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Phase Equilibria with Supercritical Carbon Dioxide for the Enzymatic Production of an Enantiopure Pyrethroid Component. Part 1. Binary Systems

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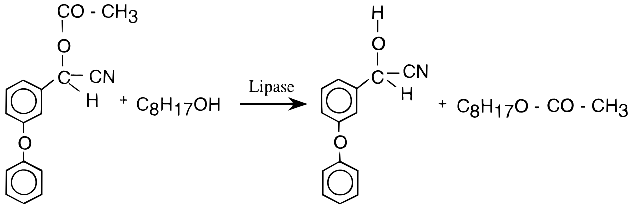
In a lipase-catalyzed kinetic resolution, racemic α-cyano-*m*-phenoxybenzyl acetate is partially converted to the *S*-enantiomer of α-cyano-*m*-phenoxybenzyl alcohol, which is an important synthon for pyrethroid insecticides. 1-Octanol acts as a reaction partner, being transformed to 1-octyl acetate. Binary phase equilibria of each reactant with supercritical carbon dioxide, the solvent for both the reaction and the subsequent product recovery by supercritical fluid extraction, were measured at a temperature of 313.15 K. The systems CO_2 + 1-octanol and CO_2 + 1-octyl acetate were investigated up to their respective critical pressures of about 17 MPa and 8.5 MPa. Maximum pressures of 20 MPa for the binary CO_2 + α-cyano-*m*-phenoxybenzyl alcohol and of 28 MPa for CO_2 + α-cyano-*m*-phenoxybenzyl acetate were applied. A high-pressure experimental apparatus was used with small samples being withdrawn from a visual equilibrium cell and analyzed online by gas chromatography. The Soave−Redlich−Kwong equation of state with the mixing rules due to Huron and Vidal was applied for correlating the measured equilibria. Deviations of correlated data from experimental results remain, in general, within the estimated accuracy of the measurements.

Introduction

Enzymes have been gaining recognition as biochemical catalysts within the last two decades. Due to the stereoselectivity of enzymes, one of the most important applications of enzymatic catalysis is preparing enantiopure compounds. They are increasingly demanded as superior pharmaceutical and agrochemical products or as their starting materials.

For a little more than a decade it has been known that some enzymes, lipases above all, work in supercritical solvents at high pressures (Randolph et al., 1985; Hammond et al., 1985). The past years have seen numerous studies mainly of lipase-catalyzed esterifications and transesterifications in supercritical CO_2 (Nakamura, 1990). Recent research shows an emphasis on enantioselective reactions such as the kinetic resolution of racemic substrates (Bornscheuer, 1993). The benefits of supercritical solvents have been exploited for a long time in well-known supercritical fluid extraction (SFE) and many of those hold with enzymatic reactions, too. Two advantages are cited most often: (1) As mass transport limitations in supercritical phases are low, reaction rates are usually higher than in organic media. (2) After most enzymatic conversions the need arises to separate products and nonconverted substrates—especially in kinetic resolutions of enantiomers where conversion is rarely extended beyond 50%. Thus, the reaction can be combined with a subsequent separation by SFE, using a single solvent.

Research on enzymatic reaction in supercritical fluids has, up to now, paid little attention to the phase equilibria of the systems. A knowledge of the phase equilibria, however, is important for two reasons: (1) solvent-to-substrate ratios as well as reaction pressure and temperature have to be fixed so that the reaction system forms a homogeneous solution all the time. Therefore, the solubility border of the reaction system has to be known—most precisely because operating very close to the solubility border is optimal in terms of reaction rates (Stephan et al., 1995). (2) For deciding about the feasibility of product recovery using SFE the phase equilibria must be known. Such information is essential in designing both the structure of a possible SFE process and fixing appropriate separation conditions.



(R/S) - CPBAc OAl (R/S) - CPBAl OAc

Figure 1. Enzymatic resolution of racemic CPBAc.

In this paper, phase equilibrium measurements of a reaction system yielding an enantiopure synthon for pesticides are presented.

Reaction System

α-Cyano-*m*-phenoxybenzyl alcohol (CPBAl) is a constituent of an important group of synthetic pyrethroids such as cypermethrin, deltamethrin, or fenvalerate. It is the *S*-enantiomer of CPBAl that accounts for the high activity of these pyrethroid insecticides. (*S*)-CPBAl can be prepared by the kinetic resolution of its racemic acetic ester (*R*/*S*)-α-cyano-*m*-phenoxybenzyl acetate (CPBAc), which is catalyzed by a lipase and can be effected in supercritical CO_2 (Stephan et al., 1995; Chrisochoou et al., 1995a,b). 1-Octanol (OAl) was used as a reaction partner that is transformed into 1-octyl acetate (OAc) (see Figure 1). After the reaction, a product mixture containing equal quantities of all four reactants has to be separated.

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Table 1. Substances Used in the Experiments

substance	CAS no.[b]	supplier	purity, mol %
carbon dioxide (CO_2)	124−38−9	Messer Griesheim	>99.995
1-octanol (OAl)	111−87−5	Aldrich	>99
1-octyl acetate (OAc)	112−14−1	Sigma	≈98
α-cyano-*m*-phenoxybenzyl alcohol (CPBAl) (racemate)	71962−66−8 (*R*) 61826−76−4 (*S*)	Inst. Org. Chemie	≈92[a]
α-cyano-*m*-phenoxybenzyl acetate (CPBAc) (racemate)	126641−87−0 (*R*) 113301−28−3 (*S*)	Inst. Org. Chemie	≈97

[a] No further purification was possible because of thermolability. [b] Supplied by the authors.

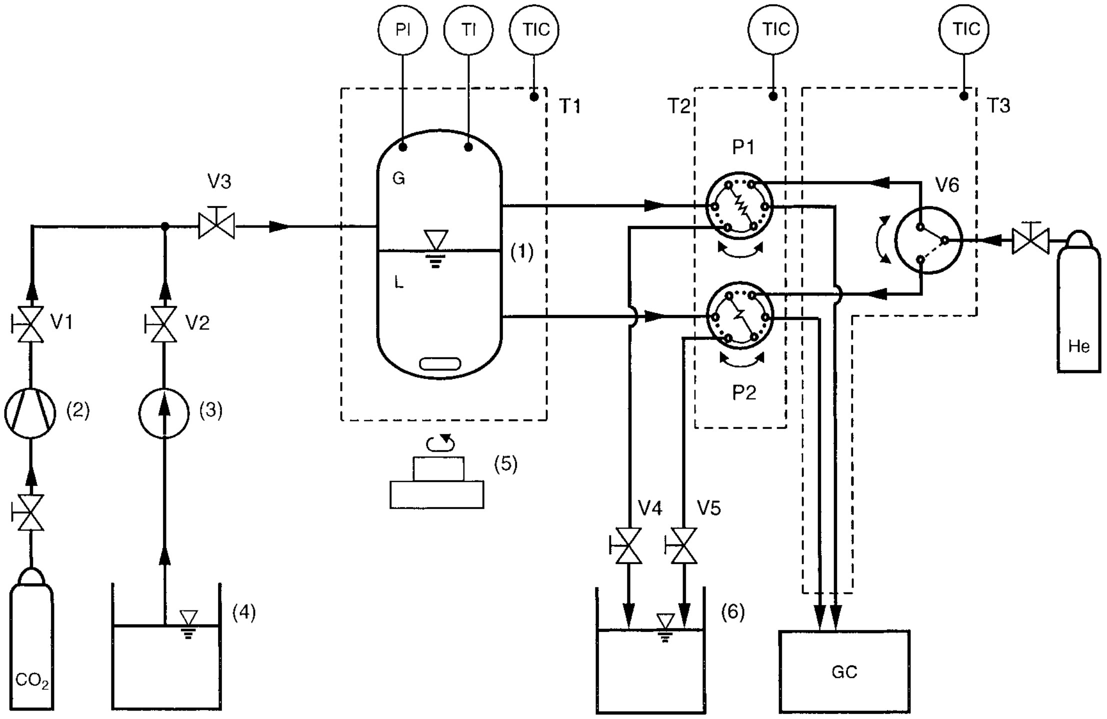

Figure 2. Apparatus for measuring high-pressure phase equilibria.

Experimental Section

Substances Used. Table 1 shows the suppliers of the substances used in the experiments together with the purities. CPBAl and CPBAc were prepared from *m*-phenoxybenzaldehyde by staff of the Institut für Organische Chemie, Universität Stuttgart, and were analyzed by mass spectroscopy. All substances were employed without further purification.

Apparatus and Procedure. A static high-pressure apparatus with online sample analysis by gas chromatography (GC) was used for the measurements in this work. Figure 2 shows the scheme of the experimental setup.

The fluid system under investigation is contained in a high-pressure equilibrium cell mounted in revolving bearings (1). It was custom-made from stainless steel, with two borosilicate windows inserted in the opposite lids, to withstand pressures of up to 30 MPa at temperatures of up to 373.15 K; its volume is about 60 cm^3. In Figure 3 the autoclave is drawn in cross-section. The cell is fed with liquid materials by a high-pressure minipump (3) and with CO_2 by a compressor (2). All the lines of the setup are of HPLC type with a maximum internal diameter of 1 mm. A magnetic stirrer (5) is used for accelerating mass exchange. The equilibrium cell is located inside an air bath with transparent polycarbonate walls (T1).

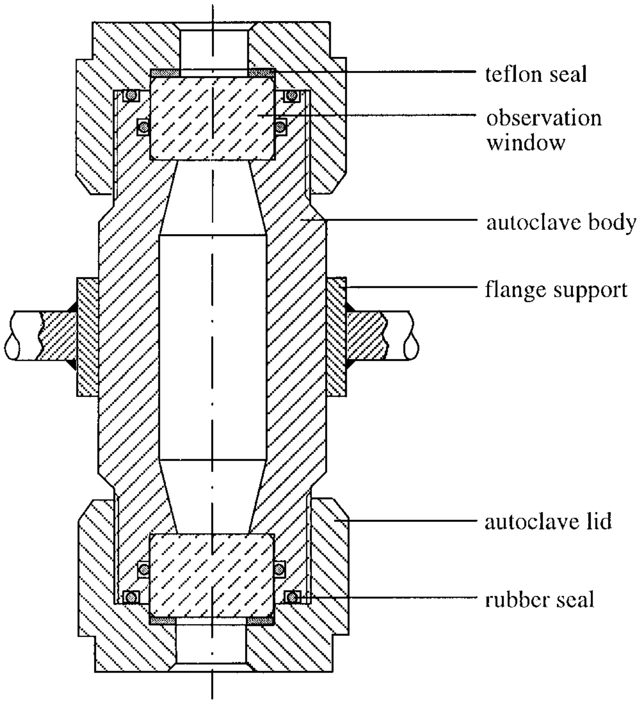

Figure 3. High-pressure visual equilibrium cell.

Measuring phase equilibria is based on withdrawing and analyzing small samples from the equilibrium phases. For sampling from the upper (gaseous) phase, valve V4 is partially opened, enabling a material flow from the cell through the sample loop (5 μL) of sample valve P1 (Rheodyne 7000, in load position). V4 being closed after a minute or so, no significant pressure drop or disturbing of the

Table 2. Technical Data and Parameters of GC Analysis

gas chromatograph	HP 5890A (Hewlett Packard)
detector	TCD, sensitivity "low", operating temperature 540.13 K
injector	packed column inlet, neddle port with septa 11 mm "low bleed", operating temperature 513.15 K
column	6 ft. × $^1/_8$ in. stainless steel, packed, stationary phase UCW-982, maximum temperature 573.13 K, phase loading 5%, solid support Chromosorb WHP, 80/100 mesh, oven temperature constant or programmed
carrier gas	helium 4.6 (purity >99,996%, Messer Griesheim), column flow rate 30 mL/min, reference flow rate 45 mL/min

Table 3. Estimated Accuracies in Mole Fraction *x* or *y*

x, *y*	absolute error	rel error, %
0.5	±0.01	2
0.1	±0.01	10
0.01	±0.002	20
0.001	±0.0005	50
0.0001	±0.0002	200

Table 4. GC Oven Temperature Programs

system	T_{init}/K	τ_{ini}/min	r/K/min	T_{fin}/K	τ_{fin}/min
CO_2 + OAl	413.15	2.0			
CO_2 + OAc	433.15	3.0			
CO_2 + XAl	323.15	1.5	25	513.15	1.0
CO_2 + XAc	443.15	1.5	15	523.15	1.0

Table 5. Experimental Data for the System CO_2 (1) + OAl (2) at 313.15 K

p/MPa	x_1	y_1	y_2	p/MPa	x_1	y_1	y_2
0.50	0.046			7.70	0.547	0.9994	0.0006
0.75	0.069			8.30	0.650	0.9989	0.0011
1.00	0.094			9.20	0.715	0.9882	0.0118
1.60	0.146			10.10	0.706	0.9800	0.0200
2.00	0.179			11.50	0.729	0.9667	0.0333
2.50	0.229			12.60	0.754	0.9486	0.0514
3.40	0.262			13.20	0.785	0.9425	0.0575
4.20	0.333			15.20	0.791	0.9239	0.0761
5.90	0.447	0.9998	0.0002				

Table 6. Experimental Data for the System CO_2 (1) + OAc (2) at 313.15 K

p/MPa	x_1	y_1	y_2	p/MPa	x_1	y_1	y_2
0.23	0.023			5.54	0.571		
0.44	0.071			6.42	0.650		
1.53	0.169			6.92	0.702		
2.60	0.275			7.32	0.735	0.9995	0.0005
3.45	0.364			7.74	0.804	0.9992	0.0008
4.57	0.465			8.28	0.875	0.9985	0.0015

Table 7. Experimental Data for the System CO_2 (1) + CPBAl (2) at 313.15 K

p/MPa	x_1	y_1	y_2	p/MPa	x_1	y_1	y_2
0.21	0.015			8.25	0.448		
0.41	0.023			9.90	0.469		
1.55	0.114			12.60	0.481	0.9990	0.0010
3.15	0.211			14.50	0.510	0.9988	0.0012
4.05	0.264			17.00	0.519	0.9987	0.0013
6.00	0.332			19.00	0.539	0.9979	0.0021
7.15	0.417			20.00	0.530	0.9983	0.0017

Table 8. Experimental Data for the System CO_2 (1) + OAl (2) at 313.15 K

p/MPa	x_1	y_1	y_2	p/MPa	x_1	y_1	y_2
0.43	0.038			12.52	0.718	0.9917	0.0083
0.91	0.087			13.85	0.729	0.9919	0.0081
1.52	0.164			15.60	0.755	0.9895	0.0105
1.98	0.184			17.20	0.770	0.9879	0.021
2.55	0.247			18.70	0.775	0.9824	0.0176
3.18	0.310			20.05	0.795	0.9818	0.0182
3.63	0.347			21.70	0.821	0.9745	0.0255
5.13	0.481			23.60	0.820	0.9702	0.0298
5.93	0.506			24.60	0.824	0.9687	0.0313
6.84	0.552			26.60	0.830	0.9550	0.0450
7.75	0.615			27.62	0.845	0.9545	0.0455
8.72	0.650			28.00	0.847	0.9515	0.0485
10.30	0.683	0.9934	0.0066				

Table 9. Critical and Pseudocritical Data of Pure Substances

component	p_c/MPa	T_c/K	source or estimation method
CO_2	7.39	304.2	Simmrock et al., 1986a
OAl	2.86	652.5	Simmrock et al., 1986b
OAc	2.13	656.3	est. (Ambrose)
CPBAl	2.90	844.0	est. (Joback-Lydersen/Fedors)
CPBAc	2.19	853.0	est. (Joback-Lydersen/Fedors)

Table 10. Experimental Vapor Pressures for CPBAc

T/K	p/kPa	T/K	p/kPa	T/K	p/kPa
473.15	0.80	493.15	2.00	512.15	3.60
477.15	0.93	494.15	2.00	517.15	4.13
480.15	1.07	496.15	2.20	522.15	5.07
484.15	1.40	497.15	2.13	524.15	5.87
487.15	1.60	499.15	2.40	527.15	6.67
489.15	1.80	503.15	2.84	533.15	7.60
491.15	1.73	507.15	3.16		

Table 11. Pure Component Parameters for the SRK Equation

component	m_i	n_i
CO_2	0.660 48	0.205 37
OAl	0.527 63	0.782 07
OAc	1.213 76	0.341 01
CPBAl	1.10	0.55
CPBAc	1.088 41	0.528 53

Table 12. Binary Interaction Parameters Due to Huron and Vidal

binary	α_{ij}	C_{ij}/[(kPa·m^3)/mol]	C_{ji}/[(kPa·m^3)/mol]
CO_2 + OAl	−0.008 018 3	27.003	−3.5994
CO_2 + OAc	−0.002 887 8	26.958	−2.0886
CO_2 + CPBAl	0.0002 19 3	49.007	−5.4978
CO_2 + CPBAc	−0.003 760 2	50.127	−4.5031

equilibrium state in the cell is incurred. Thus several samples of the same state can be drawn successively. (Withdrawing ten samples consecutively at a system pressure of 10 MPa accounts for a pressure drop of no more than 0.05 MPa.) By switching P1 to the inject position the sample loop is flushed by a helium flow, transporting the sample to the GC for analysis. (For that purpose, the sample line is soldered to the GC injector.) After that, the sample valve P1 is switched back to the load position. For withdrawing the next sample, V4 is opened again and the sample loop of P1 is purged by a new flow from the cell. Thus any helium, trapped in the sample loop when P1 is switched back from "inject" to "load" after the previous sampling, is swept out of the system before diffusing via the cell. Sampling from the lower (liquid) phase works analogously—valve V5 and sample valve P2 (Rheodyne 7410, sample loop 1 μL) have to be actuated with switching valve V6 being in the lower position. Both the sample valves and the helium-transporting lines are wrapped in heating-tapes (T2, T3). As all of the four substances OAl, OAc, CPBAl, and CPBAc are of low volatility it must be ensured that all sample components are evaporated and are in the gaseous state when entering the GC.

The temperature in the equilibrium cell is measured by a platinum resistance thermometer and is assumed to be measured within an accuracy of about ±0.1 K. A pressure transducer is used to regulate the pressure in the apparatus. The accuracy of the pressure measurement is estimated to be better than ±0.03 MPa. The air bath temperature is controlled by a digital PID controller. A temperature stability of about ±0.2 K is achieved, yielding a constancy inside the equilibrium cell of better than ±0.01 K.

The samples are analyzed using a GC with a thermal conductivity detector (TCD). The technical data of the GC are given in Table 2 together with the parameters that were constant for all analyses. The oven temperature was adjusted for each binary system investigated to stay at a fixed temperature or to run a one-ramp course. The TCD signal was processed by an electronic integrator (Hewlett-Packard HP 3396A). GC analysis was calibrated using various amounts of pure substances injected into the GC with precision syringes (Hamilton). As proposed by Traub and Stephan (1990), calibration results were fitted to second-order polynomials correlating molar quantity N_i and peak area A_i:

$$N_i = \beta_i A_i + \gamma_i A_i^2$$

with relative deviations generally not exceeding 2%. The calibration procedure was performed when investigations on a binary system were started and was repeated at the end of these measurements. Relative deviations between these two calibrations were usually less than 1%.

As CPBAl is thermolabile, it decomposes into hydrogen cyanide and *m*-phenoxybenzaldehyde during GC analysis, producing two peaks on the chromatogram. For calibration, the *m*-phenoxybenzaldehyde peak is used.

Usually, about 5−10 samples were drawn and analyzed for measuring each equilibrium phase at a constant temperature and a fixed pressure. For determining the quantities of every sample component, and thus the composition of the sample, all calibration functions were used interpolatively. The mean value of these measurements was calculated to yield the result. An estimation of the overall accuracy is delivered by the standard deviation of the single measurements. In order to improve accuracy it was necessary, especially with phases containing less than 1 mol % of a component, to increase the number of measurements up to 20. Table 3 gives an overview of the estimated accuracies depending on the molar fractions.

Due to the fact that CPBAl is analyzed from one of its decomposition products the error for the CPBAl molar fractions is assumed to be somewhat higher.

Results

Phase equilibria of the the four binary systems of CO_2 and one of the reactants OAl, OAc, CPBAl, and CPBAc were measured at a constant temperature of 313.15 K. Whereas the binaries CO_2 + OAl and CO_2 + OAc could be investigated up to their critical points, due to the pressure limit of the apparatus this was not possible for the systems CO_2 + CPBAl and CO_2 + CPBAc. Table 4 contains the GC oven temperature programs used for the different systems. The results of the measurements are presented in Tables 5−8.

Correlation with the SRK Model

For data correlation the cubic equation of state proposed by Redlich and Kwong (1949) and modified by Soave (1972 and 1979) is used (SRK equation). To account for interactions between the mixture components, the mixing rules developed by Huron and Vidal (1979) are applied (HV mixing rules). The SRK equation with the HV mixing rules has been validated by a series of authors to be an adequate tool for correlating high-pressure phase equilibria of systems with supercritical components (e.g., Traub and Stephan (1990) and Chrisochoou et al. (1995a,b). The SRK equation is employed in the following form:

$$p = \frac{RT}{\bar{V} - b} - \frac{a}{\bar{V}(\bar{V} + b)} \tag{2}$$

For a pure component *i* the parameters *a* and *b* are defined as

$$a = a_i = a_{c,i}\alpha_i(T) \tag{3}$$

$$b = b_i = b_{c,i} \tag{4}$$

with

$$a_{c,i} = \Omega_a \frac{R^2 T_{c,i}^{\ 2}}{p_{c,i}} \tag{5}$$

$$b_{c,i} = \Omega_b \frac{R T_{c,i}}{p_{c,i}} \tag{6}$$

$$\alpha_i(T) = 1 + \left(1 - \frac{T}{T_{c,i}}\right)\left(m_i + \frac{n_i}{T/T_{c,i}}\right) \tag{7}$$

Besides the critical data ($p_{c,i}$, $T_{c,i}$) the parameters of the temperature function (m_i, n_i) due to Soave (1979) are needed for each substance. (Ω_a and Ω_b are constants.)

In treating mixtures of $i = 1, ..., K$ components, parameters *a* and *b* are calculated from the pure substance parameters a_i and b_i:

$$a = b\sum_{i=1}^{K} x_i \left[\frac{a_i}{b_i} - \frac{1}{\ln 2}\left(\frac{\sum_{j=1}^{K} x_j C_{ji} b_j \exp(-\alpha_{ji} C_{ji}/(RT))}{\sum_{j=1}^{K} x_j b_j \exp(-\alpha_{ji} C_{ji}/(RT))}\right)\right] \tag{8}$$

$$b = \sum_{i=1}^{K} x_i b_i \tag{9}$$

Each pair of substances *i*, *j* in the mixture requires a set of three interaction parameters ($\alpha_{ij} = \alpha_{ji}$, C_{ij}, C_{ji}), as implied by the HV mixing rules. Parameter values are fitted to phase equilibria of the mixture under investigation.

Experimental critical data were retrieved from the literature for CO_2 and OAl (Simmrock et al., 1986a,b), while no data could be found for OAc. Both CPBAl and CPBAc do not have a critical point, as they decompose at temperatures of about 330 K and 530 K, respectively. Therefore, the critical data of OAc were estimated by group contribution methods (Reid et al., 1987); for CPBAl and CPBAc pseudocritical data were calculated using the same estimation methods. Table 9 presents the (pseudo)critical data of all five substances that were used.

The Soave parameters m_i, and n_i were obtained by fitting them to vapor pressure data for the pure substances by employing a least squares algorithm. Data for CO_2 and OAl were taken from the literature (VDI-Wärmeatlas, 1994; Boublik, 1973); a set of vapor pressure data was calculated for OAc by the Antoine equation (Gmehling et al., 1982). No experimental data or calculation method could be found for CPBAl and CPBAc. Therefore, about 20 vapor pressures for CPBAc were measured in this work. The results are given in Table 10. We used a standard

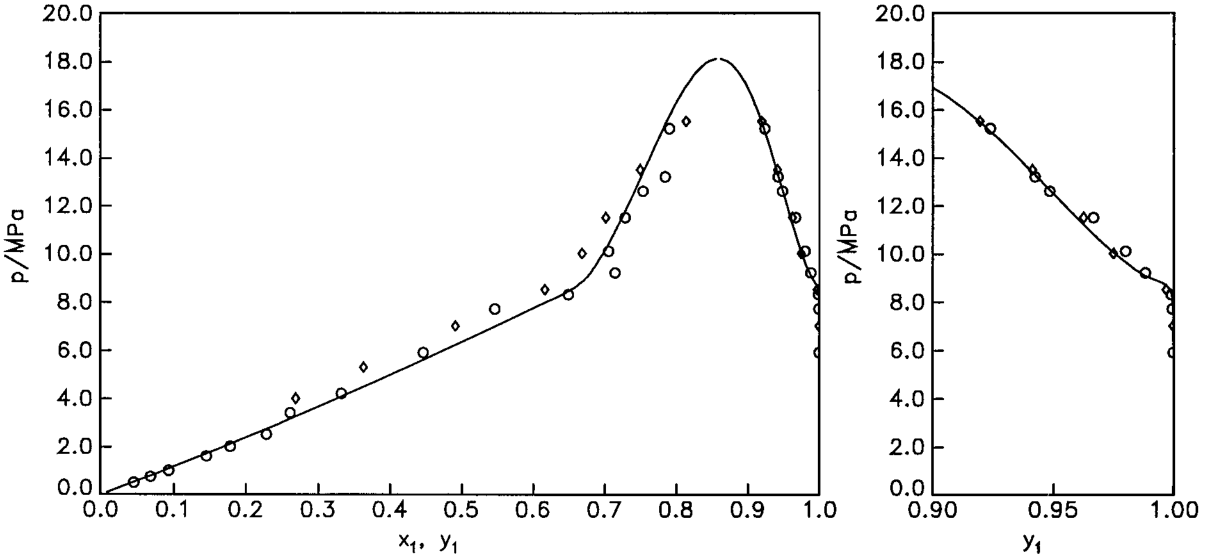

Figure 4. Phase equilibrium of CO_2 (1) + OAl (2) at 313.15 K: (◇) Weng and Lee (1992); (○) experimental, this work; (—) SRK model.

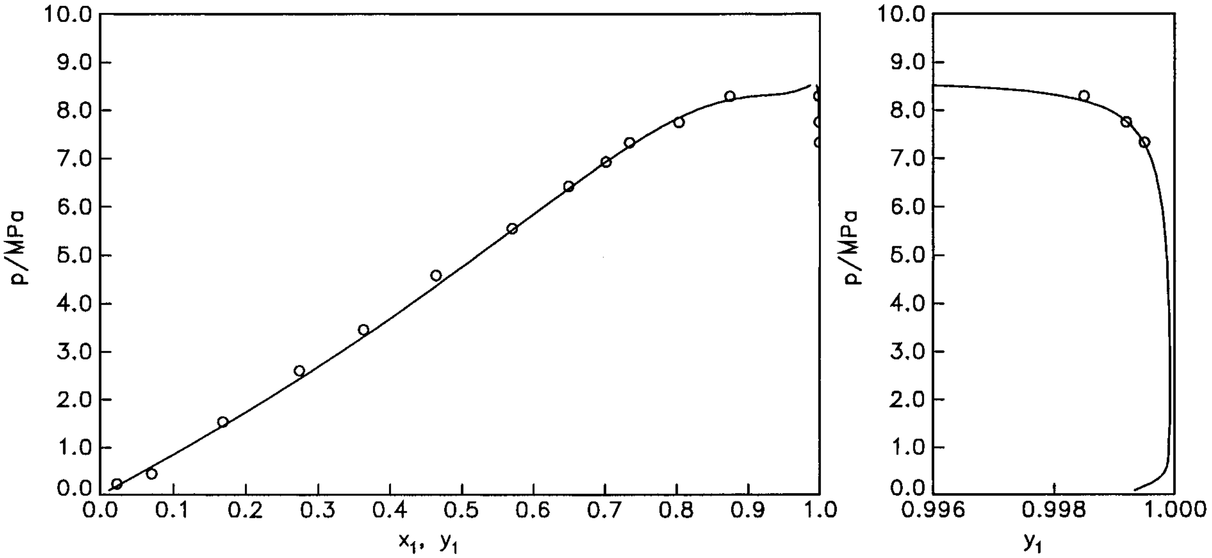

Figure 5. Phase equilibrium of CO_2 (1) + OAc (2) at 313.15 K: (○) experimental, this work; (—) SRK model.

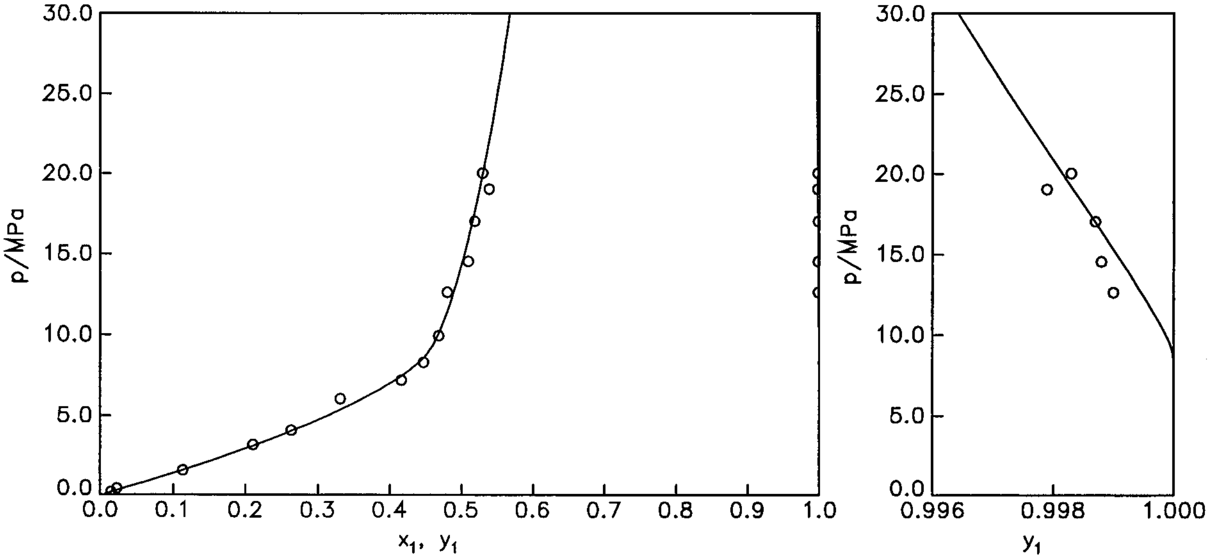

Figure 6. Phase equilibrium of CO_2 (1) + CPBAl (2) at 313.15 K: (○) experimental, this work; (—) SRK model.

isoteniscope apparatus, only replacing the usual thermostating liquid water by a high-temperature thermal oil (Marlotherm S). The accuracies of the temperature and pressure measurements were about ±0.3 K and ±0.1 kPa, respectively. Due to the restrictions of the vacuum pump and the tightness of the apparatus, the minimum pressure

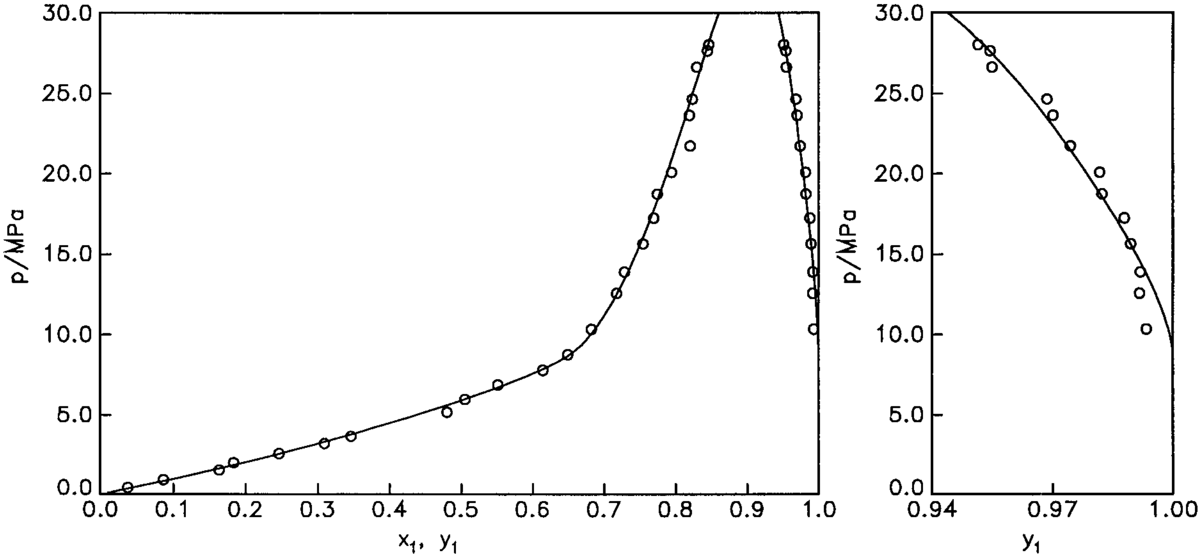

Figure 7. Phase equilibrium of CO_2 (1) + CPBAc (2) at 313.15 K: (○) experimental, this work; (—) SRK model.

to be attained was 0.3 kPa. Considering the low volatility of CPBAc, temperatures of more than 473.15 K were required in the apparatus. CPBAl is of similar low volatility but decomposes at temperatures far below 373.15 K. Hence, no measurements could be done with CPBAl. Instead, its Soave parameters were estimated to be roughly the average of those of CPBAc and of the similar component α-cyano-*m*-phenoxybenzaldehyde. In Table 11 the parameters m_i and n_i are given for all substances.

Then, the HV parameters α_{ij}, C_{ij}, and C_{ji} of the four binaries CO_2 + OAl, CO_2 + OAc, CO_2 + CPBAl, and CO_2 + CPBAc were fitted to the experimental data of this work. The data of Weng and Lee (1992) for the binary CO_2 + OAl were also included in the fitting procedure. A least squares algorithm without weights was used. Table 12 contains the HV interaction parameters that were obtained.

Figures 4−7 show the phase equilibria as calculated with the parameters of Tables 11 and 12 in comparison with the experimental data.

The liquid phase measurements of the CO_2 + OAl system presented here show relative deviations of more than 10% from those of Weng and Lee (1992), (see Figure 4). In contrast, measurements of the gaseous phases agree within less than 5%. As a consequence, SRK model calculations for the bubble point line show an average relative deviation of about 5% from the measurements. For equilibrium points of the dew point line, the difference between measured and calculated data is about the estimated accuracy of the experiments. The critical point seems to be “overshot” by the model predictions.

For the system CO_2 + OAc, the agreement of measured and calculated data is better than the estimated errors incurred in measuring, even in the vicinity of the critical region (see Figure 5).

In the case of the CO_2 + CPBAl and CO_2 + CPBAc binaries, model calculations match experimental data of the liquid phases within estimated accuracies of the measurements (see Figures 6 and 7). The same is true for the gaseous phases, except for pressures below 15 MPa where greater deviations are obvious.

Conclusions

The phase equilibrium measurements performed in this work show that the high-pressure apparatus used is an appropriate means of investigating systems containing supercritical carbon dioxide and components of very low volatility.

The experimental results for all four binary systems investigated could be described well by the SRK equation with the HV mixing rules. The fact that some of the SRK model’s parameters had to be estimated did not impair the quality of the model predictions.

This work has produced basic information about the phase equilibria of the reaction system treated, as phase equilibria of all binary subsystems of a reactant and the solvent CO_2 are known. But for fixing operating conditions to execute the reaction close to the solubility border and for designing the structure of the subsequent product recovery process, this information is, of course, not sufficient. There, a knowledge of the phase equilibria of ternary subsystems and the complete reaction system is indispensable.

For this purpose, it is necessary to check the SRK equation with the model parameters determined in this work for its ability to predict the phase behavior of the complete five-component system. To this end, the SRK model has to be completed by providing the missing interaction parameters (HV mixing rules) for the binary systems excluding CO_2. This will be done in future work, and model calculations will be compared to measurements of ternary and five-component systems.

Literature Cited

Bornscheuer, U. *Reaktionstechnische Untersuchungen zur enzymatischen Racematspaltung verschiedener 3-Hydroxyester in unkonventionellen Lösungsmittelsystemen*; Fortschr.-Ber. VDI, Reihe 17 N. 87; VDI-Verlag: Düsseldorf, 1993.

Boublik, T.; Fried, T.; Hala, E. *The Vapour Pressures of Pure Substances*; Elsevier Publishing Co.: Amsterdam, 1973.

Chrisochoou, A.; Schaber, K.; Bolz, U. Phase Equilibria for enzyme-catalyzed reactions in supercritical carbon dioxide. *Fluid Phase Equilib.* **1995a**, *108*, 1−14.

Chrisochoou, A.; Stephan, K.; Winkler, S.; Schaber, K. *Chem.-Ing.-Tech.* **1995b**, *67*, 1153 (in German).

Gmehling, J.; Onken, U.; Grenzheuser, P. *Vapor-liquid Equilibrium Data Collection. Carboxylic Acids, Anhydrides, Esters*; Chemistry Data Series, Vol. I, Part 5; DECHEMA: Frankfurt/Main, 1982.

Hammond, D. A.; Karel, M.; Klibanov, A. M.; Krukonis, V. J. Enzymatic Reactions in Supercritical Gases. *Appl. Biochem. Biotechnol.* **1985**, *11*, 393−400.

Huron, M.-J.; Vidal, J. New mixing rules in simple equations of state for representing vapour-liquid equilibria of strongly non-ideal mixtures. *Fluid Phase Equilib.* **1979**, *3*, 255−271.

Nakamura, K. Biochemical reactions in supercritical fluids. *Tibtech* **1990**, October, 288−292.

Randolph, T. W.; Blanch, H. W.; Prausnitz, J. M.; Wilke, C. R. Enzymatic catalysis in a supercritical fluid. *Biotechnol. Lett.* **1985**, *7*, 325−328.

Redlich, O.; Kwong, J. N. S. On the thermodynamics of solutions. V: An equation of state. Fugacities of gaseous solutions. *Chem. Rev.*

1949, *44*, 233−244.

Reid, R. C.; Prausnitz, J. M.; Poling, B. E. *The Properties of Gases and Liquids* 4th ed.; McGraw-Hill: New York, 1987; Chapter 1.

Simmrock, K. H.; Janowsky, R.; Ohnsorge, A. *Critical data of pure substances*; Chemistry Data Series, Vol. II, Part 1; DECHEMA: Frankfurt/Main, 1986a.

Simmrock, K. H.; Janowsky, R.; Ohnsorge, A. *Critical data of pure substances*; Chemistry Data Series, Vol. II, Part 2; DECHEMA: Frankfurt/Main, 1986b.

Soave, G. Equilibrium constants from a modified Redlich-Kwong equation of state. *Chem. Eng. Sci.* **1972**, *27*, 1197−1203.

Soave, G. S. Application of a cubic equation of state to vapour-liquid equilibria of systems containing polar compounds. *Inst. Chem. Eng. Symp. Ser.* **1979**, *56*, 1.2/1−1.2/16.

Stephan, K.; Winkler, S.; Chrisochoou, A. Supercritical Solvents for Enzymatic Reactions and Product Recovery. *Proc. Int. Symp. Biochem. Eng. 3rd*, Stuttgart **1995**, 84.

Traub, P.; Stephan, K. High-pressure phase equilibria of the system CO_2 - water - acetone measured with a new apparatus. *Chem. Eng. Sci.* **1990**, *45*, 751−758.

Verein Deutscher Ingenieure - GVC (Ed.). *VDI-Wärmeatlas*; Abschnitt Db45; VDI-Verlag: Düsseldorf, 1994.

Weng, W. L.; Lee, M. J. Phase equilibrium measurements for the binary mixtures of 1-octanol plus CO_2, C_2H_6 and C_2H_4. *Fluid Phase Equilib.* **1992**, *73*, 117−127.

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