples of interest to industry and governmental agencies because use of reagents of this type avoids many of the problems for organic liquid extractants.

### **What is Supercritical Fluid Extraction ?**

Supercritical fluids have been investigated since last century, with the strongest commercial interest initially focusing on the use of supercritical toluene in petroleum and shale oil refining during the 1970s. Supercritical water is also being investigated as a means of destroying toxic wastes, and as an unusual synthesis medium. The biggest interest for the last decade has been the applications of supercritical carbon dioxide, because it has a near-ambient critical temperature ( $31^{\circ}\text{C}$ ), thus biological materials can be processed at temperatures around  $35^{\circ}\text{C}$ . The density of the supercritical  $\text{CO}_2$  at around 200 bar pressure is close to that of hexane, and the solvation characteristics are also similar to hexane; thus, it acts as a non-polar solvent. Around the supercritical region,  $\text{CO}_2$  can dissolve triglycerides at concentrations up to 1% mass. The major advantage is that a small reduction in temperature, or a slightly larger reduction in pressure, will result in almost the entire solute precipitating out as the supercritical conditions are changed or made sub critical. Supercritical fluids can produce a product with no solvent residues. Examples of pilot and production scale products include decaffeinated coffee, cholesterol-free butter, low-fat meat, evening primrose oil, squalene from shark liver oil, etc. The solvation characteristics of supercritical  $\text{CO}_2$  can be modified by the addition of an entrainer, such as ethanol, however some entrainer remains as a solvent residue in the product, negating some of the advantages of the "residue-free" extraction.

Supercritical fluid extraction (SFE) is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids as the extracting solvent. Extraction is usually from a solid matrix, but it can also be from liquids. SFE can be used as

a sample preparation step for analytical purposes, or on a larger scale to either strip unwanted material from a product (e.g. decaffeination) or collect a desired product (e.g. essential oils). Carbon dioxide (CO<sub>2</sub>) is the most used supercritical fluid, sometimes modified by co-solvents such as ethanol or methanol. Extraction conditions for supercritical CO<sub>2</sub> are above the critical temperature of 31°C and critical pressure of 74 bar. Addition of modifiers may slightly alter this. Supercritical extraction mostly uses carbon dioxide at high pressure to extract the high value products from natural materials. Unlike other processes, the extraction process leaves no solvent residue behind. Moreover the CO<sub>2</sub> is non-toxic, non-flammable, odorless, tasteless, inert, and inexpensive. Due to its low critical temperature 31°C, carbon dioxide is known to be perfectly adapted in food, aromas, essential oils and nutraceutical industries.

### **Supercritical fluid**

A supercritical fluid is any substance at a temperature and pressure above its critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Additionally, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". Supercritical fluids are suitable as a substitute for organic solvents in a range of industrial and laboratory processes. Carbon dioxide and water are the most commonly used supercritical fluids, being used for decaffeination and power generation, respectively. CO<sub>2</sub> is the kind of extraction solvents for botanicals. It leaves no toxic residue behind. Its extraction properties can be widely and precisely manipulated with subtle changes in pressure and temperature.

There are 2 types of CO<sub>2</sub> extraction -

#### **Low pressure cold extraction**

It involves chilling CO<sub>2</sub> at 35-55<sup>0</sup>F and pumping it through the plant material at between 800-1500 psi.

#### **Supercritical extraction**

It involves heating the CO<sub>2</sub> to above 87<sup>0</sup>F and pumping it above 1100 psi. Usually, this is between 6000-10000 psi. Supercritical fluid CO<sub>2</sub> can best be described as a dense fog when CO<sub>2</sub> is used in a dense liquid state. Low-pressure CO<sub>2</sub> is often the best method for producing high quality botanical extracts. CO<sub>2</sub> loading rate in this state means that you have to pump many volumes of CO<sub>2</sub> through botanical. The loading rate is typically 10-40

volumes. For this reason, it is important to have pumped CO<sub>2</sub>, which has a much faster loading rate 2-10 volumes and a wide range of uses.

### Properties of supercritical fluid

- (i) Supercritical fluids have highly compressed gases, which combine properties of gases and liquids in an intriguing manner.
- (ii) Supercritical fluids can lead to reactions, which are difficult or even impossible to achieve in conventional solvents.
- (iii) Supercritical fluids have solvent power similar to light hydrocarbons for most of the solutes. However, fluorinated compounds are often more soluble in supercritical CO<sub>2</sub> than in hydrocarbons; this increased solubility is important for polymerization.
- (iv) Solubility increases with increasing density (that is with increasing pressure). Rapid expansion of supercritical solutions leads to precipitation of a finely divided solid. This is a key feature of flow reactors.
- (v) The fluids are commonly miscible with permanent gases (e.g. N<sub>2</sub> or H<sub>2</sub>) and this leads to much higher concentrations of dissolved gases than can be achieved in conventional solvents.

In general terms, supercritical fluids have properties between those of a gas and a liquid.

**Table 1: Critical properties for some components commonly used as supercritical fluids**

Critical properties of various solvents (Reid et al., 1987)				
Solvent	Molecular weight (g/mol)	Critical temperature (K)	Critical pressure MPa (atm)	Critical density (g/cm <sup>3</sup> )
Carbon dioxide (CO <sub>2</sub> )	44.01	304.1	7.38 (72.8)	0.469
Water (H <sub>2</sub> O) (acc. IAPWS)	18.015	647.096	22.064 (217.755)	0.322
Methane (CH <sub>4</sub> )	16.04	190.4	4.60 (45.4)	0.162

Cont...

Solvent	Molecular weight (g/mol)	Critical temperature (K)	Critical pressure MPa (atm)	Critical density (g/cm <sup>3</sup> )
Ethane (C <sub>2</sub> H <sub>6</sub> )	30.07	305.3	4.87 (48.1)	0.203
Propane (C <sub>3</sub> H <sub>8</sub> )	44.09	369.8	4.25 (41.9)	0.217
Ethylene (C <sub>2</sub> H <sub>4</sub> )	28.05	282.4	5.04 (49.7)	0.215
Propylene (C <sub>3</sub> H <sub>6</sub> )	42.08	364.9	4.60 (45.4)	0.232
Methanol (CH <sub>3</sub> OH)	32.04	512.6	8.09 (79.8)	0.272
Ethanol (C <sub>2</sub> H <sub>5</sub> OH)	46.07	513.9	6.14 (60.6)	0.276
Acetone (C <sub>3</sub> H <sub>6</sub> O)	58.08	508.1	4.70 (46.4)	0.278

**Table 2: Density, diffusivity and viscosity for typical liquids, gases and supercritical fluids**

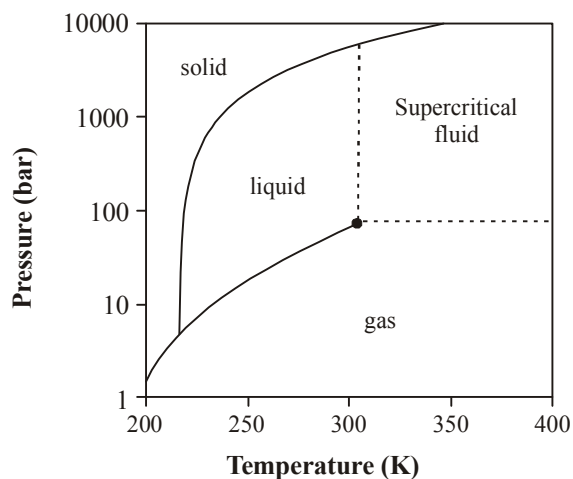
Comparison of gases, supercritical fluids and liquids			
	Density (kg/m <sup>3</sup> )	Viscosity (μPa·s)	Diffusivity (mm <sup>2</sup> /s)
Gases	1	10	1-10
Supercritical fluids	100-1000	50-100	0.01-0.1
Liquids	1000	500-1000	0.001

All supercritical fluids are completely miscible with each other so for a mixture, a single phase can be guaranteed, if the critical point of the mixture is exceeded. The critical point of a binary mixture can be estimated as the arithmetic mean of the critical temperatures and pressures of the two components,

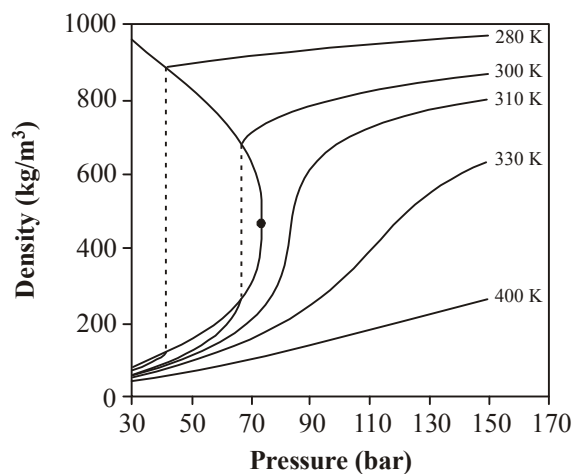
$$T_{c(\text{mix})} = (\text{Mole fraction A}) \times T_{cA} + (\text{Mole fraction B}) \times T_{cB}. \quad \dots(1)$$

For greater accuracy, the critical point can be calculated using equations of state, such as the Peng Robinson, or group contribution methods. Other properties, such as density, can also be calculated using equations of state.

## Phase diagram



**Fig. 1: Carbon dioxide pressure-temperature phase diagram**



**Fig. 2: Carbon dioxide density-pressure phase diagram**

Figs. 1 and 2 show projections of phase diagrams. In the pressure-temperature phase diagram (Fig. 1), the boiling separates the gas and liquid region and ends in the critical point, where the liquid and gas phases disappear to become a single supercritical phase.

This can be observed in the density-pressure phase diagram for carbon dioxide, as shown in Fig. 2. At well below the critical temperature, e.g. 280 K, as the pressure increases, the gas compresses and eventually (at just over 40 bar) condenses into a much denser liquid,

resulting in the discontinuity in the line (vertical dotted line). The system consists of 2 phases in equilibrium, a dense liquid and a low-density gas. As the critical temperature is approached (300 K), the density of the gas at equilibrium becomes denser, and that of the liquid lower. At the critical point, (304.1 K and 7.38 MPa (73.8 bar)), there is no difference in density, and the 2 phases become one fluid phase. Thus, above the critical temperature, a gas cannot be liquefied by pressure. At slightly above the critical temperature (310 K), in the vicinity of the critical pressure, the line is almost vertical. A small increase in pressure causes a large increase in the density of the supercritical phase. Many other physical properties also show large gradients with pressure near the critical point, e.g. viscosity, the relative permittivity and the solvent strength, which are all closely related to the density. At higher temperatures, the fluid starts to behave like a gas, as can be seen in Fig. 2. For carbon dioxide at 400 K, the density increases almost linearly with pressure.

Many pressurized gases are actually supercritical fluids. For example, nitrogen has a critical point of 126.2 K (-147°C) and 3.4 MPa (34 bar). Therefore, nitrogen in a gas cylinder above this pressure (or compressed air) is actually a supercritical fluid. These are more often known as permanent gases. At room temperature, they are well above their critical temperature, and therefore, behave as a gas, similar to CO<sub>2</sub> at 400 K above. However, they cannot be liquefied by pressure unless cooled below their critical temperature.

### **Supercritical fluid extraction process**

The system must contain a pump for the CO<sub>2</sub>, 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<sub>2</sub> can then be cooled, re-compressed and recycled, or discharged to atmosphere.

### **Pumps**

Carbon dioxide is usually pumped as a liquid, usually below 5°C and a pressure of about 50 bars. The solvent is pumped as a liquid as it is then almost incompressible. As a supercritical fluid, much of the pump stroke will be "used up" in compressing the fluid, rather than pumping it. For small-scale extractions (up to a few grams/minute), reciprocating CO<sub>2</sub> pumps or syringe pumps are often used. For larger scale extractions, diaphragm pumps are most common. The pump heads will usually require cooling, and the CO<sub>2</sub> 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 bars, and most extractions are conducted at less than 350 bar. However, sometimes, higher pressures will be needed, such as extraction of vegetable oils, where pressures of 800 bars 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 oil or electrically heated jacket for larger vessels. Care must be taken if rubber seals are used on the vessel, as the CO<sub>2</sub> may dissolve in the rubber, causing swelling, and the rubber will rupture on depressurization.

## **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 backpressure 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<sub>2</sub> 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.

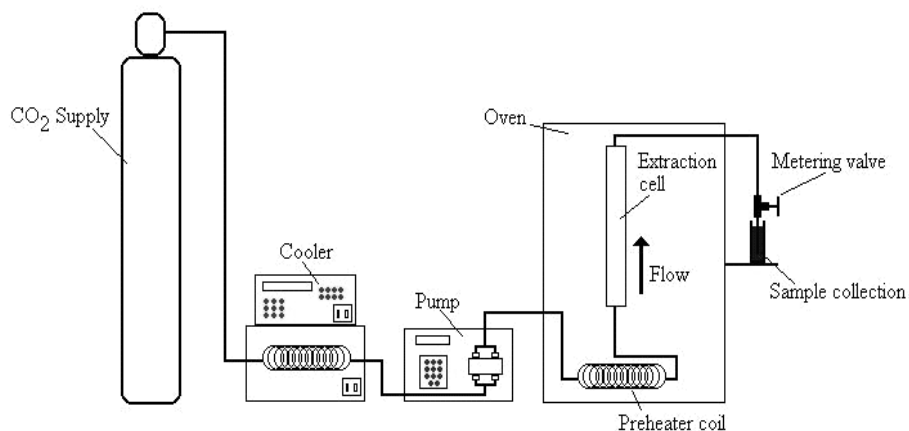
## **Collection**

The supercritical solvent is passed into a vessel at lower pressure than the extraction vessel. The density, and thus, dissolving power, of supercritical fluids varies sharply with pressure, and hence, the solubility in the lower density CO<sub>2</sub> 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<sub>2</sub> can be recycled or depressurized to atmospheric pressure and vented. For analytical SFE, the pressure is usually dropped to atmospheric, and now the gaseous carbon dioxide is bubbled through the solvent to trap the precipitated components.

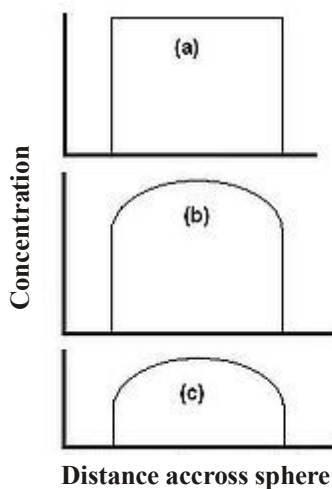
## **Heating and cooling**

This is an important aspect. The fluid is cooled before pumping to maintain liquid conditions, and 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 hair dryer. For larger systems, the energy required during each stage of the process can be calculated using the thermodynamic properties of the supercritical fluid.



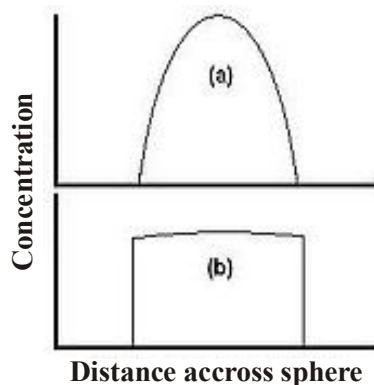
**Fig. 3: Schematic diagram of SFE apparatus**



**Fig. 4: Concentration profiles during a typical SFE extraction**

There are two essential steps to SFE, transport (by diffusion or otherwise) from with the solid particles to the surface, and dissolution in the supercritical fluid. Other factors, such as diffusion into the particle by the SF and reversible release such as desorption from an

active site are sometimes significant, but not dealt in detail here. Fig. 4 shows the stages during extraction from a spherical particle where at the start of the extraction, the level of extractant is equal across the whole sphere Fig. 4(a). As extraction commences, material is initially extracted from the edge of the sphere, and the concentration in the center is unchanged Fig 4(b). As the extraction progresses, the concentration in the center of the sphere drops as the extractant diffuses towards the edge of the sphere Fig. 4(c).

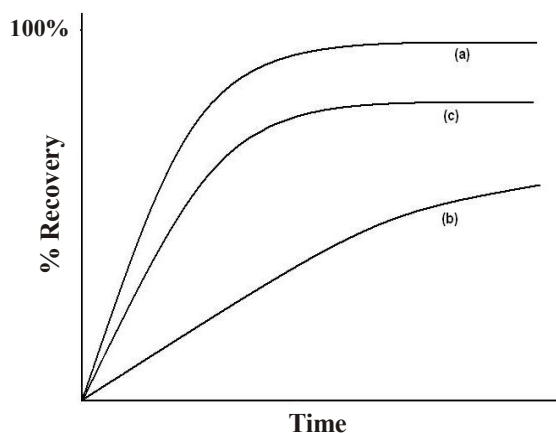


**Fig. 5: Concentration profiles for (a) diffusion limited and (b) solubility limited extraction**

The relative rates of diffusion and dissolution are illustrated by two extreme cases in Fig. 5. Fig. 5(a) shows a case where dissolution is fast relative to diffusion. The material is carried away from the edge faster than it can diffuse from the center, so the concentration at the edge drops to zero. The material is carried away as fast as it arrives at the surface, and the extraction is completely diffusion limited. Here, the rate of extraction can be increased by increasing diffusion rate, for example raising the temperature, but not by increasing the flow rate of the solvent. Fig. 5(b) shows a case where solubility is low relative to diffusion. The extractant is able to diffuse to the edge faster than the solvent can carry it away, and the concentration profile is flat. In this case, the extraction rate can be increased by increasing the rate of dissolution, for example by increasing flow rate of the solvent.

The extraction curve of % recovery against time can be used to elucidate the type of extraction occurring. Fig. 6(a) shows a typical diffusion controlled curve. The extraction is initially rapid, until the concentration at the surface drops to zero, and the rate then becomes much slower. The % extracted eventually approaches 100%. Fig. 6(b) shows a curve for solubility limited extraction. The extraction rate is almost constant, and only flattens off towards the end of the extraction. Fig. 6(c) shows a curve where there are significant matrix effects, where there is some sort of reversible interaction with the matrix, such as desorption

from an active site. The recovery flattens off, and if the 100% value is not known, then it is hard to tell that extraction is less than complete.



**Fig. 6: Extraction profile for different types of extraction**

### **Optimization**

The optimum will depend on the purpose of the extraction. For an analytical extraction is to determine, say, antioxidant content of a polymer, then the essential factors are complete extraction in the shortest time. However, for production of an essential oil extract from a plant, then quantity of CO<sub>2</sub> used will be a significant cost, and "complete" extraction is not required; a yield of 70 - 80% perhaps being sufficient to provide economic returns. In another case, the selectivity may be more important, and a reduced rate of extraction will be preferable if it provides greater discrimination. Therefore, few comments can be made, which are universally applicable. However, some general principles are outlined below.

### **Maximizing diffusion**

This can be achieved by increasing the temperature, swelling the matrix, or reducing the particle size. Matrix swelling can sometimes be increased by increasing the pressure of the solvent, and by adding modifiers to the solvent. Some polymers and elastomers in particular are swelled dramatically by CO<sub>2</sub>, with diffusion being increased by several orders of magnitude in some cases.

### **Maximizing solubility**

Generally, higher pressure will increase solubility. The effect of temperature is less certain, as close to the critical point, increase in the temperature causes a decrease in density,

and hence, dissolving power. At pressures well above the critical pressure, solubility is likely to increase with temperature. Addition of low levels of modifiers (sometimes called entrainers), such as methanol and ethanol, can also significantly increase solubility, particularly of more polar compounds.

### **Optimizing flow rate**

The flow rate of CO<sub>2</sub> should be measured in terms of mass flow rather than by volume because the density of the CO<sub>2</sub> changes according to the temperature both; before entering the pump heads and during compression. Coriolis flow meters are best used to achieve such flow confirmation. To maximize the rate of extraction, the flow rate should be high enough for the extraction to be completely diffusion limited (but this will be very wasteful of solvent). However, to minimize the amount of solvent used, the extraction should be completely solubility limited (which will take a very long time). Flow rate must therefore be determined depending on the competing factors of time and solvent costs, and also capital costs of pumps, heaters and heat exchangers. The optimum flow rate will probably be somewhere in the region where both solubility and diffusion are significant factors.

### **Advantages**

#### **Environmental improvement and reduced product contamination**

SFE is an alternative to liquid extraction using solvents such as hexane or dichloromethane. There will always be some residual solvent left in the extract and matrix, and there is always some level of environmental contamination from their use. In contrast, carbon dioxide is easy to remove simply by reducing the pressure, leaving almost no trace, and it is also environmentally benign. The use of SFE with CO<sub>2</sub> is approved by the Soil Association for organic products. The CO<sub>2</sub> used is largely a by-product of industrial processes or brewing, and its use in SFE does not cause any extra emissions.

### **Selectivity**

The solvent strength of a supercritical fluid can be varied by change in the pressure and a lesser extent in the temperature.

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<sub>2</sub> at higher pressures, and then phospholipids can be removed by adding ethanol to the solvent.

## Speed

It is a fast process and completed in 10 to 60 minutes. It 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.

## Purity

A supercritical fluid can be separated from analyte by simply releasing pressure.

Supercritical fluids are cheap, inert and nontoxic. Thus, they are readily disposed off after an extraction is completed by allowing them to evaporate into the atmosphere.

## Recovery

Recovery of analytes becomes simple.

## REFERENCES

1. Gennaro, A. R. Remington. The Science and Practice of Pharmacy 20<sup>th</sup> Ed. Vol. 2<sup>nd</sup> p. 750
2. S. J. Carter, Cooper and Gunn's Tutorial Pharmacy, p. 1477
3. B. K. Sharma Instrumental Methods of Chemical Analysis. P.C-21 to C-40.
4. D. A. Skoog, F. J. Holler and, Crouch R. Stanley, Instrumental Analysis. Indian Ed., pp. 936-945
5. Edit Székely, Supercritical Fluid Extraction, Budapest University of Technology and Economics. <http://sunny.vemt.bme.hu/sfe/angol/supercritical.html>. Retrieved 2007-11-20.
6. Supercritical Fluid Extraction, Density Considerations, <http://eng.ege.edu.tr/~otles/SupercriticalFluidsScienceAndTechnology/Wc488d76f2c655.htm>. Retrieved 2007-11-20.
7. A. A. Clifford (2007-12-04), Calculation of Thermodynamic Properties of CO<sub>2</sub> using Peng Robinson equation of state, Critical Processes Ltd. [http://www.criticalprocesses.com/Calculation % 20 of % 20 density, % 20 enthalpy % 20 and % 20 entropy % 20 of % 20 carbon % 20 dioxide.htm](http://www.criticalprocesses.com/Calculation%20of%20density,%20enthalpy%20and%20entropy%20of%20carbon%20dioxide.htm)

8. Y. Tanaka and O. Takeshi, Extraction of Phospholipids from Salmon Roe with Supercritical Carbon Dioxide and an Entrainer. *J. Oleo Sci.*, Japan Oil Chemists Society, **53(9)**: 417–424 (2004).
9. Tsung Min Kuo and Harold Gardner (Eds.) *Lipid Biotechnology*, Marcel Dekker Inc. New York (2002) pp. 663-687.
10. Calculation of Density, Enthalpy and Entropy for Supercritical Carbon Dioxide with Examples, [http://www.criticalprocesses.com/Calculation % 20 of % 20 density, % 20 enthalpy % 20 and % 20 entropy % 20 of % 20 carbon % 20 dioxide.htm](http://www.criticalprocesses.com/Calculation%20of%20density,%20enthalpy%20and%20entropy%20of%20carbon%20dioxide.htm). Retrieved, 12-17 (2007).
11. Clifford, Tony, *Fundamentals of Supercritical Fluids*, Oxford Science Publications, Oxford (1999).
12. H. J. Vandenberg et al., Analytical Extraction of Additives from Polymers, *Analyst* 122: 101R–115R, doi: 10.1039/a704052k, <http://www.rsc.org/Publishing/Journals/AN/article.asp?doi=a704052k> (1997)
13. Supercritical Fluid Extraction, Density Considerations, [http://eng.ege.edu.tr/~otles/Supercritical Fluids Science and Technology/Wc488d76f2c655.htm](http://eng.ege.edu.tr/~otles/Supercritical%20Fluids%20Science%20and%20Technology/Wc488d76f2c655.htm). Retrieved, 01-04 (2008).

*Accepted : 20.02.2010*