

SEPARATIONS TECHNOLOGY

Pharmaceutical and Biotechnology
Applications

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Table 4.1. Supercritical Fluids

Advantages	Disadvantages
Applicable to low temperature extraction of high boiling or temperature sensitive compounds.	High pressure process, management and operators may be used to processes operated at near ambient conditions.
Extract is readily removed from the solvent.	Plant costs are comparatively high.
Solvent is easily recovered for reuse.	Supercritical fluid plants require high pressure vessels and pipework.
Reduced loss of heat-sensitive components.	Sophisticated process control systems are adviseable.
Solvent is approved as food ingredient.	Maintenance costs could be relatively high as a consequence of sophistication.
No toxic solvent residues are contributed from the solvent.	Full chemical engineering and design data are not yet mature.
High purity of commercial carbon dioxide available.	Many variants are already patented.
Readily available and comparatively inexpensive solvent.	
Some extractions could not be made by other techniques.	
Very pure products in relatively few steps.	

applications. It is hoped that through examination of some of the theoretical aspects of supercritical fluids, along with the current commercially viable applications, that some possibilities can be suggested for the pharmaceutical industry. In addition, several studies have been conducted successfully on pharmaceutically related compounds involving the modeling and economics of process scale systems. Review

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of these applications should provide a guideline as to the utility of supercritical fluid techniques, for potential exploitation by the pharmaceutical industry.

THEORY

Definition of a Supercritical Fluid

A supercritical fluid is obtained when a substance is brought to temperature and pressure above or near its critical point. Supercritical fluids are neither gases nor liquids, but possess properties of both states of matter. For example, CO₂ exists as a solid (dry ice) at -85°C and sublimates directly to the gas phase at ambient conditions. Hence, liquid CO₂ exists only under pressure and between -56°C and 31°C. However, at even higher pressures (≥ 72.9 atm) and above the critical temperature (31.3°C), CO₂ exists in its supercritical state.

In the supercritical state properties such as density, viscosity, and vapor-liquid equilibrium ratios become strongly temperature dependent at a given pressure. Subtle variations in temperature and pressure can produce wide variations of density, particularly in the region of the critical point. This affords the ability to control solubility of solutes in the supercritical fluid. The control of solute solubility in a supercritical fluid, by manipulating pressure and temperature, is the basis of both process and analytical SFE and SFC. This is discussed in the next section.

Solubility

Pressure, Temperature, and Density

The solvating power of supercritical fluids is highly dependent on the density of the fluid. High fluid density in the supercritical fluid is achieved as a result of the high pressures created by the system rather than by intermolecular attraction as with liquids. Supercritical fluids, in general, exhibit lower viscosities and permit greater solute diffusivities, than those associated with liquids. In addition, no surface tension is associated with supercritical fluids, permitting greater wettability of sample matrices. By varying the temperature and pressure on the fluid, the density of the supercritical fluid can be controlled. This allows selective manipulation of the solvating power of the fluid. In general, higher densities lead to increased solubility.

Temperature plays a dual role in supercritical fluid separation technology, since it also influences the vapor pressure of solutes. Temperature can also play an important role in the desorption of

analytes from a matrix or sorbent. Higher temperatures also decrease density and, therefore, increase mass transfer coefficients of the solutes in the fluid. In SFE enhanced mass transfer permits greater permeation of the sample matrix by the supercritical fluid, leading to more efficient extractions. For SFC the result is more frequent and rapid equilibrium of solute partitioning into and out of the stationary phase, thereby achieving higher chromatographic resolution than obtainable with liquids.

By analyzing the solubility trends for the respective components in a mixture, a set of conditions can be ascertained that can provide selective extraction or separation of the individual solutes. This often occurs in what is referred to as the crossover region. The crossover point for a single analyte occurs at the pressure where there is a change in the temperature dependence of the solubility. Below this pressure, solubility is dependent on the density of the supercritical fluid. Under these conditions a decrease in temperature leads to an increase in solubility. Above this pressure solubility is also dependent on solute vapor pressure. At this pressure, raising the temperature results in an increase in the vapor pressure and, hence, an increase in solubility. Therefore, for two solutes (being chromatographed or extracted) whose crossover regions occur at difference pressures, it becomes possible to separate the two solutes by varying the temperature or pressure under which the separation is conducted.

Cosolvent Effects

Some solutes may be quite soluble in a supercritical fluid at a given density, but when the same conditions are used in SFE or SFC, their elution may be substantially impaired due to solute-matrix interactions. This is particularly true for polar solutes which may adsorb to active surface sites in the matrix or chromatographic packing in a column. To overcome this problem, organic cosolvents (also called entrainers or modifiers) are frequently added to the supercritical fluid to enhance the solvating power over that achieved with the neat fluid. Also, those cosolvents compete with active sites in the sample matrix or chromatographic packings, thereby enhancing the extraction or chromatography of the desired components. Many low molecular weight polar organic solvents, such as ethanol, are miscible in CO₂ up to a specific mole fraction at a given pressure and temperature. For example, Brunner and Peter (1982) demonstrated that the addition of 10 percent ethanol to CO₂ increased the solubility of palm oils under supercritical conditions by a factor of 20 times as compared to neat CO₂. It should be noted that the amount of cosolvent used in conjunction with the supercritical fluid is much less than is required in conventional liquid processes.

Additional information on the physicochemical properties and behavior of supercritical fluids can be found by consulting several well known texts (Penninger 1985; McHugh 1986; Johnston 1989; Lee and Markides 1990; Bruno 1991).

APPLICATIONS OF SUPERCRITICAL FLUIDS

General Industrial Applications

Specific applications mentioned in this chapter should not be considered as an exhaustive listing either of current or potential uses of supercritical fluids, but simply as examples from which analogies can be drawn in formulating decisions about the use of supercritical fluids in pharmaceutical process separations. It is apparent that more industrial applications will be forthcoming as more physicochemical data is available for predicting solubilities of various classes of pharmaceutical compounds and as models for scaling separations to the process scale continue to improve.

Decaffeination

Probably the best-known application of supercritical fluids is for the decaffeination of coffee. The CO₂ decaffeination process was discovered and developed by Kurt Zosel and the Max Planck Institute in Germany. The process was patented in the 1970's and licensed to Kaffee HAG and General Foods (Zosel 1978). In the separation of caffeine from coffee beans, Zosel recommended extraction of the hydrated beans with CO₂ (Zosel 1981). The first commercial-scale plant to be operated in Germany (designed for the decaffeination of coffee) became operational in the late 1970s with a capacity of 30,000 metric tons/year.

Nestle, a major food company also holds patents for the extraction of the coffee aroma, using CO₂. The soluble coffee is then rearomatized just prior to packing by back addition of the volatile fraction. Far less flavor is lost, than in the conventional processing technique (Pictet et al. 1968).

The large scale decaffeination of coffee demonstrates that supercritical fluids can be used to provide selective isolation of alkaloids from a natural product. It is apparent that this would not only be applicable to the isolation of an alkaloid from coffee, but that the potential exists for applying the technology to a wide variety of other separation needs involving alkaloid, and other extraction and isolation processes within the pharmaceutical industry.

Hops

The first major plants to use CO₂ for the removal of flavor components from hops, were built in Australia and England (Clarke and Mailer 1981) to provide a product for the beer brewing industry. Initially, liquid CO₂ was used as the solvent (Wheldon 1981). A process utilizing supercritical fluid CO₂ was eventually developed in West Germany at, surprisingly, the Coal Research Institute and HAG AG (Vitzthum 1971). In 1981 a plant was commissioned for the extraction of the aroma from hops in Germany, which became operational in 1982. Plants in England and Germany are capable of processing one fifth of their respective nation's annual hop crop, whereas the Australian plant can handle over half of that country's annual production. This capability is important since the hops themselves are difficult to store in their harvested state, due to their tendency to deteriorate. Carbon dioxide derived extracts store better and are superior as flavoring agents. Supercritical fluid based hop plants are utilized for the extraction of other products, at the conclusion of the hops harvesting season. Today, there are several supercritical CO₂ based hops plants, processing products in the Yakima Valley region of the State of Washington.

A related application of SFE is the isolation of oils from brewer's grains that can be utilized as antifoaming agents in brewing processes. These oils are normally extracted and lost during the brewing process. Refortification with the supercritical fluid extract is but one example of adding a "natural" CO₂ extract back into a production process producing a foodstuff.

Other Applications

Some other references to additional applications of supercritical fluids are included in Table 4.2. These examples illustrate the wide potential that exists for supercritical fluid separations. Although many have not yet reached commercial application, they deserve serious consideration from the pharmaceutical industry as viable alternatives in ecologically compatible manufacturing processes.

Selected Pharmaceutical Applications

Supercritical Fluid Extraction

Extraction of Natural Products. Just as liquid and supercritical fluid CO₂ may be used in the extraction of foods, they may also be used to extract drugs and related precursors. Other pharmaceutically related applications are as a reaction media for chemical and enzyme catalyzed synthesis and for the purification of synthetic organic

Table 4.2. Application References

Supercritical fluids as media for enzymatic reactions	Randolph et al. (1985); Taniguchi et al. (1987a); Kamihira et al. (1987c); Russel and Beckman (1991a, 1991b)
Sterilization	Kamihira et al. (1987b); Taniguchi et al. (1987b)
Enzymatic analysis for pesticide residues	King and France (1991); Nam and King (1994)
Enzyme synthesis of aspartame precursors	Kamihira et al. (1987c)
Decaffeination of coffee	Vizthum and Hubert (1975); Asche (1980)
Production of low alcohol wines	Berger et al. (1981)
Fractionation of vegetable and cottonseed oils	Coenen and Kriegel (1984); List et al. (1984)
Extraction of spices	Avagimov et al. (1980); Brannolte (1982)
Essential oils flavors and aromas from plants	Stahl et al. (1982); Coenen and Kriegel (1984); Krukonis (1984); Caragay (1981); Moyler (1985); Williams (1981); Temelli et al. (1988)
Extraction of food colors	Degnan et al. (1991)
Regenerating lubricating oils	Coenen et al. (1980, 1982)
Enzyme catalysis to manufacture high value oils from lower value oils	Nakamura (1987)

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Pyrethrin insecticides from pyrethrum	Stahl and Schultz (1980); Sims (1982)
Alcohol from fermentation broths	De Filippi (1982)
Reduction of fat in potato chips	Hannigan (1981)
Regeneration of spent bleaching clays	King et al. (1992)
β -Carotene from liquid solvents	Chang and Randolph (1991)
Removal of fat and cholesterol from milk	Process for removing . . . (<i>The Cheese Reporter</i> 1989); Bhaskar et al. (1993)
Fat from meats	King et al. (1989)
Removal of nicotine and tars from tobacco	Roselius et al. (1970)
Extraction of aroma from tobacco dust	Luganskaya et al. (1967)
Dry cleaning of garments	Maffei (1971)
Extraction of paraffins and extraction of liquid fuels from coal	Williams (1981); Demitrelis et al. (1984)

chemicals. The isolation of drugs from fermentation broths may be possible in certain instances using selected supercritical fluids.

Supercritical fluid techniques are being examined in the biochemical and pharmaceutical industries as a result of the needs for separating temperature sensitive compounds using physiologically inert solvents.

The number of applications of supercritical fluid extractions has been limited, in part due to the fact that the preferred solvent,

CO₂, does not always provide the required selectivity. Biomolecules generally have low solubilities in supercritical fluids and often occur in supercritical fluids, as trace components in the presence of high concentrations of lipid coextractants. Some additional selectivity can be obtained, by using cosolvents. Further purification steps may still be required, including the removal of the cosolvent from the extract.

The following are but a few examples of the application of supercritical fluid extraction to compounds of pharmaceutical interest. Some of the reported studies are concerned with solubility studies in supercritical fluids, or in analytical SFE and SFC studies on compounds of pharmaceutical interest. These studies, although not strictly process in scale or scope, suggest potential applications in the pharmaceutical industry and invite further research investigations.

Tocopherol concentrates are utilized in various applications as antioxidants and vitamin supplements. The recovery and purification of tocopherols from natural substances, such as vegetable oils, has been accomplished using SFE, coupled with preparative scale SFC (King 1993a). In a two-step process the tocopherols were isolated and transferred from the SFE stage, and deposited at the head of a preparative column, containing silica sorbent. The tocopherols were then eluted from the preparative column via SFC, using supercritical CO₂ in order to isolate the enriched alpha, beta, gamma, and delta fractions. Recoveries of approximately 75 percent were reported, based on the original concentration in the starting oil. A schematic depiction of the device used in these experiments is given in Figure 4.1. Enrichment factors are shown in Table 4.3. Results demonstrated that the additional enrichment afforded by SFC is significant, and achieves a result, not possible by SFE alone.

Taxol (MW = 853.9) is a natural product, exhibiting anti-cancer properties, which can be isolated from *Taxus brevifolia* (Northwestern pacific yew tree) as well as other botanicals of the *Taxus* genus. It has been used in the treatment of breast, ovarian, and other forms of cancer. Harvesting taxol from *Taxus brevifolia* is somewhat problematic (Borman 1991). Since taxol occurs in small amounts in the bark of *Taxus brevifolia* [50 to 100 mg/kg bark (Zuer 1988)], it is estimated that the bark of two 60-year-old trees would be required for the treatment of a single patient (Caruana 1991). Obviously, extraction of taxol from such a natural source is not a long term solution. Synthesis of taxol has been demonstrated using a compound derived from extracts of the leaves of *Taxus brevifolia* as a precursor (Denis et al. 1988). More recently, Chun et al. (1994) described the extraction of taxol, baccatin III and 10-deacetyl baccatin III used as precursors of an efficient semi-synthetic method for producing taxol. The leaves of the tree (or

Figure 4.1. Schematic of SFE/SFC used for the production of tocopherol concentrates. (Source: King et al. (1993a) with permission)

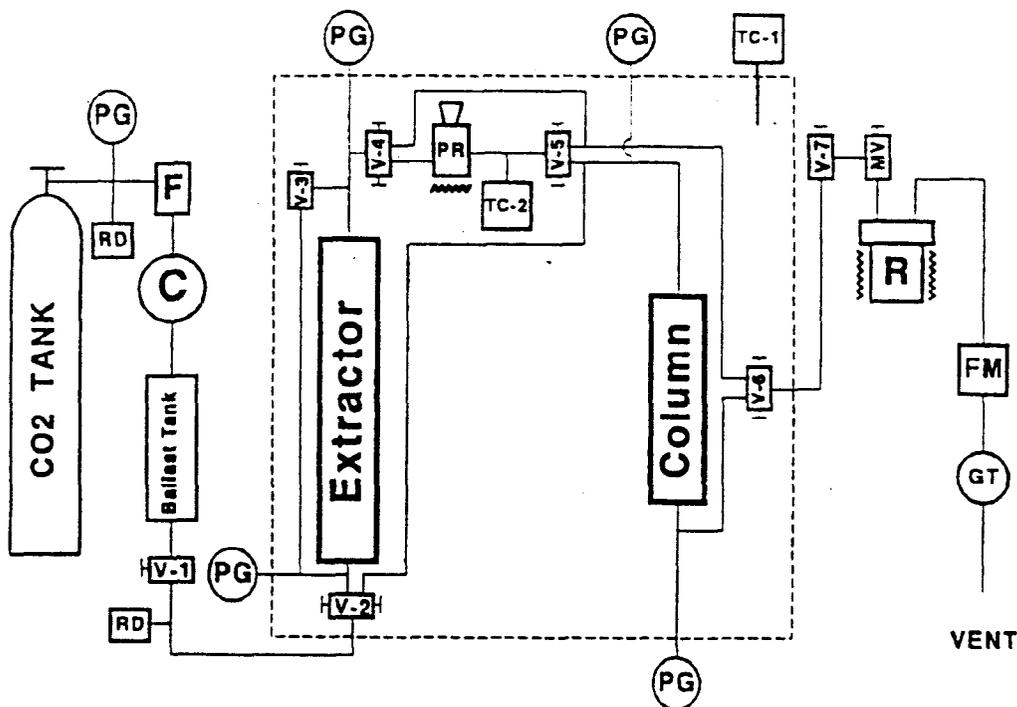


Table 4.3. Enrichment of Tocopherols from Soybean Flakes

	SFE	SFE/SFC
alpha	4.33	12.1
beta	1.83	2.4
gamma	3.94	15.0
delta	3.75	30.8

Source: King et al. 1993a with permission.

possibly plant tissue culture) being a renewable resource may prove to be an alternative solution (Witherup et al. 1990; Caruana 1991).

The application of SFE for the extraction of taxol from the *Taxus brevifolia* bark has been investigated (Jennings et al. 1992). Ground bark was alternately extracted with neat supercritical CO₂ and CO₂ with ethanol as a cosolvent. The experiments were conducted isothermally at 318 K and the amount of taxol extracted from the bark was shown

to increase with increasing pressure for both neat supercritical CO₂ and supercritical CO₂/ethanol. In both cases, taxol was more selectively recovered from the bark using supercritical extraction compared to the liquid extraction method. The CO₂/ethanol mixtures were the most selective of all, providing the highest percentage of taxol per weight of total extractants. Although the supercritical fluid extraction conditions were not optimized in this study, the ability of supercritical fluid extraction to provide a purer extract than that obtained with liquid extraction was demonstrated.

In addition, coextracted materials that were present in the supercritical fluid extract were more amenable to separation by liquid chromatography than the coextractants recovered via liquid extraction. This is of significance when one considers the economics for the total isolation of natural products. Recently, (King 1993b) has demonstrated that 85 percent recovery of taxol is possible from yew heartwood via SFE.

Monocrotaline is valuable as a precursor in the production of the semisynthetic pyrrolizidine alkaloids for use as anticancer agents (Gelbaum et al. 1982). It can be obtained from the seeds of *Crotalaria spectabilis*. The conventional method for isolating monocrotaline from *Crotalaria spectabilis* is quite difficult and expensive. Schaeffer et al. (1989a, 1989b) have demonstrated the extraction of monocrotaline from *Crotalaria spectabilis* using supercritical CO₂ with ethanol as a co-solvent.

By conducting experiments in the temperature-solubility crossover region, an attempt was made to find a set of conditions that would allow the selective separation of coextracted lipids from the monocrotaline (Schaeffer et al. 1988). A 22 percent increase in monocrotaline purity was obtained, however, the monocrotaline content of the total extracted mass was still only 49 percent.

Additional experiments were conducted (Schaeffer 1989b) to evaluate the feasibility of conducting SFE in conjunction with a bed of cation-exchange resin used for selectively trapping the alkaloid moieties in the presence of supercritical CO₂. By incorporating the ion-exchange column, the extracted lipid components can be removed by washing, leaving the monocrotaline behind for selective elution from the column (Gelbaum 1982).

To verify this, supercritical CO₂ modified with ethanol, and with water, was used in separate experiments, to extract *Crotalaria spectabilis* seeds. The experiments were conducted as follows; the seeds were extracted with the supercritical fluid, the solute-laden supercritical effluent was passed (still at system temperature and pressure) through the cation-exchange column (in the H⁺ form). The column effluent was then depressurized into a collection vessel, leaving the monocrotaline

adsorbed onto the cation-exchange resin. Some lipid material was also trapped on the resin, however, the cation-exchange column was then washed with ethanol:water 95:5 (v:v), which removed the adsorbed lipid materials. No monocrotaline was detected in the ethanol wash. The cation-exchange column was then washed with 1 N NH_4OH , thereby releasing the adsorbed monocrotaline.

Using both ethanol and the water modified supercritical CO_2 , monocrotaline purities of >95 percent were obtained. No lipid material was detected in the adsorbed fraction wash.

The significance of the above process lies in the applicability not only for the specific isolation of monocrotaline but to a wide variety of compounds from plants and other matrices that could be treated with SFE. It follows that this process could also be extended to the use of an anion-exchange resin for the removal of various acidic drugs from supercritical fluids.

The use of SFE in combination with ion-exchange columns for trapping extracted analytes was used to design a prospective process for the production of monocrotaline on the kilogram scale (Dyken et al. 1990). Computer modeling was performed to study the production process, utilizing data from Schaeffer's previous work (Schaeffer 1989b). Information was obtained related to predicted production levels, and an economic analysis was performed. It was concluded that SFE in conjunction with ion-exchange, affords a low-energy, moderate temperature recovery process, that provides an end product of high purity. The resulting information suggested that the final extracted product must have a high value in order for supercritical processing to be cost-effective.

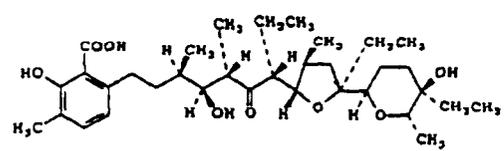
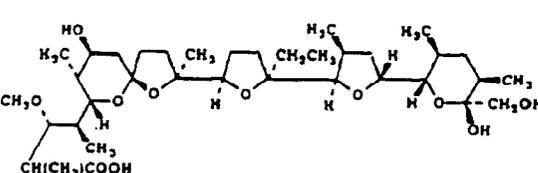
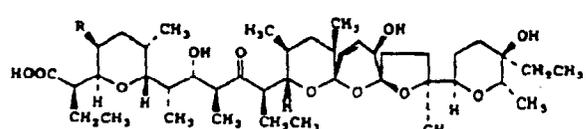
Vindoline and catharanthine are used as precursors in the biosynthetic pathways of high value dimeric indole alkaloids, such as vinblastine and vincristine, that are used in chemotherapeutic treatment of acute leukemia and Hodgkin's disease (Lee et al. 1992). However, Lee and coworkers, using only CO_2 , were able to demonstrate the successful extraction of both alkaloids. It seems clear that further studies including the utilization of modifiers would certainly result in similar results to other studies where alkaloid substances were successfully isolated. In fact, other alkaloids that have been isolated by SFE, include morphine and quinine (Ndiomu and Simpson 1988).

Antibiotics. *Penicillin.* Antibiotics present a unique challenge for SFE due to their structural attributes (i.e., high molecular weight, high polarity, low volatility, and thermal lability). The β -lactam antibiotics represent the largest group of commercially available antimicrobial agents, of which penicillin's are one chemical type. Penicillin V is the most widely used of the penicillin derivatives. The classical separation

and purification process for antibiotics includes a series of solvent extractions and precipitations culminating with crystallization of the product (Florey et al. 1949). Additional separation techniques such as ion-exchange, metal complexation, and chromatographic processes may be necessary for compounds which are difficult to crystallize. These purification processes can take up to 60 processing steps and account for as much as 80 percent of the expense of an antibiotic production operation (Ko et al. 1991). Supercritical fluid extraction could reduce the cost of the separation and purification process, by possibly extracting the antibiotic directly from the fermentation broth or by purifying the solid precipitate.

Polycyclic Ethers. The solubility of the sodium salts of the polycyclic ether class of veterinary antibiotics (Figure 4.2) have been examined (Maxwell et al. 1992). The purpose of the study was to see if SFE could be used in the analysis of incurred antibiotic residues in edible tissues. The solubility data obtained however is of value for separation and purification purposes in pharmaceutical production. The solubility of four polyether antibiotics (lasalocid, monensin, narasin, and salinomycin) were measured in neat CO_2 and cosolvent modified CO_2

Figure 4.2. The structure of the four polycyclic ether antibiotics and their equilibrium solubilities at 80°C in pure and modified CO_2 . (Source: Maxwell et al. (1992) with permission)

	Molecular Weight Sodium Salt	Equilibrium Solubility (mol/l) at 80°C, 400 bar
 <p style="text-align: center;">Lasalocid</p>	$\text{C}_{34}\text{H}_{53}\text{O}_5\text{Na}-612$	2.3×10^{-4} ($\text{CO}_2/1\% \text{ MeOH}$)
 <p style="text-align: center;">Monensin</p>	$\text{C}_{36}\text{H}_{61}\text{O}_{11}\text{Na}-692$	5.7×10^{-5}
 <p style="text-align: center;">R = CH_3, Narasin</p>	$\text{C}_{43}\text{H}_{71}\text{O}_{11}\text{Na}-786$	1.4×10^{-3}
<p style="text-align: center;">R = H, Salinomycin</p>	$\text{C}_{42}\text{H}_{69}\text{O}_{11}\text{Na}-772$	7.4×10^{-3}

(methanol and water) (Table 4.4). Attempts to correlate the molecular structure with solubility trends were frustrating due to lack of physicochemical data on the solutes.

Table 4.4. Solubility Parameters for the Polycyclic Ether Antibiotics in Modified and Pure Carbon Dioxide

Pressure (bar)	CO ₂ Density (mol/ℓ)	Solubility	
		Mol/ℓ	Mole Fraction
Lasalocid			
CO ₂ + 1% MeOH/80°C			
152	10.07	7.00×10^{-5}	6.95×10^{-6}
207	13.60	5.00×10^{-5}	3.68×10^{-6}
279	16.44	6.55×10^{-5}	3.98×10^{-6}
344	18.17	1.17×10^{-4}	6.44×10^{-6}
382	18.93	1.37×10^{-4}	7.22×10^{-6}
390	19.08	2.34×10^{-4}	1.23×10^{-5}
Monensin			
CO ₂ /80°C			
201	13.60	1.23×10^{-5}	9.04×10^{-7}
277	16.40	1.57×10^{-5}	9.57×10^{-7}
309	17.15	3.39×10^{-5}	19.77×10^{-7}
408	18.85	5.71×10^{-5}	30.29×10^{-7}
CO ₂ + 1% MeOH/80°C			
185	12.36	7.59×10^{-6}	6.14×10^{-7}
243	15.22	4.35×10^{-5}	2.86×10^{-6}
316	17.48	1.20×10^{-4}	6.87×10^{-6}
402	19.30	2.08×10^{-4}	1.08×10^{-5}
CO ₂ + H ₂ O ¹ /80°C			
182	12.01	7.68×10^{-6}	6.35×10^{-7}
242	14.49	8.02×10^{-6}	5.55×10^{-7}
316	16.30	1.43×10^{-5}	8.77×10^{-7}
401	17.71	2.54×10^{-5}	1.43×10^{-6}

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Solubility			
Pressure (bar)	CO ₂ Density (mol/l)	Mol/l	Mole Fraction
Narasin			
CO ₂ /60°C			
195	16.30	2.6×10^{-4}	1.59×10^{-5}
206	16.65	3.3×10^{-4}	1.98×10^{-5}
264	18.20	4.3×10^{-4}	2.36×10^{-5}
274	18.40	4.4×10^{-4}	2.39×10^{-5}
332	19.35	7.0×10^{-4}	3.62×10^{-5}
401	20.25	1.12×10^{-4}	5.53×10^{-5}
CO ₂ /70°C			
175	13.65	3.10×10^{-4}	2.27×10^{-5}
241	16.48	4.80×10^{-4}	2.91×10^{-5}
314	18.18	8.40×10^{-4}	4.62×10^{-5}
405	19.55	1.26×10^{-3}	6.44×10^{-5}
CO ₂ /80°C			
141	8.80	1.61×10^{-4}	1.82×10^{-5}
217	14.35	2.69×10^{-4}	1.87×10^{-5}
276	16.35	5.31×10^{-4}	3.24×10^{-5}
364	18.15	1.35×10^{-3}	7.43×10^{-5}
CO ₂ + H ₂ O ¹ /60°C			
181	14.81	3.9×10^{-4}	2.63×10^{-5}
184	14.94	5.7×10^{-4}	3.82×10^{-5}
243	16.88	2.23×10^{-3}	1.32×10^{-4}
311	18.34	4.48×10^{-3}	2.44×10^{-4}
401	19.72	6.29×10^{-3}	3.19×10^{-4}
Salinomycin			
CO ₂ /70°C			
185	14.23	2.42×10^{-4}	1.70×10^{-5}
249	16.73	9.86×10^{-4}	5.39×10^{-5}
318	18.25	2.37×10^{-3}	1.30×10^{-4}
391	19.38	2.92×10^{-3}	1.51×10^{-4}

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Pressure (bar)	CO ₂ Density (mol/ℓ)	Solubility	
		Mol/ℓ	Mole Fraction
CO ₂ /80°C			
142	8.90	2.32×10^{-4}	2.61×10^{-5}
202	13.60	6.35×10^{-4}	4.67×10^{-5}
256	15.80	1.77×10^{-3}	1.12×10^{-4}
273	16.30	2.05×10^{-3}	1.26×10^{-4}
318	17.35	4.71×10^{-3}	2.71×10^{-4}
395	18.65	7.36×10^{-3}	3.95×10^{-4}
CO ₂ + H ₂ O ¹ /60°C			
175	14.53	2.57×10^{-4}	1.77×10^{-5}
184	14.94	5.7×10^{-4}	3.82×10^{-5}
243	16.88	2.23×10^{-3}	1.32×10^{-4}
313	18.38	3.24×10^{-3}	1.76×10^{-4}
326	18.60	5.74×10^{-3}	3.09×10^{-4}
404	19.75	5.82×10^{-3}	2.95×10^{-4}

¹Water-saturated CO₂ (experimental)

Source: Maxwell et al. (1992) with permission.

Solvent Removal from Antibiotics. The production of antibiotics as well as in the synthesis of organic drug substances often require solvents to purify the final product. Purification of the final product also requires the removal of organic solvents used in their production. Traditionally this is accomplished by vacuum drying the final product; however, traces of the solvent can be left behind in the antibiotic. Experiments were conducted by Kamihira et al. (1987a) to investigate the potential for solvent removal from antibiotics by SFE using neat CO₂. The residual amounts of organic solvents left in penicillin G and streptomycin are shown in Table 4.5.

Differences in the final residual amount of solvent remaining in the antibiotics were observed to be dependent upon the specific compound. It appeared that the results obtained were due to differences in the affinity of each antibiotic for a specific solvent. Dramatic results were shown, even when a twofold weight ratio of solvent to antibiotic was used (sufficient to immerse the antibiotic). However, supercritical CO₂ was still effective in removing the solvent.

Water was also used as a cosolvent to increase the rate and extent of solvent removal. The extraction efficiency for the solvent approximately

Table 4.5. Relative Remaining Activity and Residual Amount of Solvents after Extraction with Supercritical CO₂*

Solvent	Penicillin G, Potassium		Streptomycin Sulfate	
	Relative Remaining Activity (%)	Residual Amount of solvent (g/g-dry matter)	Relative Remaining Activity (%)	Residual Amount of Solvent (g/g-dry matter)
Methanol	94	$<1 \times 10^{-5}$	101	9×10^{-5}
Ethanol	100	2×10^{-5}	99	4×10^{-5}
Acetone	100	5×10^{-5}	98	8×10^{-5}
Isopropyl alcohol	100	6×10^{-5}	97	7×10^{-5}
Ethyl acetate	103	$<1 \times 10^{-5}$	106	$<1 \times 10^{-5}$
<i>n</i> -Butyl acetate	ND	0.032	ND***	ND
<i>n</i> -Butyl alcohol	ND	0.222	ND	ND

*Extraction conditions: 35°C, 200 atm, 2 hr

**Initial amount of solvent: 2.0–2.2 g/g-dry matter

***ND: Not Determined

Source: Kamihira et al. (1987a) with permission

doubled, compared to the use of neat CO₂. Note, that the use of a supercritical fluid resulted not only in rapid removal of the organic solvent, but produced in most cases no loss in enzymatic activity.

Supercritical Fluid Chromatography

Potential Uses. As noted previously, the greater diffusivity of solutes in a supercritical fluid allows larger chromatographic resolution to be obtained, relative to that obtained using liquid chromatographic methods. Some process scale chromatography is accomplished using the normal phase mode, hence SFC using CO₂ could be a suitable replacement for the nonpolar solvents used in this mode of chromatography. In addition, the SFC literature indicates that many compounds of pharmaceutical interest can be chromatographed (Xie et al. 1992). Considering these factors, it is worthwhile to consider several select possibilities of applying SFC to compounds of pharmaceutical interest.

Chiral Separations. Chromatographic separation of enantiomers on the preparative and process scales to isolate optically pure compounds is becoming an increasingly important (Francotte and Junker-Buchheit 1992; Blum and Kumar 1994).

The cost and time required for developing a chiral synthetic method may be prohibitive, especially early in development when the final success of the project has yet to be determined. The investment of resources to develop and implement a chromatographic separation in order to produce pure chiral compound may provide savings in time and capital. SFC, using CO₂, has the potential to be a viable and lower cost alternative to LC in preparative and process scale chiral separations.

The unique selectivity and superior chiral resolution obtained on the analytical scale by SFC compared to LC is well documented for analytical separations (Lee et al. 1990; Brugger et al. 1991; Marti et al. 1991; Xie et al. 1992). Preparative separation of several enantiomers of optically active drug substances have been recently presented (Blum and Kumar 1994). The unique selectivity offered by SFC, documented for analytical-scale separations, was also observed in preparative-scale separations as well. A schematic of typical preparative SFC equipment that was utilized is shown in Figure 4.3. Preparative-scale separations were achieved for warfarin, propranolol, and another Merck-produced drug (Figures 4.4–4.6).

Additional Pharmaceutical Applications of Supercritical Fluids

Supercritical fluids are useful in other areas of pharmaceutical manufacturing besides separation and extraction alone. Two such

Figure 4.3. Schematic of preparative scale SFC equipment used for chiral separations. (Source: Blum and Kumar (1994) with permission)

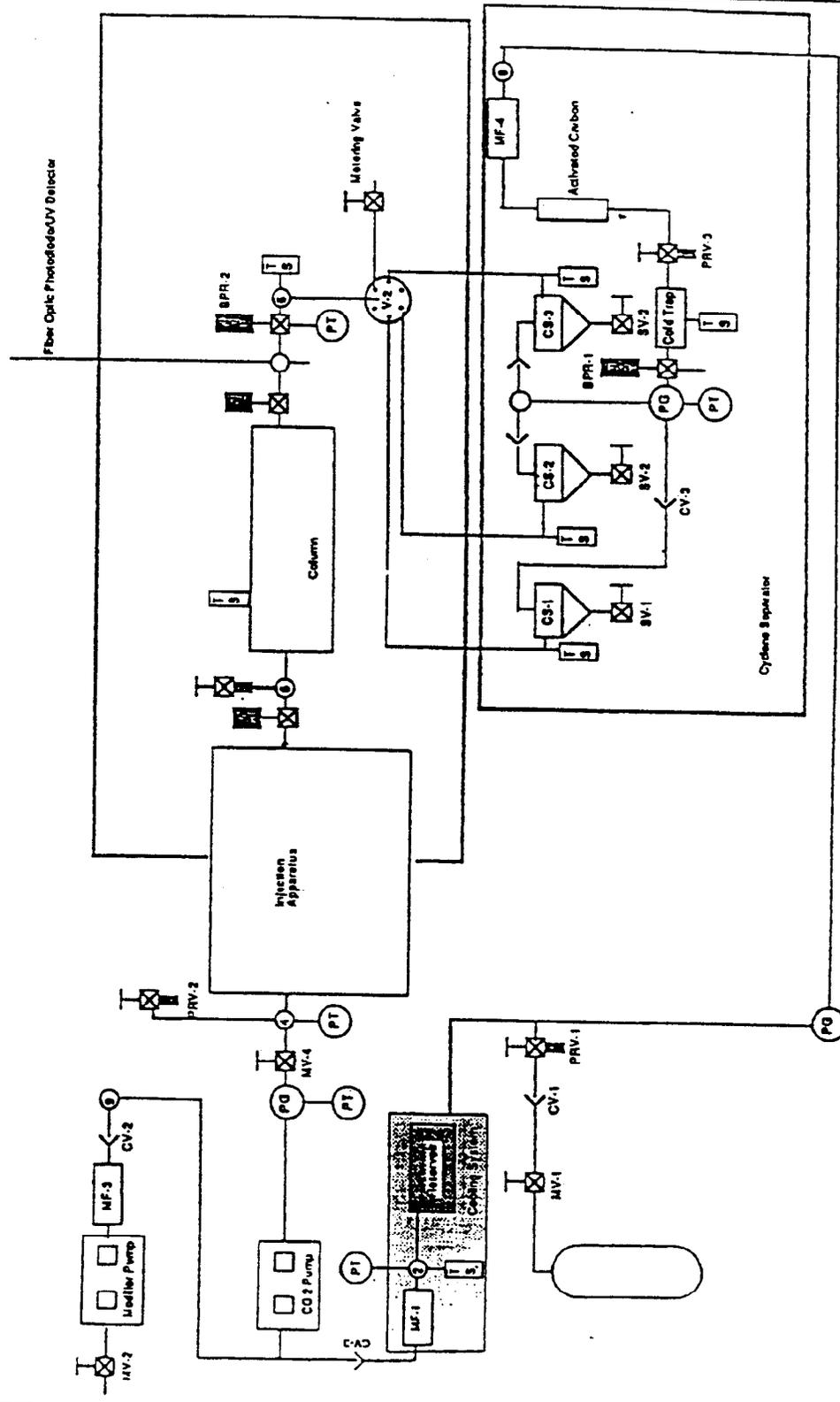
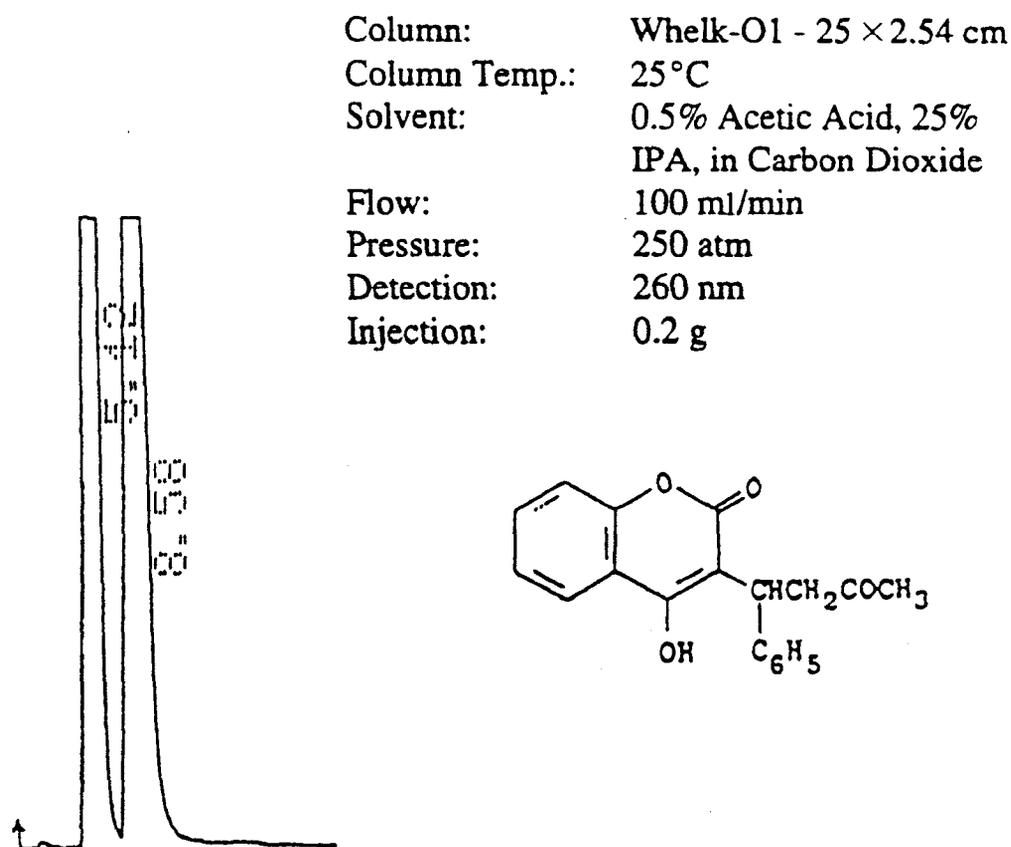


Figure 4.4. Preparative SFC of Warfarin. (Source: Blum and Kumar (1994) with permission)

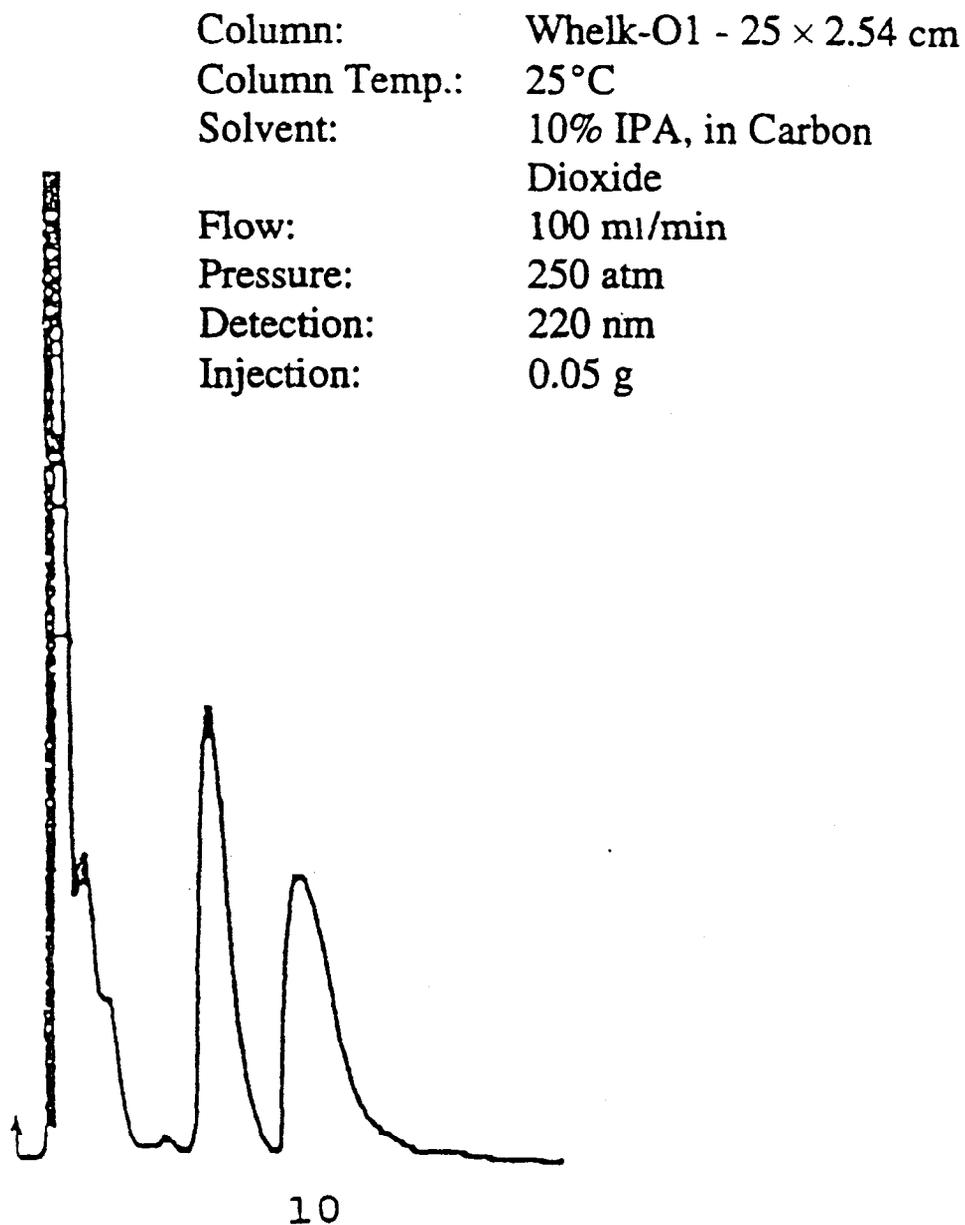


operations are described below to illustrate the diversity of supercritical fluid technology.

Micronization of Drug Delivery Particles. In order to obtain drug-loaded polymeric particles for the delivery of drug substances, processes must be developed involving their precipitation from organic solvents. In these methods, surfactants and bead suspending agents are utilized in organic solvents, that must then be removed from the microspheres, in order to allow their use. Methods currently used producing these microspheres, are spray drying and melt pressing, followed by micronization. However, both of these methods involve the application of heat which may affect the stability of drug substances.

A process known as rapid expansion of supercritical solutions (RESS) (Debenedetti et al. 1993; Srinivasan and Elliot 1992) can provide an alternative method for coprecipitating biodegradable polymers with drug substances. As noted previously, supercritical fluids exhibit no surface tension, hence they are able to "wet" particles more completely without the need for surfactants. This allows the supercritical

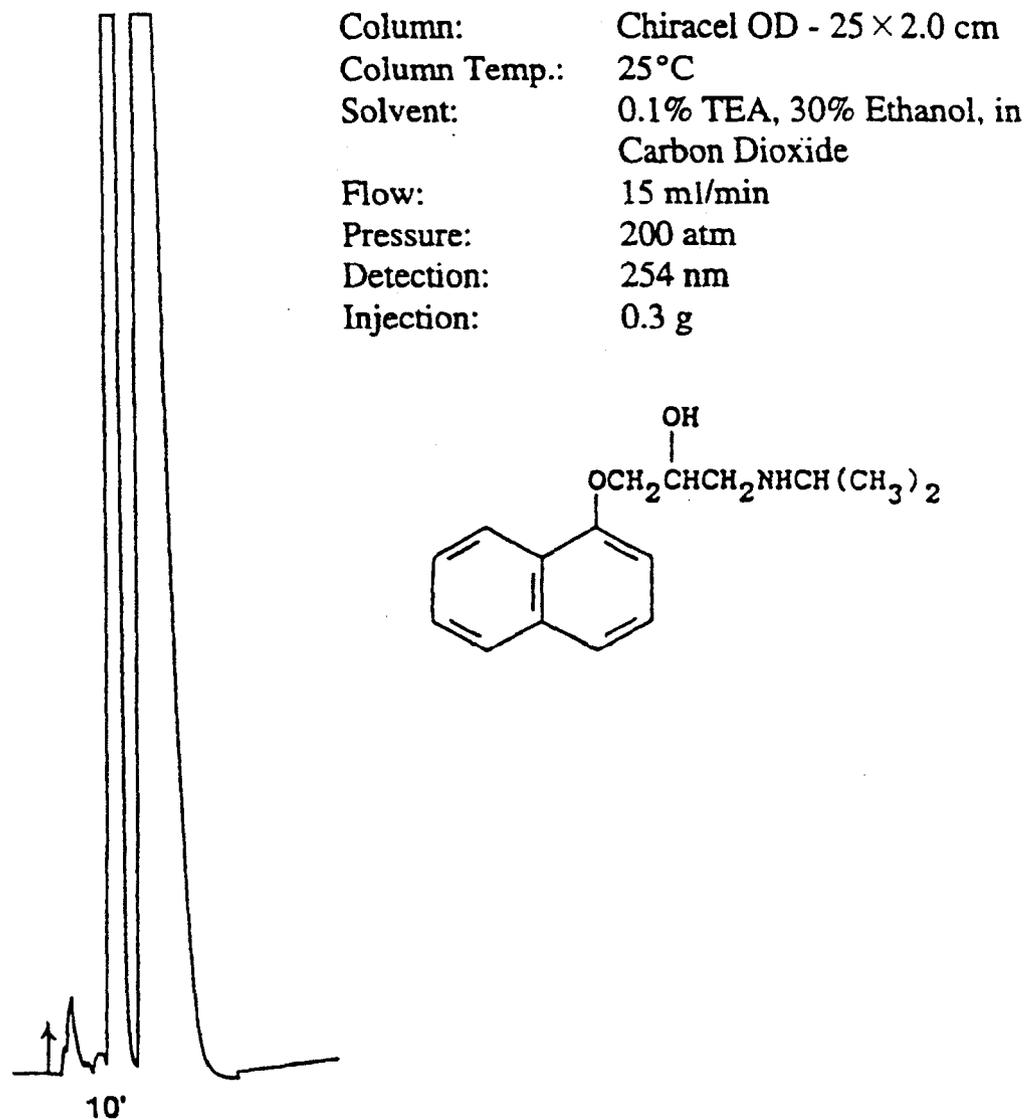
Figure 4.5. Preparative SFC of Propranolol. (Source: Blum and Kumar (1994) with permission)



fluid to effectively infuse a dissolved drug substance into the microspheres. The drug is then precipitated in situ by reducing pressure. By selecting supercritical fluids with sufficiently low critical temperatures, this process has the capability of producing high quality, solvent-free, drug-laden microspheres for biomedical use.

Sterilization. Supercritical fluids have also been applied in the sterilization of enzymes (Kamihira et al. 1987b) and blood plasma powder (Taniguchi and Suzuki et al. 1987b), as an alternative to the use of heat, ethylene oxide, radiation, microwaves, and other methods. These

Figure 4.6. Preparative SFC of DMPC. (Source: Blum and Kumar (1994) with permission)



methods compromise the integrity of biologically active products, and can cause degradation of the active components. Supercritical fluid treatment is capable of accomplishing sterilization, and conserving bioactivity while not degrading thermally labile compounds.

The above study examined the influence of supercritical CO₂ under both wet and dry conditions and with/without organic cosolvents. Moist SFE conditions were shown to be more effective than dry conditions, for the sterilization of the target microorganisms. This is illustrated by the data provided in Table 4.6. The addition of organic modifier caused a dramatic improvement in the results obtained under dry conditions, while under moist conditions, a further small

Table 4.6. Sterilizing Effect of Supercritical CO₂ at 200 atm and 35°C

Microorganism	Ratio of Living Cells	
	Wet Cells*	Dry Cells*
Baker's yeast	5.4×10^{-7}	0.50
<i>E. coli</i>	7.2×10^{-6}	0.047
<i>S. aureus</i>	1.5×10^{-5}	0.037
<i>A. niger</i> (conidia)	1.2×10^{-5}	0.88
<i>B. subtilis</i> (endospore)	0.47	0.99
<i>B. stearothersophilus</i> (endospore)	1.07	0.80

*Water content: wet cells, 70–90%; dry cells, 2–10%

Source: Kamihira et al. (1987b) with permission

improvement was observed when a cosolvent was used in conjunction with CO₂ vs. sterilization with neat CO₂ (Table 4.7). Table 4.8 shows the effect of sterilization on the activity of enzymatic preparations. Not only was sterilization shown to be effective, but activity was essentially preserved in each case.

Morphological examination of the microorganisms after sterilization indicated that some cells had burst, but the death of the cells did not seem to be due to cell disruption. Rather it was concluded that the death of the microbial cells was due to inactivation of some enzyme via a pH decrease and/or extraction of intracellular material, such as phospholipids (provided cosolvents were used, since phospholipids are negligibly soluble in CO₂). It was concluded that sterilization by supercritical CO₂ is applicable to biologically active and heat-sensitive products and holds promise as an alternative to current methods.

SUPERCritical PROCESS EQUIPMENT

Processing in high pressure extraction, can only be optimized from the thermodynamic point of view, if the relevant thermophysical properties are known (Hederer 1985). Unfortunately, there has been little impetus to experiment with SFC on this scale, due to the lack of theory or

Table 4.7. Effect of an Entrainer on Supercritical CO₂ Treatment^a

Microorganism	Ratio of Living Cells					
	Wet cells			Dry cells		
	CO ₂	CO ₂ + EtOH ^b	CO ₂ + AcOH ^b	CO ₂	CO ₂ + EtOH ^b	CO ₂ + AcOH ^b
<i>A. niger</i> (conidia)	1.2×10^{-5}	$<2.3 \times 10^{-6}$	$<7.9 \times 10^{-6}$	0.88	1.2×10^{-6}	$<1.6 \times 10^{-5}$
<i>B. subtilis</i> (endospore)	1.07	0.62	0.43	0.80	0.49	0.43

^aTreatment conditions: 200 atm, 35°C, 2 hr

^bEthanol (EtOH) or acetic acid (AcOH) was added to CO₂ at a weight ratio of 2% or 0.5%, respectively

Source: Kamihira et al. (1987b) with permission

Table 4.8. Sterilization of an Enzyme Preparation with Supercritical CO₂

Enzyme	Microorganism	Enzyme Activity (%)	Ratio of Living Cells
α-Amylase	<i>E. coli</i>	121	5.2×10^{-5}
	Baker's yeast	135	3.6×10^{-3}
Lipase	<i>E. coli</i>	88	8.9×10^{-5}
	Baker's yeast	78	4.7×10^{-3}

Treatment conditions: 200 atm, 35°C, 2 hr

Source: Kamihira et al. (1987b) with permission

models, whereas little can be done to model these processes, until more experimental information is available. For SFE the successful design and implementation of plants for decaffeination and hop extraction have provided the initiative for further investigation. This section will primarily concern itself with existing models for SFE processes in the literature.

Construction and Modeling

A supercritical fluid separation process according to Körner (1985) is comprised of a separation stage (with or without recycling of the supercritical fluid), a stage for the removal of the required extract, and a system for modifying pressure and/or temperature. The following questions need to be answered:

- Can thermodynamic data adequately predict solubility?
- Are laboratory tests required?
- Is a cosolvent needed?

A reputable and experienced supercritical fluid plant contractor should be engaged to be responsible for basic engineering, including design, in order to avoid accidents. It is recommended that prior to initiating construction, a contract manufacturer's pilot plant facilities be used to verify process parameters, confirm assumptions, and detect weak points. At that time the cost versus expected profit can be estimated.

Because of the diversity of products that can be extracted by SFE, production plants may vary in size (i.e., for highly valued pharmaceutical products extractors with volumes as small as 50 ℓ could be considered commercial scale). However (for example), in the removal of caffeine from coffee, the extractors would have to be several cubic meters in dimension. A point in reference is the General Foods plant in Houston, TX, which can process approximately 100 cubic meters of material (McHugh 1994).

Since most of the products to be extracted are often solids, continuous charging and discharging of the plant must be viable. Since there are no lock-hopper systems available for such high pressures, the product to be extracted must be admitted and discharged from the extractors at atmospheric pressure after extraction. Currently, most supercritical fluid processes are batch rather than continuous (Paulaitis et al. 1983). The challenge is to develop a semicontinuous mode of operation. Supercritical fluid extraction plants can operate with only one extractor, but most are equipped with several extractors to permit the batch process to be semicontinuous. Since most pharmaceutical production processes are themselves batch processes; this is not as critical as it might be in the chemical or food industries.

Some modeling as well as empirical information is available to evaluate process-scale supercritical fluid separations. For example, Eggers and Tschiersch (1980) have described the essential criteria underlying the design of a plant for the recovery of an extract from a supercritical CO_2 extraction of a natural product. Details of equipment and components as well as thermodynamic analyses and methods of optimization can be found in this reference.

Ramchandran et al. (1992) has examined the use of SFE process control, based on deviation from linear behavior for supercritical systems. Nonlinear process control (based on approximate models) uses adjustable parameters to mimic process behavior. This allows the engineer to look at the entire process in real time, using multivariable decoupling and process nonlinearities to select the appropriate control action. Computer programs have also been developed which use thermodynamic data in conjunction with mathematical models to simulate supercritical extraction, in lieu of pilot plant studies (Cesari et al. 1989).

A procedure has been developed by Colussi et al. (1992) that can be used with several equations of state for understanding the SFE process. In this procedure, the enthalpy balance along the extraction column is computed since small temperature changes in the supercritical fluid can significantly effect the resultant separation. Cubic equations of state, used for demonstrating this program, were constrained to pass through the critical point of the fluid. The resulting distortion of the isotherms near the critical point, using this program, suggest

that a much more complicated thermodynamic model is required. The program was demonstrated to be reliable; since simulation by this or any other model is dependent upon the thermodynamic model chosen to describe the chemical system.

Recently, Mukhopadhyay and Raghuram Rao (1993) have used a modified covolume-dependent (CVD) mixing rule to predict the solubility of mixed solids in pure and mixed supercritical fluids. The CVD mixing rule assumes that in a dilute supercritical mixture, the probability of a molecule interacting with another depends on the fraction of the surface it can "see" of the other molecule, rather than its mole fraction. Using the Peng-Robinson equation of state (EOS), solubility predictions could be performed from pure component solubility data. Because of the complex, highly compressible, and asymmetric nature of supercritical systems, a cubic EOS is required. Peng-Robinson EOS has been shown to predict as well as more complicated perturbed hard-sphere EOS and hence was selected as the model for the above study. The required pure component properties needed are van der Waals molecular volume, dipole moment, molecular weight, and molar volume. This predictive model was shown to be effective for the preliminary screening of process design parameters. Solubilities in modified CO₂, as well as crossover separation parameters were correctly predicted using this model.

In the industrial production of a chemical commodity, separation process can account for 40–70 percent of the production cost. Supercritical fluid extraction facilities usually involve large investments. For example, a new extraction plant for decaffeination of 32 m³/day of whole, green coffee beans can represent an investment in the range of 15 million European currency units (ECU). Nevertheless, operation costs of about 0.35 ECU/kg are acceptable (Leyers et al. 1991). There are two factors which significantly influence the investment cost for a supercritical plant.

1. The licensing royalties
2. The high pressures (1,000 to 6,000 psig) associated with supercritical fluid systems increase the capital investment significantly and only products selling for more than \$1/lb are likely to justify the manufacturing cost (Humphrey 1987)

Velo et al. (1994) showed that in some cases, standard equipment can be used, thereby making supercritical fluid a more reasonable alternative to conventional processes, provided plant design is optimized.

Additional energy is required for plants that require depressurization of the extractors in order for them to be charged and discharged.

Körner (1985) described a cascading system which minimized pressure loss, thereby reducing costs due to repeated repressurizations. Similarly, more energy is required when separation of the extract from the supercritical fluid is accomplished by reducing the pressure, rather than by means of modifying temperature, using water washing systems, or adsorption traps. Since most equipment necessary is well designed and has been tested under similar conditions, no additional technical risks are involved in designing and implementing an SFE plant. Because of compression costs, it is desirable to operate at as low a pressure as possible. However, it is important to remember that generally higher densities equate with higher solute solubilities outside the crossover region, resulting in less extraction time and lower cost. Also, operation in this pressure range, can lower production costs since higher solute concentrations will result in a reduced recycle rate of supercritical fluid.

Separation of the extract from the fluid phase is desirable by using as small a decrease in pressure as possible, to minimize recompression costs. It is preferred to keep pressure constant and alter temperature, but sometimes a pressure change is necessary. By determining the region where solute solubility is most effected by pressure, recompression costs can be minimized.

One advantage of SFE over conventional extraction with liquid solvents is the ease by which the extract can be separated from the solvent. The ratio of the saturated vapor pressure of the solvent to the solute is approximately 10^3 larger for the supercritical CO_2 /solute system over their liquid/solute counterparts. Therefore, it is possible to obtain a higher grade product, solvent free, in virtually a single extraction operation by SFE. Recycling the supercritical fluid can be used to improve separations. As components become concentrated in the fluid phase, they are transferred to a separator where a change in pressure and/or temperature causes a loss in solvating power and the extract is deposited. The discharge gas is recompressed and then recycled to the extraction vessel for reuse.

The National Institute of Standards and Technology (NIST) has reported an applied study which focusses on the economics of an SFE process. Called SEPSOL (Bruno 1992), it is applicable for the extraction of natural products from aqueous or wet matrices. In specific, β -carotene a high cost, and low volume chemical was justified economically. One limitation to this design, as with many other SFE designs is the batch mode of operation, resulting in additional costs compared to a continuous process. Naturally, the tendency would be to minimize the number of times the vessel is opened and maximize the length of the run and increase productivity. In the case of β -carotene, longer residence times would translate into degradation of

and make for a lower quality product. Alternatively, a lock-hoper flash tank may be considered as a viable option. Collection of the carotenoid product occurs in a stabilizing oil matrix directly, and hence is suitable for commercial use. In situ process analyzers were suggested to monitor solute concentration and quality and to provide automated process control.

The above examples on the utilization of SFE in commercial processes not only demonstrate its applicability, but show that the construction and implementation of supercritical fluid plants can be done in a manner that does not attenuate the cost of the resultant process or products. Hence, the value of supercritical extracts are sufficient to accommodate the high capital outlays, while still keeping the cost per unit of product, at least low enough to maintain a competitive edge in the market. However, it is not anticipated that supercritical fluid technology will always be able to provide a sufficient economic advantage in all the process cases, to justify implementation. Although much work has been accomplished in the area of supercritical separation processes, commercial success in the future will depend upon the reliable design, simulation, and scale-up operation of actual processing plants.

Safety

The safety of supercritical fluids for process-scale separations can be rationalized on the basis that they reduce the amount of highly flammable organic solvents which are used in conventional liquid extraction processes. Even with the use of small quantities of organic solvents as cosolvents, the pressure of CO₂ reduces the possibility of a flammable explosion. There is limited, if any, toxicity associated with the use of supercritical CO₂, if adequate ventilation is provided to prevent the possibility of asphyxiation, since the safe exposure limits for CO₂ are relatively high.

Due to the concomitant high pressures associated with the utilization of supercritical fluids in relation to pilot and commercial applications, it is prudent to consider the possible dangers and appropriate precautions which should be addressed to minimize loss potential. As in any other process, hazard analysis should be performed to detect all relevant failure paths for a given system. Since many supercritical processes are new, and limited data is available from which to create reliable models, non-traditional hazard analyses has been deemed appropriate (Randhava and Calderone 1985). Some fluids such as nitrous oxide, deserve careful examination, since its use in the presence of organic solutes represents a potentially explosive hazard (Raynie 1993).

CONCLUSION

Many current applications of SFE and SFC have been discussed in this chapter and potential applications have been identified. The successful implementation of this technology in the food processing industry suggests that the pharmaceutical industry should add SFE and SFC to their arsenal. The increases in the number of patents indicates a growing interest in these versatile solvents. More physicochemical data and improved modeling schemes (Rizvi 1994) are needed to assist the engineer/scientist in implementing this technology in industry. In summary, supercritical fluids as a process tool hold great promise for future applications in the pharmaceutical industry.

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