Supercritical Extraction of *Crotalaria spectabilis* in the Cross-Over Region

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Chimowitz and Pennisi (1986) have recently described a novel process for the separation and purification of components from mixtures of solutes in supercritical fluids. Their process was based on operation in the multicomponent temperature-solubility cross-over region. For a single solute, the cross-over point $(\partial y/\partial T)_P = 0$ represents the pressure at which there is a change in the temperature dependence of the solubility. Below this pressure, solubility is principally dependent on solvent density and a decrease in the temperature leads to an increase in the density and hence the solubility. Above this pressure, solubility is principally dependent on solute sublimation pressure. Raising the temperature leads to an increase in the sublimation pressure and therefore an increase in solubility. In the case of two solutes, each solute exhibits a cross-over point. If these points are at different pressures, the system temperature may be manipulated at an intermediate pressure causing one of the solutes to fall out of solution. Chimowitz and Pennisi demonstrated that this effect could be used to separate mixtures of 1,10-decanediol and benzoic acid using supercritical carbon dioxide. They also recommended a processing scheme for the separation of multicomponent mixtures, but did not study any multicomponent systems experimentally.

We have recently studied the extraction of monocrotaline (Schaeffer et al., 1988), a pyrrolizidine alkaloid of chemotherapeutic interest, from the seeds of *Crotalaria spectabilis* using supercritical carbon dioxide and carbon dioxide-ethanol mixtures. In our study, the crushed seeds of *Crotalaria spectabilis* were extracted at temperatures ranging from 308.15 to 328.15 K and at pressures ranging from 8.86 to 27.41 MPa with carbon dioxide containing up to 10 mol % ethanol. Our results indicated that the single-stage supercritical extraction process could not be used to isolate monocrotaline since the lipid material is always extracted with the monocrotaline. However, if the extract is assumed to consist of two components only—namely, monocrotaline and lipid material, crossover regions at different pressures could be identified in our work, Figure 1. The present work was, therefore, undertaken to examine the feasibility of isolating pure monocrotaline from a complex extract using cross-over phenomena. It differs from the work of Chimowitz and Pennisi (1986) in that the solute is a multicomponent system consisting of the polar monocrotaline and a series of nonpolar lipids.

Experimental

The apparatus used in this investigation is shown schematically in Figure 2. It has been described in detail in our previous work (Schaeffer et al., 1988). Pressurized carbon dioxide was filtered, liquified in an ice bath, and fed into an Eldex dual-head metering pump. The cosolvent from a 250 mL graduated cylinder was also filtered and entered the Eldex pump. Both the solvent and cosolvent were pumped to the desired system pressure and fed into an extraction vessel which was packed with alternating layers of plant material and glass beads and immersed in the primary constant temperature bath. Equilibrium between the supercritical phase and the plant material was achieved in this vessel.

Unlike our previous study, the mixture in the present study then passed through a second constant temperature bath—an Exacal EX-100 UHP constant temperature bath, where the temperature was controlled and monitored. The section of tubing immersed in the second bath could be isolated to determine the composition of the material deposited due to the temperature change. The undeposited material was then depressurized in a heated micrometering valve and the solute and a portion of the cosolvent were collected in a collection vessel immersed in an ice bath. The depressurized gas then passed successively through a Perkin-Elmer flame ionization gas chromatograph (for determination of the gas composition), and through a Precision Scientific wet test meter (for gas volume totalization). The extracts were quantitatively analyzed for monocrotaline content using a proton NMR technique developed by Molyneaux et al. (1979). From the measurements of gas volume, cosolvent concentration, total mass of solute and monocrotaline mass, the

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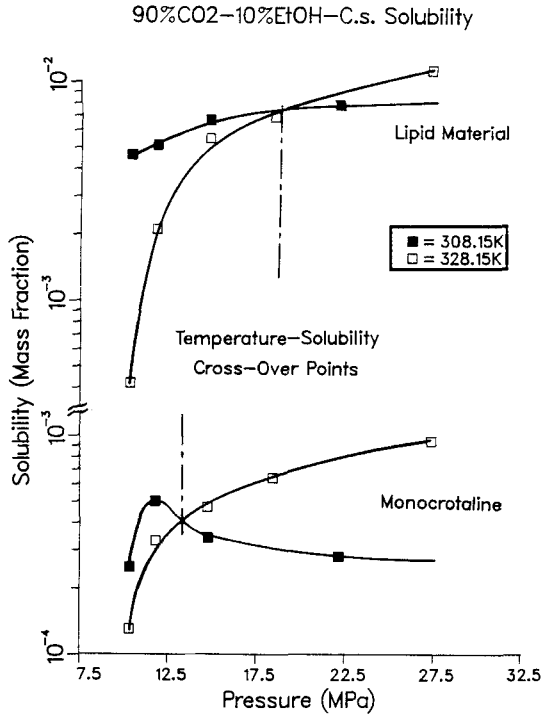

Figure 1. Monocrotaline and lipid material solubility in the extraction of *Crotalaria spectabilis* with 90% carbon dioxide–10% ethanol versus pressure.

solubilities of monocrotaline and of the lipid material (by difference) could be calculated.

Results and Discussion

Typical results from our study of the carbon dioxide–ethanol–*Crotalaria spectabilis* system are shown in Figure 1. In Figure 1, the solubilities of monocrotaline and the lipid seed material (considered as a single pseudocomponent) in carbon dioxide–ethanol mixtures (90 mol % carbon dioxide–10 mol % ethanol) are plotted as a function of pressure at 308.15 and 328.15 K. As expected, the monocrotaline and lipid solubilities increase with increasing pressure and ethanol concentration. However, at 308.15 K, the monocrotaline solubility reaches a maximum at 12 MPa and decreases thereafter. This is unlike the behavior observed in binary and ternary systems and may be a consequence of the complexity of the carbon dioxide–ethanol–*Crotalaria spectabilis* system (Schaeffer et al., 1988). The lipid material exhibits a cross-over point for the two isotherms at 17.0

Table 1. Equilibrium Solubilities of Monocrotaline and Lipid Material Extracted from *Crotalaria spectabilis* Using 90 mol % Carbon Dioxide–10 mol % Ethanol Mixtures

Temperature (K)	308.15	328.15
Pressure (MPa)	14.77	14.77
Ethanol Concentration	10.0	10.0
Mass Fraction Lipids in Extract $\times 10^4$	67.1	55.3
Mass Fraction Monocrotaline in Extract $\times 10^4$	3.4	4.7

MPa, whereas the monocrotaline exhibits a cross-over point at 13.0 MPa. Therefore, at say 15.0 MPa, a decrease in the temperature from 328.15 to 308.15 K should cause pure monocrotaline to be deposited from the supercritical fluid stream. Our experimental results are presented in Tables 1 and 2. Table 1 shows the equilibrium solubilities of monocrotaline and lipid material in a mixture of 90 mol % carbon dioxide–10 mol % ethanol at 14.77 MPa and at two temperatures (308.15, 328.15 K). The yield–purity characteristics for a deposition process based on a temperature change from 328.15 to 308.15 K at 14.77 MPa are presented in Table 2. It is obvious that, although the actual and calculated yields of monocrotaline are in satisfactory agreement in spite of the complexity of the system, a considerable amount of lipid material is also deposited. The reason for this is obvious. The lipid material is a mixture of many components, each of which has a cross-over point. A fractionation of the lipid material occurs when the temperature is changed from 328.15 to 308.15 K. Nevertheless, the deposition process is able to enhance the purity of the monocrotaline by a factor of 1.86, Table 2.

An alternative to the deposition process is a process in which the temperature is raised in the second stage to deposit the lipid

Table 2. Yield and Purity Enhancement Factors for Monocrotaline and Lipids in Supercritical 90 mol % Carbon Dioxide–10 mol % Ethanol Mixtures at 14.77 MPa for a Temperature Decrease from 328.15 to 308.15 K

	Expected	Experimental
Yield of Monocrotaline*	1.3×10^{-4}	1.5×10^{-4}
Yield of Lipids*	0	9.5×10^{-4}
Purity Enhancement Factor of Monocrotaline in Solid Phase**	∞	1.86
Concentration of Monocrotaline in Solid Phase after Temp. Change (Mass %)	100.0	13.6

*Yield = mass of solid deposited/mass of fluid phase
**Purity enhancement factor = ratio of mass of monocrotaline/mass of lipids in the deposited phase to that in the feed

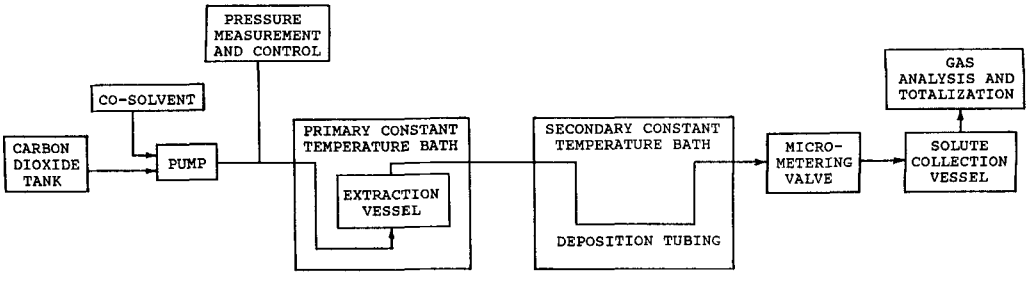

Figure 2. Schematic diagram of the apparatus used in this study.

Table 3. Yield and Purity Enhancement Factors for Monocrotaline and Lipids in Supercritical 90 mol % Carbon Dioxide–10 mol % Ethanol Mixtures at 14.77 MPa for a Temperature Increase from 308.15 to 363.15 K

	Expected*	Experimental
Yield of Monocrotaline	0	0.2×10^{-4}
Yield of Lipids	$>11.8 \times 10^{-4}$	64×10^{-4}
Purity Enhancement Factor of Monocrotaline in Supercritical Phase**		18.7
Concentration of Monocrotaline in Supercritical Phase after Temp. Change (Mass %)		48.6

*The expected values were calculated assuming a monotonic relationship between solubility and temperature.

**Purity enhancement factor = ratio of mass of monocrotaline/mass of lipids in the supercritical fluid after deposition to that before deposition

material. Operating the extraction vessel at 308.15 K and the deposition bath at 363.15 K, resulted in the yield–purity characteristics presented in Table 3. The deposited material was analyzed and found to contain very little monocrotaline. If a linear relationship between monocrotaline solubility and temperature is assumed over this small temperature range, the deposited phase should contain lipid material only. The presence of a small amount of monocrotaline as shown in Table 3 could be due to a change in the monocrotaline solubility–temperature relationship with increasing temperature. The purity enhancement for monocrotaline in the supercritical phase was found to be 18.7 (or ten times greater than that found in the previous case). The advantage of this process is therefore that the supercritical stream can be depressurized to yield a product which is 49% monocrotaline by mass.

Conclusions

Utilization of the temperature-solubility cross-over region exhibited by solutes in supercritical fluids is a promising isolation technique in multicomponent separations. However, when the substrate is a complex biological material consisting of a large number of components, multiple separations may be necessary to isolate pure components. In addition, the location of the deposition region will require a great deal of experimentation with the system of interest.

Literature Cited

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