EQUILIBRIUM SOLUBILITIES OF β-CAROTENE IN SUPERCRITICAL CARBON DIOXIDE

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ABSTRACT

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Equilibrium solubilities of pure crystalline β-carotene in supercritical CO_2 have been measured using an LDC Analytical SPA extractor **. The apparatus features a recirculating loop with an in-line UV detector. Quantitation by UV absorbance permits the detection of lower solubilities than can normally be obtained gravimetrically. The values reported were obtained at temperatures from 40 to 70 °C and pressures from 200 to 500 bar. The measured mole fractions ranged from 9.0×10^{-9} at 40 °C and 275 bar to 1.9×10^{-6} at 70 °C and 400 bar. Solubilities are also reported for β-carotene in mixtures of CO_2 and 1 wt.% ethanol, 1 wt.% methanol and 1 wt.% methylene chloride, at 70 °C and various pressures. Addition of the cosolvent increased the solubility in every case, with the largest increase occurring in a mixture of CO_2 and 1 wt.% ethanol. Pure component properties for β-carotene were estimated, and the experimental data were correlated to a modified Peng–Robinson equation of state. The model predictions compare well with the experimental data.

INTRODUCTION

β-Carotene is a lipid-soluble yellow- to orange-red pigment present in the flowers, fruit and roots of plants as well as in fungi and bacteria. This large organic molecule has a molecular weight of 536.9 and is illustrated in Fig. 1. It is a precursor to vitamin A in human and animal metabolism. β-Carotene is used in the food processing industry to impart a uniform color and some vitamin A activity to a wide variety of food products. In addition, recent clinical studies have indicated that β-carotene may play a significant role in the prevention of many forms of cancer (Menkes et al., 1986). Such findings may help to increase the market for this compound as a food additive, since the public perception of food products enriched with cancer preventatives should be very positive.

At the present time, the bulk of the β-carotene used commercially is manufactured by chemical synthesis. However, plant materials containing large amounts of β-carotene, such as carrots and palm oil, offer the possibility of obtaining "natural" β-carotene through extraction with non-toxic, supercritical CO_2. In addition, industrial production of β-carotene by fermentation has been reported, the β-carotene being isolated by a process of solvent washings and crystallizations (Ninet and Renaut, 1979). Alternatively, extraction with supercritical CO_2 may allow the isolation of "natural" β-carotene directly from the fermentation broth.

Data pertaining to the solubility of β-carotene in supercritical CO_2 are limited. For example, Favati et al. (1988) have studied the extraction of carotene and lutein from alfalfa leaf protein concentrates with supercritical CO_2. Using a dynamic single-pass system, they investigated the extractability of carotene at 40 °C in the pressure range 100–700 bar. Stahl et al. (1988) have also investigated the extraction of carotenoids from tomatoes, paprika, carrots and powdered algae, using an in-line microextractor coupled with thin-layer chromatography. They performed the experiments with supercritical CO_2 and trifluoromethane at 40 °C and various pressures. In both of

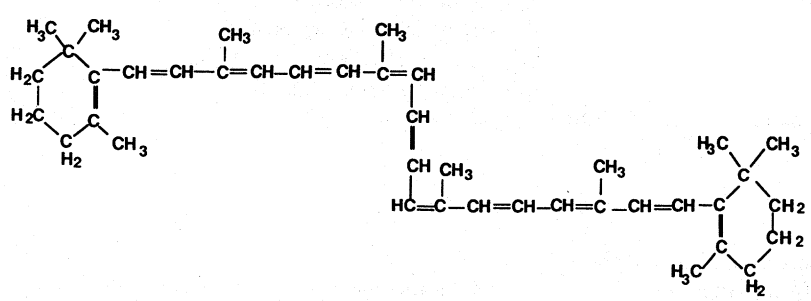

Fig. 1. Molecular structure of β-carotene.

these studies, however, the natural products were used as substrates, in which carotenoids are present in small amounts. Also, lipid-like materials present in the natural products, highly soluble in CO_2, may act as entrainers and actually enhance the solubility of β-carotene over its true equilibrium value.

This study was therefore initiated to measure the equilibrium solubility of pure crystalline β-carotene in supercritical CO_2, and in CO_2 modified with small amounts of various cosolvents. Solubilities were measured at temperatures in the range 40–70 °C and pressures in the range 200–450 bar, conditions appropriate for the extraction of β-carotene from natural products. These measurements were incorporated into a thermodynamic model, as reported herein, and the model was used in the design optimization of an SCE process to extract β-carotene with supercritical CO_2 (Cygnarowicz and Seider, 1990). Optimal designs were obtained that are economically promising.

EXPERIMENTAL

Extraction procedure

The solubility measurements were made using a Sample Preparation Accessory (SPA), manufactured by LDC Analytical (Riviera Beach, FL), modified as shown in Fig. 2. Carbon dioxide (SFC grade, Scott Specialty Gases, Plumsteadville, PA) from the supply tank was cooled to a liquid state and then compressed to the desired pressure by a constant pressure pump. The compressed CO_2 was preheated to the desired temperature before being introduced into the oven. When the system was charging the CO_2 flowed from the loop control valve through the recirculation pump and switching valve and into the extractor, which had been loaded with β-carotene (Type IV, crystalline from carrots, Sigma Chemical Co., St. Louis, MO). The extractor was a 5 ml 316 stainless steel cup with a porous bottom and lid with frit sizes of 2 or 5 μm. After leaving the extractor, the CO_2 stream flowed through a high pressure cell of a variable wavelength UV monitor.

Once the desired pressure in the oven had been reached, the extraction loop was isolated from the supply tank by turning the loop control valve, the recirculation pump was activated, and the fluid was allowed to flow through the closed loop. The progress of the extraction was monitored by the UV detector, which was set to a wavelength of 466 nm for β-carotene. The absorbance could not be monitored at the UV maximum (450 nm), as the detector in the SPA would saturate at higher pressures. The solute and solvent were equilibrated when the UV absorbance reading leveled off. The value was recorded, and the pressure adjusted to the next set point. Then

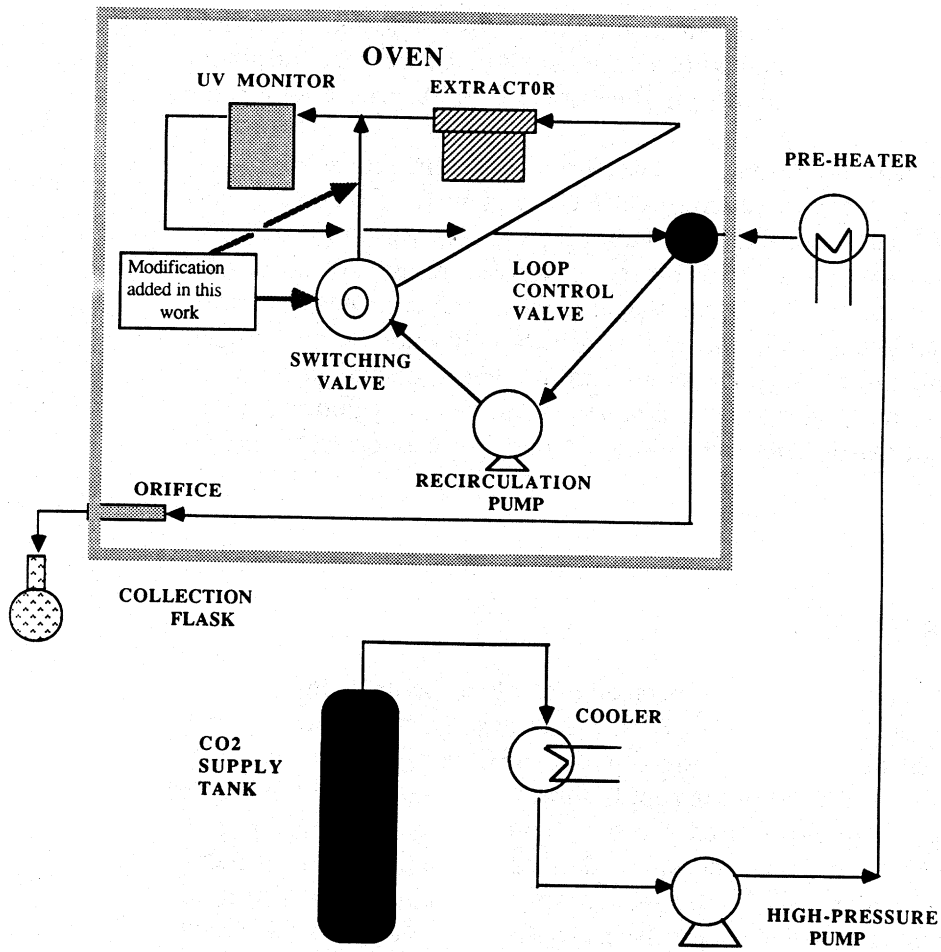

Fig. 2. Experimental apparatus used to measure solubilities in supercritical CO_2.

either the same procedure was repeated, or the solute collection process, described in the next section, was initiated.

The absorbance readings from the UV detector in the oven had to be corrected for the change in density (and refractive index) of the CO_2 with changes in pressure. This was accomplished by zeroing the monitor with no sample in the extractor and the CO_2 at tank pressure, and recording the change in the absorbance reading as the pressure was increased. A polynomial relationship was established between the change in the baseline and the pressure, and a correction was added to the absorbance readings recorded for the β-carotene/CO_2 mixtures.

To validate the absorbance measurements obtained with the UV detector in the SPA, several experiments were conducted in which the solute was collected after equilibrium had been established. In this procedure, after the solute and solvent had equilibrated, the switching valve was turned so as to bypass the extractor, and the loop control valve was opened to the orifice. In this mode, fresh CO_2 entered the loop to sweep out the CO_2/β-carotene stream at a constant pressure.

After it had been passed through the heated orifice, the CO_2/β-carotene stream was bubbled into a 10 ml volumetric flask which was half full of hexane (UV grade, Burdick and Jackson, Muskegon, MI). At the orifice, the resulting drop in pressure caused the CO_2 to evaporate and the β-carotene to precipitate into the hexane. During the collection procedure, the absorbance reading on the SPA was again monitored as it decreased on replacement of the β-carotene/CO_2 stream with fresh CO_2. Once the absorbance had dropped to its zero level, the loop control valve was temporarily returned to the position for charging the loop, and the solvent between the switching valve and the entrance to the extractor was flushed. The absorbance rose quickly, and returned to zero as the remaining CO_2/β-carotene mixture was eluted. At this point, the solute collection process was terminated and the loop control valve returned to the position for charging the loop. The extraction pressure was adjusted to the next set point, and the process was repeated. The amount of solute collected was determined by the solute quantitation procedure described below.

Solute quantitation

A calibration curve for β-carotene in hexane was determined by preparing solutions of known concentration and reading their absorbance using a Beckman DU-70 spectrophotometer. The concentration was a linear function of the absorbance, indicating adherence to the Beer–Lambert law. The molar extinction coefficient for β-carotene in hexane was determined to be 8.16×10^4 (1000 cm^2 mol^{-1}). This compares favorably with the literature values, which range from 10.1×10^4 to 13.4×10^4 (Zechmeister and Polgar, 1943).

To convert the absorbance readings given by the UV detector in the SPA to concentrations, it was assumed that the absorbance of β-carotene in CO_2 is similar to that in hexane. Thus, the molar extinction coefficient described above was used in the Beer–Lambert law to determine the concentration of β-carotene in CO_2. (The cell pathlength was 0.2 cm.)

TABLE 1

Critical points for CO_2 mixtures [a]

Solvents	T_c (°C)	P_c (bar)
Pure CO_2	31.2	73.7
1 wt.% methanol	27.7	77.9
1 wt.% ethanol	27.1	78.8
1 wt.% methylene chloride	33.6	74.7

[a] Computed by the method of Heidemann and Khalil (1980) using the SRK equation of state.

The collected solute was quantified by diluting the β-carotene/hexane solution to a known volume and reading its absorbance. The UV absorbance indicated the moles extracted, and since the volume of the extraction loop was factory calibrated as 16.5 ml, calculation of the concentration (mol l^{-1}) of β-carotene in CO_2 was straightforward.

Extraction with CO_2/cosolvent mixtures

To investigate the effect of the addition of cosolvents on the β-carotene solubility, cylinders of CO_2 with 1 wt.% methanol, 1 wt.% ethanol and 1 wt.% methylene chloride were obtained (SFC grade CO_2, Scott Specialty Gases, Plumsteadville, PA). The critical pressures and temperatures of the mixtures were calculated using the Soave–Redlich–Kwong equation of state, with interaction coefficients from Reid et al. (1977), and the method of Heidemann and Khalil (1980) to ensure operation in the supercritical region. The values thus computed are shown in Table 1. The β-carotene extraction procedure with the mixed solvents was the same as that described for pure CO_2.

Since the solubility of β-carotene in all of the CO_2 mixtures is sufficiently high to saturate the UV monitor in the SPA, only the solute collection procedure could be used to quantify the solubility. When methanol was used as the cosolvent, it formed a second liquid phase in the solute collection vessel. The β-carotene remained in the hexane phase, and hence was easily separated from the methanol. Ethanol and methylene chloride are miscible with hexane, but these cosolvents did not pose a problem since their absorbance cut-offs are well below the maximum absorbance of β-carotene.

RESULTS AND DISCUSSION

The SPA device was designed principally to prepare analytic concentrates for subsequent analysis by HPLC (Nair and Huber, 1988), but with minor

modifications it is well suited to the measurement of solid equilibrium solubilities. It offers two features that are particularly advantageous for solubility measurements: a recirculating loop, and an in-line UV detector.

The recirculation loop ensures adequate contact time between the solvent and the solute. Many experimental solubilities are obtained using the "dynamic" method (McHugh and Krukonis, 1986) in which the solvent flows through the solute in a single pass. However, unless the solvent flow rate is extremely low, the system may not equilibrate, and the solubilities measured will be below their equilibrium values. The in-line UV detector allows the progress of the extraction to be monitored, and the contact time for equilibrium to be accurately established. This is an improvement over other recirculation methods (King et al., 1983), in which the time needed to reach equilibrium is determined by a trial and error procedure.

As originally designed, however, the SPA cannot be used to collect the solute after equilibrium is established without further extracting the solute from the sample cell. In its original configuration, when removing the solute/solvent stream, the fresh solvent flows through the extractor, where additional β-carotene is extracted. To overcome this limitation, a switching valve and bypass tee were added to the instrument, enabling fresh solvent to enter the system and elute the solvent/solute stream at a constant pressure without contacting the solute (Fig. 2). This modification allowed the absorbance readings taken from the UV monitor in the SPA to be verified.

A comparison of the results from the absorbance readings of the UV detector in the SPA with those obtained by the solute-collection procedure indicates good agreement at high pressures. There is poor agreement, however, at low pressures. The errors are probably due to the extremely small amounts of solute collected at lower pressures (e.g. 26.8×10^{-6} g at 200 bar and 70 °C). Even minute losses during collection have a substantial impact on the error. Unless otherwise noted, the values reported herein were obtained from absorbance readings of the UV monitor in the SPA.

These studies were carried out at 40, 60 and 70 °C and at pressures between 200 and 500 bar. The measured concentrations of β-carotene were converted to mole fractions using the Schmidt–Wagner equation for the density of CO_2 (Bruno and Svoronos, 1989). These values are presented in Table 2 and plotted in Fig. 3. At least two experiments were run at each temperature, and the relative errors ranged from 10 to 60%. The larger errors were obtained at the low pressures, where only small amounts of β-carotene were solubilized. The results demonstrate that the mole fractions are low (2.54×10^{-6}), even under the most favorable conditions (70 °C and 439 bar). Structurally, β-carotene is an unsaturated hydrocarbon chain bounded on either end by cyclohexene rings (see Fig. 1). The lack of polar moieties in the molecular structure would suggest that the solubility of β-carotene in

TABLE 2

Mole fractions of β-carotene in supercritical CO_2 at 40, 60 and 70 °C

Temperature (°C)	Pressure (bar)	CO_2 density (mol l^{-1})	$(Y_b \times 10^7)$ [a]
40	275	20.38	0.090
	314	20.86	0.223
	384	21.59	0.658
	415	21.87	1.02
60	236	17.55	0.429
	273	18.45	0.724
	309	19.02	0.954
	348	19.50	1.52
	373	19.93	2.72
70	212	15.51	1.95
	249	16.73	3.33
	287	17.65	6.23
	328	18.43	10.00
	358	18.91	12.50
	400	19.49	19.10
	439	19.95	25.4

[a] Y_b is the mole fraction of β-carotene in CO_2.

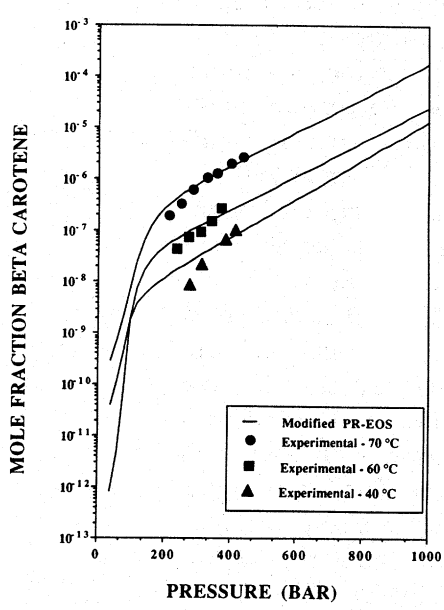

Fig. 3. Mole fractions of β-carotene in supercritical CO_2 as a function of pressure at 40, 60 and 70 °C. Experimental data and theoretical estimates.

TABLE 4

Enhancement factors E at 70 °C

Solvents	CO_2 density (mol l^{-1})	
	17	19
1 wt.% ethanol	4.7	2.4
1 wt.% methylene chloride	3.5	1.9
1 wt.% methanol	2.1	1.8

where Y_t is the mole fraction of solute in the CO_2/cosolvent mixture, and Y_b is the mole fraction of solute in CO_2. The enhancement factor for cholesterol was shown to range from 2.4 using 3.5 wt.% ethanol to 7.2 using 3.5 wt.% methanol.

The results of the present study of the solubility enhancement of β-carotene in CO_2 with 1 wt.% methanol, 1 wt.% ethanol and 1 wt.% methylene chloride are shown in Table 3. In these experiments, the measurements were made at 70 °C and pressures of between 180 and 375 bar. Solubility increases were obtained with each cosolvent over the entire pressure range. At the highest densities reported (approximately 19 mol l^{-1}), the mole fractions varied from 3.75×10^{-6} for CO_2 with 1 wt.% ethanol to 2.77×10^{-6} for CO_2 with 1 wt.% methylene chloride.

Enhancement factors, shown in Table 4, were computed at solvent densities of 17 and 19 mol l^{-1} by interpolation of the data in Table 3. At the low density, the enhancement factor is largest for ethanol (4.7) and smallest for methanol (2.1). At the higher density, ethanol continues to show the greatest enhancement, but the values for methanol and methylene chloride are nearly identical.

The solubility enhancement is due to complex interactions between the β-carotene, the cosolvents, and CO_2. Dobbs et al. (1987) have demonstrated that the solubility in supercritical media is related to dipole and inductive forces, and acidity and basicity, among other factors. However, without further experimentation, quantitative explanations cannot be given for the differences in enhancement among the cosolvents studied.

Finally, it is important to consider the stability of the β-carotene samples used in these experiments. β-Carotene undergoes a *cis* isomerization reaction which is accelerated by heat and light, with the *cis* isomer impurities observed to induce a melting point decrease and an additional absorbance peak in the UV region (Marty and Berset, 1986). However, the presence of the *cis* isomer does not significantly affect the location and size of the UV maximum. Although the laboratory samples have undergone some isomerization, since all-*trans* β-carotene is very unstable, commercial crystals con-

TABLE 3

Mole fractions of β-carotene in CO_2/cosolvent mixtures at 70 °C

Pressure (bar)	CO_2 density (mol l^{-1})	(Y_b or $Y_t \times 10^7$) [a,b]
β-Carotene–CO_2		
212	15.51	1.95
249	16.73	3.33
287	17.65	6.23
328	18.43	10.00
358	18.91	12.50
400	19.49	19.10
439	19.95	25.4
β-Carotene–CO_2–1 wt.% ethanol		
223	15.92	9.6
249	16.73	19.5
316	18.22	25.2
374	19.14	37.5
β-Carotene–CO_2–1 wt.% methylene chloride		
234	16.28	12.8
247	16.67	13.3
312	18.16	21.7
370	19.08	27.7
β-Carotene–CO_2–1 wt.% methanol		
180	13.92	3.98
268	17.26	9.62
330	18.47	15.60
373	19.12	30.60

[a] Y_b is the mole fraction of β-carotene in CO_2.
[b] Y_t is the mole fraction of β-carotene in the CO_2/cosolvent mixtures.

supercritical CO_2 should be fairly high. However, although the compound is non-polar, its molecular weight is large (536.9), a factor known to inhibit solubility in supercritical CO_2 (Dandge et al., 1985).

Because of the low solubility measured for β-carotene in supercritical CO_2, further experiments were conducted with mixtures of CO_2 and various cosolvents. The use of small amounts of cosolvents to increase the solubility of solutes other than β-carotene has been investigated (Joshi and Prausnitz, 1984; Wong and Johnston, 1986; Dobbs et al., 1987). Generally, it has been found that the solubility increases significantly with the addition of cosolvents, the increase being dependent on the cosolvent and solute. For example, Wong and Johnston (1986) measured the solubilities of cholesterol, stigmasterol and ergosterol in supercritical CO_2 and computed the enhancement factor E:

$$E = \frac{Y_t}{Y_b}$$

tain some degradation products. In this respect, the samples used in these measurements are comparable to commercial crystals.

Estimation of β-carotene properties

Modeling of solid–supercritical fluid phase equilibrium using an equation of state requires both pure component and mixture solubility data. In general, the critical temperature and pressure and the acentric factor are needed to estimate the a and b parameters in the equation of state. For large molecules, like β-carotene, the critical temperature cannot be measured, since these species decompose at lower temperatures. Instead, group contribution techniques, such as the Lydersen (1955) or Ambrose (1979) methods, may be used to estimate "pseudo-critical" properties. These methods utilize the normal boiling point, typically obtained from vapor pressure data.

Initial attempts to measure the low vapor pressures of β-carotene were unsuccessful (Bruno, 1989a), although 10^{-11} torr was reported as an upper bound at 30 °C. In the absence of experimental measurements, the UNIFAC group contribution method (Jensen et al., 1981) was used to estimate the vapor pressures of the pure solid. It should be noted that Schaeffer et al. (1988) followed this approach for several pyriolizidine alkaloids and reported good agreement with experimental measurements. For β-carotene, UNIFAC vapor pressure estimates, which range from 4.6×10^{-11} torr at 30 °C to 6.6×10^{-7} torr at 70 °C, were used to compute the coefficients of the Antoine equation

$$\log_{10}(P^{\mathrm{sat}}, \text{mm Hg}) = A - \frac{B}{T(^\circ\mathrm{C}) + C} \tag{1}$$

It should be noted that the estimate at 30 °C is on the order of the experimental bound. The normal boiling point was extrapolated using the Antoine equation (499.7 K), and the critical properties were estimated by the Lydersen method. Since there is considerable uncertainty in these estimates, they were adjusted to minimize the differences between the experimental and predicted solubilities (described below), with the interaction coefficients equal to zero. The original and adjusted values are presented in Table 5.

Subsequent to these calculations, Bruno (1989b) has reported several vapor pressure measurements. These were used in place of the UNIFAC estimates with no significant improvements in the solubility estimates. Although the Antoine equation is not recommended for use at these low pressures, the insensitivity of the solubility estimates means that a more accurate equation is not warranted.

TABLE 5

Adjusted β-carotene properties [a]

T_c(°C)	374.7 (401.3)
P_c (bar)	15.2 (8.36)
ω	0.411 (4.80)
V (cm^3 mol^{-1})	536.8 [b]
Antoine coefficients:	
A	19.52
B	7395
C	217.7

[a] The entries in parentheses have been estimated using the Lydersen method.
[b] Weast, R.C. (Ed.), 1983. CRC Handbook of Chemistry and Physics.

Phase equilibrium model

To predict the solubility of β-carotene in supercritical CO_2, the compressed gas model (Prausnitz, 1969) is used. In this approach, the fugacity of species i in the supercritical phase is set equal to the fugacity of the pure solid, giving

$$y_i P \Phi_i = P_i^{\text{sat}} \Phi_i^{\text{sat}} \exp\left[\frac{V_i\left(P - P_i^{\text{sat}}\right)}{RT}\right] \tag{2}$$

where y_i is the mole fraction of species i in the supercritical phase, P is the pressure, Φ_i is the fugacity coefficient of species i in the supercritical phase, P_i^{sat} is the vapor pressure of pure species i, Φ_i^{sat} is the fugacity coefficient of species i at its vapor pressure, V_i is the molar volume of the solid (assumed to be independent of pressure), R is the gas constant, and T is the temperature. Normally, Φ_i^{sat} is unity, since the vapor pressures are very low. Solving for the mole fraction of species i in the supercritical phase gives

$$y_i = \frac{P_i^{\text{sat}} \exp\left[\frac{V_i\left(P - P_i^{\text{sat}}\right)}{RT}\right]}{P\Phi_i} \tag{3}$$

The modified Peng–Robinson equation of state of Panagiotopoulos and Reid (1986) was used to compute Φ_i. This model includes a second interaction coefficient for each binary pair, k_{ji}, and a composition-dependent term. The mixing rules for the a coefficient are

$$\begin{aligned} a &= \sum_{i=1}^{N_c} \sum_{j=1}^{N_c} x_i x_j a_{ij} \\ a_{ij} &= \sqrt{a_i a_j}\left[1 - k_{ij} + \left(k_{ji} - k_{ij}\right) x_i\right] \end{aligned} \tag{4}$$

TABLE 6

Interaction coefficients for the modified Peng–Robinson equation of state for the system $CO_2(1)$–β-carotene(2)

T(°C)	k_{12}	k_{21}
40	0.112	−0.016
60	0.102	−0.001
70	0.078	−0.086

When $k_{ij} = k_{ji}$, eqns. (4) are the mixing rules for the Peng–Robinson equation. This relatively minor modification significantly improves the predictions of the liquid phase compositions for mixtures such as acetone/CO_2 and water/CO_2 (Panagiotopoulos and Reid, 1987).

The interaction coefficients k_{ij} and k_{ji} were computed for several temperatures. Values for each temperature were determined by minimizing

$$f = \frac{1}{N} \sum_{i=1}^{N} \left(\frac{y_i - y_i^{\exp}}{y_i} \right)^2 \tag{5}$$

where y_i are the mole fraction estimates (eqn. (3)), $y_i^{\exp}$ are the measured mole fractions, and N is the number of data points. The resulting interaction coefficients for the CO_2/β-carotene system are given in Table 6, and the theoretical and experimental mole fractions are compared in Fig. 3. Good agreement is obtained, especially at the higher temperatures.

CONCLUSIONS

Measurements of the equilibrium solubilities of β-carotene in supercritical CO_2 indicate that favorable conditions for extraction are at 70 °C and pressures in excess of 400 bar. Although the solubilities are low under these conditions, the addition of cosolvents in low concentrations significantly enhances the solubilities. Of the three cosolvents tested, ethanol provided the greatest enhancement. The modified Peng–Robinson equation of state (Panagiotopoulos and Reid, 1987), with interaction coefficients fitted to the solubility data, provided reliable solubility estimates.

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LIST OF SYMBOLS

A, B, C	coefficients in the Antoine equation, eqn. (1)
a	mixture parameter in the modified PR-EOS
a_i	parameter for species i in the modified PR-EOS
E	enhancement factor
k_{ij}	interaction coefficient between species i and j in the modified PR-EOS
N_c	number of chemical species
P	pressure (bar)
P_c	critical pressure (bar)
P_i^{sat}	vapor pressure of solid species i (mm Hg, bar)
R	universal gas constant
T	temperature (°C, K)
T_c	critical temperature (°C, K)
V_i	molar volume of solid species i
y_i	predicted mole fraction of species i in CO_2
y_i^{exp}	experimental mole fraction of species i in CO_2
Y_b	mole fraction of β-carotene in CO_2
Y_t	mole fraction of β-carotene in CO_2/cosolvent mixture

Greek letters

Φ_i	fugacity coefficient of species i in supercritical CO_2
Φ_i^{sat}	fugacity coefficient of species i at its vapor pressure
ω	acentric factor

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