MEASUREMENTS AND CORRELATION OF EFFECT OF COSOLVENTS ON THE SOLUBILITIES OF COMPLEX MOLECULES IN SUPERCRITICAL CARBON DIOXIDE

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Abstract—A new transparent microscale circulation-type high pressure equilibrium cell with on-line sampling was devised. With this apparatus, experimental solubility of molecularly complex species such as steroids (cholesterol, stigmasterol and ergosterol) and fatty acids (palmitic acid and stearic acid) in supercritical carbon dioxide(sc-CO_2) were measured. Also, to find an appropriate substance for enhancing both the polarity and the solubility power of the sc-CO_2 solvent, we arbitrarily selected three polar substances such as acetone, methanol and water and the effect of these cosolvents on the solubility of solutes in sc-CO_2 are examined. The supercritical phase equilibrium data of solute-cosolvent-sc-CO_2 systems were quantitatively correlated using a new equation of state based on the lattice fluid theory incorporated with the concept of multibody interaction. We found that the addition of tracer amount of acetone or methanol to sc-CO_2 enhances the solubility of all solutes about thirty to sixty times when compared with the case of pure sc-CO_2. However, for the case of cosolvent water, no further enhancement of the solubility of solutes was realized. Also, the versatile fittability of the equation of state proposed in this work was demonstrated with the newly measured ternary supercritical equilibrium data.

Key words: *Supercritical Fluids, Carbon Dioxide, Cosolvents, Biomolecules, Cholesterol, Stigmasterol, Ergosterol*

INTRODUCTION

Carbon dioxide is one of the most widely used solvent to date in supercritical fluid extraction technology by virtue of its manageable supercritical conditions and cheap price. However, it is frequently pointed out that carbon dioxide has no polarity and accordingly it is not a good solvent for extracting polar organic substances by the supercritical fluid extraction [1]. Thus recently, a possibility of an addition of small portion of polar fluid such as acetone to sc-CO_2 for the purpose of enhancing its polarity has been tested [2-4]. However, due to an intrinsic difficulty of experimental measurement of the effect of tracer amount of cosolvent and the difficulty of thermodynamic understanding of these complex ternary systems, further quantitative examination of the cosolvent effect with respect to sc-CO_2 remains as an unsolved topic in the field of supercritical fluid technology. When it is possible to use sc-CO_2 solvent even for extraction of polar substances, the supercritical fluid extraction process can widely extend its applicability to the separation and purification of various natural products.

In the present study, attention has placed on the quantitative phase equilibria measurement of the effect of cosolvent (e.g., acetone, methanol, and water etc.) on the solubilizing capacity of sc-CO_2 for molecularly complex large molecules such as cholesterol, stigmasterol, ergosterol, palmitic acid and stearic acid. Also, to design supercritical extraction equipment, experimental solubility data can reliably be correlated. However, due to the complex nature of such supercritical fluid systems, existing equations of state such as cubic ones are frequently errorneous and cannot be used

reliably for the thermodynamic correlation of those supercritical phase equilibrium data. Thus, toward the goal of providing a new reliable model for modeling of supercritical phase equilibrium data of complex systems, our effort of data correlation based on a new equation of state presented elsewhere by the present authors [5-6] were quantitatively discussed.

EXPERIMENTAL

1. Experimental Systems

The solubilities of solutes in sc-CO_2 tested in the present work are the biomolecules exist in natural products; cholesterol, ergosterol, stigmasterol, palmitic acid and stearic acid. It is known to us that these systems are thermally unstable and show very low solubility in sc-CO_2 alone. In order to obtain phase equilibrium data, we purchased high purity (above 99.9%) solutes from Sigma Chemical Co. and used directly without further purification. Some important physical properties were summarized in Table 1. CO_2 and candidate cosolvents (acetone, methanol, and water) are HPLC grade (above 99.99%).

2. Equilibrium Cell and Experiment

As shown in Fig. 1, a new micro-volume circulation type experimental apparatus was originally designed and constructed in the present work. Also, the detail drawing of the microsize viewcell was shown in Fig. 2. The viewcell made by Pyrex glass tube (0.2 ml internal volume) and the whole volume of the circulation loop including the viewcell was about 3 ml. When we control the circulation flowrate of moving sc-CO_2 phase by 0.5 ml/min, it took about 6 minutes for one circulation. To check the time required for equilibration, we took the sample through on-line sampling device and analyzed by HPLC as a function of the circu-

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Table 1. Physical properties of solutes

Name, formule	Mol. wt.	b.p. ℃	m.p. ℃	Density (g/cm³)	n_D	Solubility
Cholesterol[a] C₂₇H₄₅OH	388.66	360d, 233[0.5]	148.5 (anh)	1.067[20/4]	-	eth, bz, chl, aa
Ergosterol C₂₈H₅₀O	402.70	488.86[b]	144.5[b]	0.7309[b]	-	eth, chl
Stigmasterol C₂₉H₄₈O	412.70	525.71[b]	170.0[b]	0.7361[b]	-	eth, ace, bz, chl
Palmitic acid[a] CH₃(CH₂)₁₄CO₂H	256.43	350, 267[100]	63	0.8527[62/4]	1.4335[60]	al, eth, bz, ace, chl
Stearic acid[a] CH₁₈H₃₆O₂	284.47	383	69-70	0.847	1.4299	al, eth

a: CRC Handbook 72ed [22].

b: The Properties of Gases and Liquids 4ed [18].
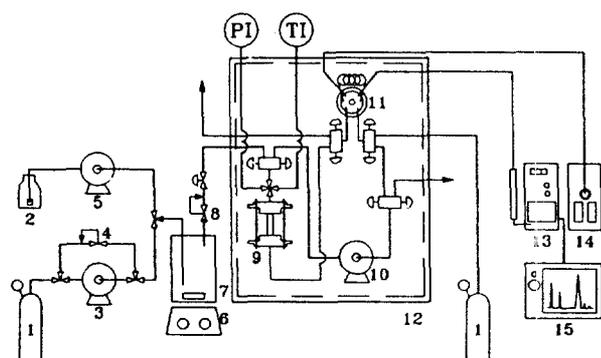
Fig. 1. Schematic diagram of microvolume circulation type equilibrium cell.

1. CO cylinder	8. Forward regulator
2. Cosolvent	9. Viewcell
3. Haskel booster	10. Circulation pump
4. Backpressure regulator	11. Sampling valve
5. Liquid pump	12. Adiabatic bath
6. Magnetic stirrer	13. HPLC detector
7. Reservoir	14. HPLC pump
	15. Integrator

lation time. It was required about 10 to 15 minutes for the equilibration. To guarantee whether the equilibrium state was satisfied or not, we repeated the pretest for three times and we adopt about 30 minutes interval for sampling data. Accordingly, we believed that the apparatus proposed in the present work can guarantee a rapid equilibration between solvent and solute phase with the consumption of the least amount of expensive solutes.

Upon our safety test of maximum allowable pressure of the viewcell, we found its allowable pressure was about 300 bar. Pressure at equilibrium state was measured by Heise guage (CMM-43776). Equilibrium temperature was controlled within ± 0.1℃ by a well insulated circulating air bath. CO₂ was pumped by gas booster (Haskel Booster 75/15) and the cosolvent was pumped by Milton Roy Constametric III. Also, by installing forward regulator (Tascom Product) the pressure in the circulation line was controlled and the circulation accomplished by Milton Roy minipump. To reduce sampling error, the in-situ multiport micro-sampling valve was installed directly on the circulation line and made it possible to sample small amount (20 μl) and connected to HPLC for further analysis. The sampled solute and solvent
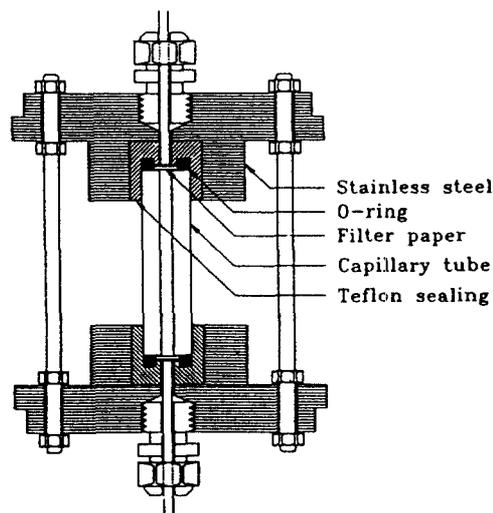

Fig. 2. Details of pyrex tube viewcell.

are directly analysed in the HPLC. The HPLC used is Milton Roy Spectromonitor-3100 and the column used is SGE-ODS2. The supercritical state solvent and solute system were analysed at the on-line mode with an auxiliation of buffer solution.

Experiment was carried out by a stepwise procedure: The viewcell was filled by solid state powdered sample solute and plugged both end by filter paper (mesh size 5 μm) to prevent an entrainment during the circulation of solvent through the cell and connected it to the line. The occurrence of the circulation was checked with our eyes. When the circulation flowrate kept less than 1 mol/min, the solute charged with excess amount stayed in position in the viewcell without any disturbance. Thus, we maintained the flowrate around 0.5 ml/min for further experiment. We found that the circulation took place well within the flowrate we selected. The mole % of CO₂ and candidate cosolvent were controlled by magnetic driven autoclave type of premixer using backpressure regulator connected to the cosolvent reservoir. While keeping a designated thermal (T, 313.15-323.15 K) and mechanical (P, 103.3-241.2 bar) equilibrium condition, the headspace supercritical solvent phase was continuously circulated until the concentration of solute in the line reach a constant value. Every experiments were repeated at least three times to guarantee a reproducibility of the data.

MODELING SUPERCRITICAL PHASE EQUILIBRIA DATA

1. A New EOS Based on Lattice Fluid Theory with Multibody Interaction

Design of supercritical fluid extraction process mainly rely on an appropriate modeling of phase equilibrium behavior of mixture system. However, modeling of supercritical phase behavior is remain to date as a significantly difficult problem due to the intrinsic nature of asymmetry, high compressibility and singular nature of mixtures near the critical region. Until present, most of modeling approaches were based on the use of equations of state. As critically reviewed by Brennecke and Eckert [7], various equations of state (EOS) such as cubic EOS, perturbed EOS, lattices fluid and scaling forms, expanded liquid treatment, empirical, and related variety of mixing rules with computer simulation were tested extensively. While all these developments have advantages

and disadvantages, it is generally accepted that the lattice theory-based EOS approach seems to provide a more theoretically-based approach than other genre of EOSs with potential for further development, especially, for supercritical fluid mixtures containing molecularly complex and large molecules.

Most recently, present authors have been placed their attention for formulating a new lattice theory-based EOS. As a result, a new EOS based on generalized nonrandom lattice hole theory was formulated in an explicit form by expanding Helmholtz free energy of full Guggenheim combinatory. They successfully applied the EOS to model various phase equilibrium behavior of complex systems at elevated pressure [8-9]. However, due to its rigorous treatment of the nonrandomness of holes as well as occupied molecules with the framework of a quasichemical approximation and multidimensional series expansion in the lattice description, the final expression of the EOS was significantly lengthy and complex for an easy use in engineering practice. Thus, currently we are trying to propose a simplified form of EOS with a legitimate introduction of the semiempirical treatment of the terms due to the nonrandom contribution [5, 10]. We present here one of the tentative EOS with omitting further detailed discussion of the theoretical aspect of the lattice theory.

Following an argument after Guggenheim [11], the configurational part of the nonrandom lattice partition function in a lattice space of coordination number z and the unit cell size V_H, may be expressed as the sum of random contribution (combinatorial) and nonrandom contribution (residual) of holes and molecules. Based on this argument, the Helmholtz free energy may be written by

$$\beta A = -\ln\Omega = -\ln g_R - \ln[g_{VR}\exp(-\beta U)]$$ (1)

Also, in general, the configurational Helmholtz free energy written in Eq. (1) can be separated by

$$A^c = A^{c(C)}{}_{combinatorial} + A^{c(R)}{}_{residual}$$ (2)

where the configurational combinatorial part $A^{c(C)}$ for the contribution of random array and residual part $A^{c(R)}$ for nonrandom array. Since one may treats separately the quasichemical approximation for the residual part of A^c, different solutions can be considered under the framework of this generalized Guggenheim combinatory and several pioneering theories of solutions are originated from Eq. (1). We have discussed in detail these previous works and their advantages and shortcomings elsewhere [5-6, 8-10].

As discussed elsewhere by the present authors [5, 10], the final expression for the configurational Helmholtz free energy for pure fluids is written by

$$\frac{A^{c(C)}}{RT} = N_1\ln(r_1\gamma_1) - N_1\ln(\widetilde{\rho_1}) + N_0\ln(1-\widetilde{\rho_1})$$
$$+ \frac{Z}{2}N_q\ln\left[1+\left(\frac{q_1}{r_1}-1\right)\widetilde{\rho}\right]$$ (3)

$$\frac{A^{c(R)}}{RT} = \frac{(Z/2)}{(RT)}(N_0+N_1q_1)\theta^2$$ (4)

where R is universal gas constant and q the surface area parameter, r the number of segment per molecule, N_1, number of species 1 and N_0 the number of holes. The overall segment fraction was defined by

$$\theta = \frac{N_1q_1}{(N_0+N_1q_1)} = \frac{(q_1/r_1)\widetilde{\rho}}{[1+(q_1/r_1-1)\widetilde{\rho}]}$$ (5)

Thus, from Eqs. (3) and (4), we can readily obtain all thermodynamic properties.

The EOS and the chemical potential for pure fluids were obtained as follows,

$$\frac{\widetilde{P}}{\widetilde{T}} = -\ln(1-\widetilde{\rho}) + \frac{Z}{2}\ln\left[1+\left(\frac{q_1}{r_1}-1\right)\widetilde{\rho}\right] - \frac{\theta^2}{\widetilde{T}}$$ (6)

$$-\frac{\widetilde{\mu_1}}{RT} = \gamma_1 - r_1\frac{\widetilde{P}}{\widetilde{T}} + \ln q_1 - \ln\theta r_1\left(1-\frac{Z}{2}\right)\ln$$
$$\left[1+\left(\frac{q_1}{r_1}-1\right)\widetilde{\rho}\right] + \frac{q_1}{\widetilde{T}}(2\theta-\theta^2)$$ (7)

where the reduced properties are given by

$$\widetilde{P} = \frac{P}{P^*}, \quad \widetilde{T} = \frac{T}{T^*}, \quad \widetilde{V} = \frac{V}{V^*} = \frac{1}{\widetilde{\rho}}, \quad P^*V_H = RT^* = \left(\frac{Z}{2}\right)\varepsilon_{11}$$ (8)

Above expression contains two molecular parameters, V^* and ε_{11}. Real pure fluids are fully characterized by the EOS with these two pure parameters determined from the basic pure thermodynamic properties as discussed in the later section. The EOS for pure fluids is basically identical with the random lattice theory based ones [12-14]. However, extension to mixed fluids are different from those previous theories.

Expression of configurational Helmholtz free energy defined in Eq. (3) and (4) can readily extend to multicomponent mixture [8-10]. However, as discussed previously, we replace the residual part for mixture by the concept of multibody as discussed by Dieters [15] and Mart and Popadopolous [16]. The concept of multibody interaction among molecular species is basically an empirical one. However, this theory qualitatively being taken into account the complex nature of molecular interaction in mixtures. Especially at elevated pressure such as supercritical state, the pairwise additivity assumption of molecular two-body interaction frequently tend to show erroneous results and accordingly one needs to correct this assumption. Based on this argument, we introduced the multibody interaction to the residual contribution to the Helmholtz free energy and reached the following EOS and chemical potential for multicomponent system. The final expressions for the EOS and the chemical potential are given by,

$$\frac{\widetilde{P}}{\widetilde{T}} = -\ln(1-\widetilde{\rho}) + \frac{Z}{2}\ln\left[1+\left(\frac{q_M}{r_M}-1\right)\widetilde{\rho}\right] - \frac{\theta^2}{\widetilde{T}}\left[1-\frac{\varepsilon_m^{(m)}}{\varepsilon_m}\theta\right]$$ (9)

$$-\frac{\widetilde{\mu_i}}{RT} = \gamma_i - r_i\frac{\widetilde{P}}{\widetilde{T}} + \ln q_i - \ln(\theta\theta_i) - r_i\left(1-\frac{Z}{2}\right)\ln\left[1+\left(\frac{q_m}{r_m}-1\right)\widetilde{\rho}\right]$$
$$+ \left(\frac{q_i\theta}{\widetilde{T}\varepsilon_M}\right) - \left(\frac{q_i\theta^2}{\widetilde{T}}\right)\left[1+(1-\theta)\frac{\varepsilon_M^{(m)}}{\varepsilon_M}\right]$$ (10)

where the relation among molecular parameters is given by

$$P^*V_H = RT^* = \left(\frac{Z}{2}\right)\varepsilon_M$$ (11)

also, q_M and r_M are given by

$$q_M = \Sigma x_i q_i$$ (12)

$$r_M = \Sigma x_i r_i$$ (13)

By an introduction of the concept of multibody interaction among molecular species, the overall interaction energy parameter ε_M is defined by

$$\varepsilon_M = \Sigma\Sigma \ \theta_j\theta_k\varepsilon_{jk} = \varepsilon_M^{(2)} - \theta\varepsilon_M^{(m)}$$ (14)
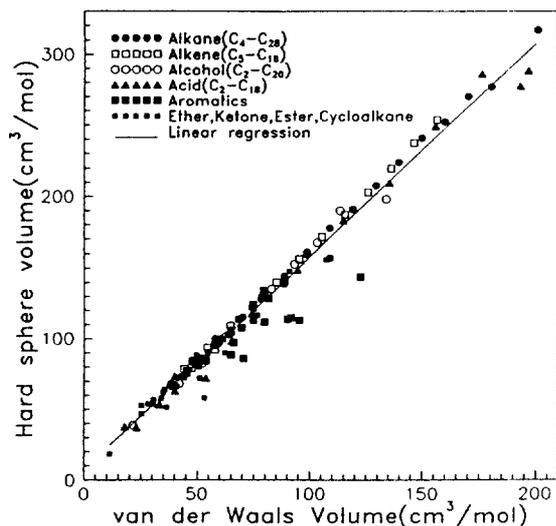
Fig. 3. Relation between van der Waals volume and molecular hardcore volume.

Table 2. Isothermal hardcore volume and energy parameters for pure fluids

Chemicals	ε_{11}/R [K⁻¹]	V^* [cm³mol⁻¹]	Temp. [K]
Carbon dioxide	83.68	37.56	313.15
	82.86	38.00	323.15
Acetone	124.14	68.06	313.15
	123.70	68.24	323.15
Methanol	185.84	39.62	313.15
	182.50	39.99	323.15
Water	395.72	18.16	313.15
	388.66	18.18	323.15
Cholesterol	98.05	324.52	-
Stigmasterol	81.97	356.42	-
Ergosterol	92.12	362.23	-
Palmitic acid	116.80	285.94	313.15
	116.70	285.95	323.15
Stearic acid	119.40	290.33	313.15
	119.53	290.97	323.15

$$\varepsilon_M^{(2)} = \Sigma\Sigma \ \theta_j\theta_k\varepsilon_{jk}^{(2)} \tag{15}$$

$$\varepsilon_M^{(m)} = \Sigma\Sigma \ \theta_j\theta_k\lambda_{jk}\varepsilon_{jk}^{(2)} \tag{16}$$

$$\varepsilon_{ij}^{(2)} = (1-\delta_{ij})\sqrt{\varepsilon^{(2)}_{ii}\varepsilon^{(2)}_{jj}} \tag{17}$$

The binary interaction energy parameter, λ_{jk}, and δ_{ij} are defined for taking into account the multibody interaction among species at an elavated pressure and these parameters were regressed from experimental data obtained in the present study.

2. Determination of EOS Molecular Parameters for Pure Fluids

There are apparently four molecular parameters in the EOS for pure fluids: z. V_H, V_1^*, and ε_{11}. However, as we did previously, we set $z=10$ and $V_H=9.75$ cm³mol⁻¹ since we found that any specific choice of these values has no significant effect to the applicability of the EOS. Thus for real pure fluids we only need to determine two independent molecular parameters, V_1^* and ε_{11} from two independent property data at given temperature.

For gases above the critical point, PVT data were fitted to the pure EOS to determine V_1^* and ε_{11} for each isotherm by the regression analysis. For cosolvent species, parameters are determined using vapor pressure and saturated liquid volume wherein the relation of VLE pressure and chemical potential for vapor and liquid phases, i.e., $P^V = P^L$ and $\mu^V = \mu^L$ were used for each isotherm. For high molecular weight solute species the parameters were determined by the different data sources depends on the availability of existing data sources. For fatty acids whose vapor pressure data are available, the method discussed previously was based. However, for steroids whose vapor pressure data are not in hand, V_1^* was determined from the relation between the hard sphere volume and van der Waals volume. Then the energy parameter ε_{11} was determined using the hard sphere volume and saturation density at boiling point. The van der Waals volumes were obtained by the molecular group contribution method of Bondi [17]. Additionally we examined a quantitative relation between V_1^* and van der Waals volume V_{VDW} at reference temperature for various hydrocarbons including the cosolvent species. As shown in Fig. 3, they have a linear relation.

For the cases of stigmasterol and ergosterol, there were no

existing data for boiling point and density. Thus, the boiling points and densities were estimated based on the group contribution method proposed in the literature [18] and the molecular parameters were determined at one reference temperature. Finally, the molecular parameters for pure systems measured in the present work were summarized in Table 2.

3. Modeling Phase Equilibria

The solutes measured in the present study are solid powders and the phase equilibria between solvents and solutes are the type of solid-vapor equilibria. In the SVE calculation, solid phase was assumed as the pure system. The equilibrium condition then becomes,

$$\mu_i^s = \mu^{sr}_i \tag{18}$$

The chemical potential in the solid phase causes difficulty in calculation since it cannot be estimated using Eq. (10). If both sides of Eq. (18) are subtracted by the chemical potential of a pure subcooled liquid, we have

$$\mu_p^s - \mu_p^o = \mu^{sr}_i - \mu_p^o \tag{19}$$

The l.h.s. of Eq. (10), then, can be expressed by the fugacity ratio,

$$\mu_p^s - \mu_p^o = RT \ \ln(f_p^s/f_p^o) \tag{20}$$

Then, by using the relation of fugacity equation for pure and fundamental thermodynamic relations, the r.h.s of Eq. (10) can alternatively be used the following relation,

$$\frac{P^{sat}\Phi^{sat}\exp\left[\dfrac{V_s(P-P^{sat})}{RT}\right]}{P\Phi_p} = \exp\left(\dfrac{\mu^{sr}_i - \mu_p^o}{RT}\right) \tag{21}$$

Results of data correlation based on the necessary equations presented in this sections were discussed in the next section.

RESULTS AND DISCUSSION

Illustratively, equilibrium solubility of three kinds of steroids (cholesterol, stigmasterol and ergosterol) and two kinds of fatty acids (palmitic acid and stearic acid) in sc-CO₂ were measured

Table 3. Adjustable binary interaction energy parameters

Chemicals		λ_{ij}	ε_{ij}	Temp. [K]
CO₂-	Cholesterol	−0.2066	0.1224	313.15
		−0.0381	0.0100	323.15
	Stigmasterol	−0.0966	0.0586	313.15
		−0.1287	0.0168	323.15
	Ergosterol	−0.0168	0.0103	313.15
		0.0503	−0.0482	323.15
	Palmitic acid	0.0179	0.0241	313.15
		0.0179	0.0241	323.15
	Stearic acid	−0.0798	0.0609	313.15
		−0.0798	0.0609	323.15
CO₂-	Acetone	0.0604	−0.0737	313.15
		0.0604	−0.0737	23.15
	Methanol	−0.2264	0.1219	313.19
		−0.2264	0.1219	323.15
	Water	0.1667	−0.0357	313.15
		0.1667	−0.0357	323.15
Cholesterol-	Acetone	−0.0781	−0.0642	313.15
		0.6959	−0.0014	323.15
	Methanol	0.7852	−0.7469	313.15
		0.1762	0.0548	323.15
	Water	1.3260	0.3778	313.15
		1.4572	0.3326	323.15
Stigmasterol-	Acetone	−0.0414	−0.0906	313.15
		−0.0287	0.0545	323.15
	Methanol	0.0379	0.1529	313.15
		−0.2054	−0.1067	323.15
	Water	0.7553	0.6731	313.15
		0.4339	0.7362	323.15
Ergosterol-	Acetone	−0.2218	−3242	313.15
		−0.2728	−0.4225	323.15
	Methanol	−0.2292	−0.1245	313.15
		−0.2123	−2385	323.15
	Water	1.5164	0.9335	313.15
		1.5717	0.9476	323.15
Palmitic acid-	Acetone	−0.2281	−0.3447	313.15
		−0.3647	−0.3957	323.15
	Methanol	−0.3064	−0.2119	313.15
		−0.4057	−1.6451	323.15
	Water	0.2597	0.4392	313.15
		0.2648	0.5021	323.15
Stearic acid-	Acetone	0.3062	−0.4766	313.15
		1.1383	−1.2618	323.15
	Methanol	0.6573	0.2903	313.15
		0.7249	0.3073	323.15
	Water	0.2896	0.3677	313.15
		0.0856	0.3294	323.15

Table 4. Pure properties used for Peng-Robinson equation of state

| Chemicals	T_c [K]	P_c [bar]	ω
Carbon dioxide	304.2	73.8	0.225
Acetone	508.1	47.0	0.309
Methanol	512.6	81.0	0.559
Water	647.3	220.5	0.344
Cholesterol	790.6	11.1	0.806
Stigmasterol	596.4	10.3	0.789
Ergosterol	651.1	10.2	0.767
Palmitic acid	777.7	13.9	0.975
Stearic acid	810.8	12.3	0.968
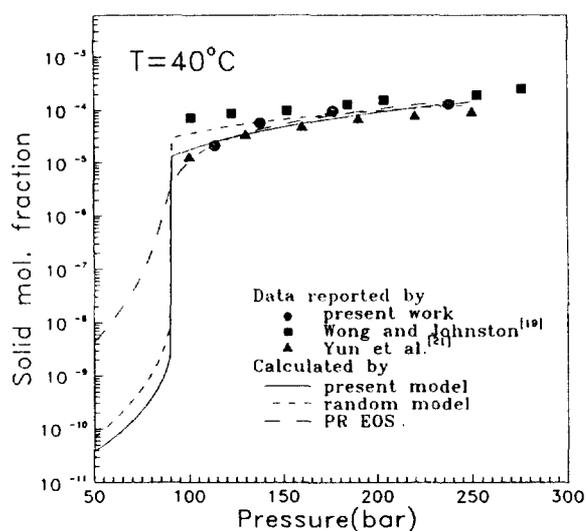
Fig. 4. Measured and calculated solubility of cholesterol in supercritical pure carbon dioxide at 313.15 K.

in the presence of trace amount of cosolvents such as acetone, methanol and water at 313.15-323.15 K and 103.3-241.2 bar. Also, based on these data, three set of two binary interaction energy parameters were obtained for each solute-CO₂-cosolvent ternary system; δ_{ij} for normal mode of binary interaction and λ_{ij} for the multibody interaction. The regression of these results are summarized in Table 3. The specific values of estimated binary interaction energy parameters δ_{ij} and λ_{ij} for the systems with cosolvent acetone and methanol stay within the reference zero, however, for the case of water they tend to deviate significantly from the

reference contrast to the expectation in the modeling process of the EOS. We believe these deviation represent that the EOS cannot exactly correlate the system contained water. Also, we compared the correlations with the Peng-Robinson EOS [22] with the equivalent basis of same number of binary adjustable parameters (2 for binary and 6 for each ternary systems) and we concluded that the present theory is at least comparable to the PR-EOS and the present EOS theory seemed to correlate these ternary systems reasonably well. For further interest readers who intend to conform the results by the PR-EOS, the critical constants and acentric factors used in the present work was summarized in Table 4 wherein the values for solutes were based on the Joback group contribution methods [18]. In comparison, calculated results based on the random case EOS [13] were also summarized in the illustrations.

The SVE data at high pressure are often controversial when one intends to discuss their accuracy and consistency since any experimental apparatus poses an intrinsic difficulty of the control of high equilibrium pressure and sampling at same system pressure. Especially, since we newly devised the microsize equilibrium cell in the present work, we took any point of experimental data with at least three times repeatedly to ensure a reliability of current data. Thus, we simply replaced the thermodynamic consistency test through comparison of data sources in the literatures [19-21]. For illustrative comparisons of present data with others, the solubility of cholesterol in pure sc-CO₂ at 313.15 K were com-
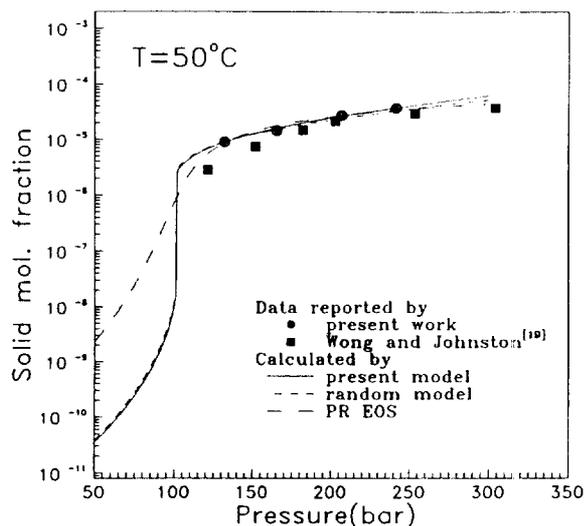
Fig. 5. Measured and calculated solubility of stigmasterol in supercritical pure carbon dioxide at 323.15 K.
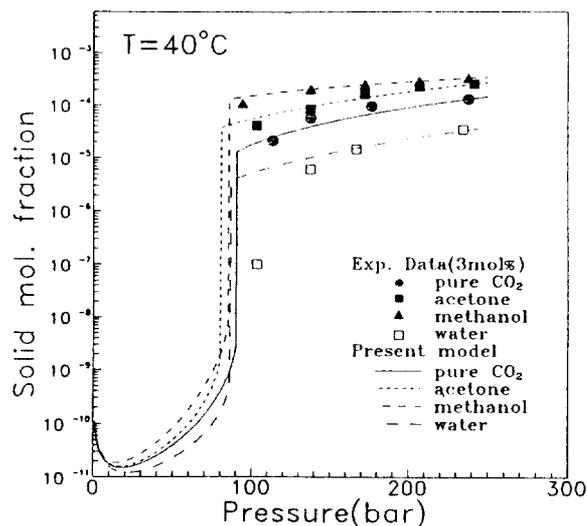

Fig. 7. Effect of cosolvents on the solubility of cholestrol in supercritical carbon dioxide at 313.15 K.
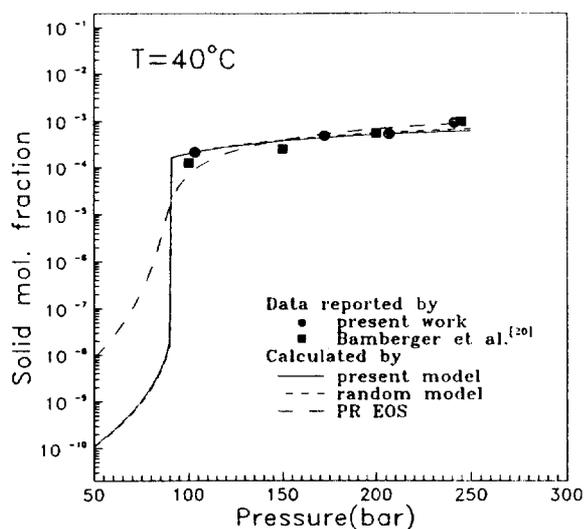
Fig. 6. Measured and calculated solubility of palmitic acid in supercritical pure carbon dioxide at 313.15 K.
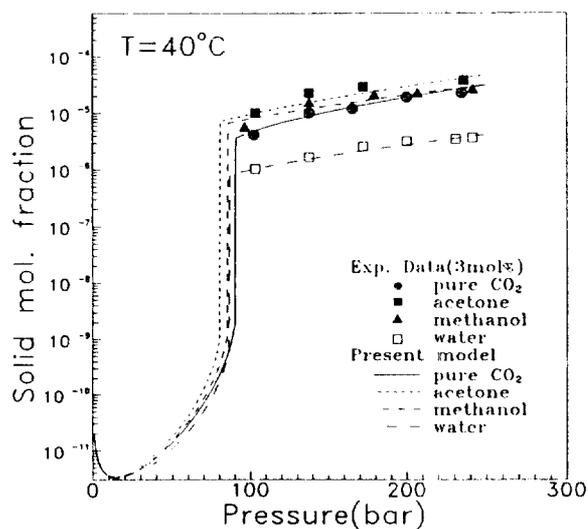
Fig. 8. Effect of cosolvents on the solubility of stigmasterol in supercritical carbon dioxide at 313.15 K.

pared with the data reported by Wong and Johnston [19] and Yun et al. [21], in Fig. 4, together with PR-EOS with two adjustable parameters for pure systems. As one can see in Fig. 4, both the present data and the EOS are acceptable for engineering practice. Similar results were obtained for the solubility of stigmasterol in pure sc-CO₂ at 323.15 K (Fig. 5) and for the system palmitic acid in sc-CO₂ at 313.15 K (Fig. 6).

In Figs. 7-9, experimental data and the correlations by the present EOS for the solubilities of three kinds of steroids (cholesterol, stigmasterol and ergosterol) in sc-CO₂ in the presence of co-solvents were shown, respectively. Expectedly we found that the presence of acetone and methanol significantly enhances the solubility of steroids compared to the case of pure sc-CO₂ while the presence of water tends to decrease significantly the solubility of steroids. Also, in Figs. 10 and 11, data and calculated results for the fatty acids (palmitic acid and stearic acid) in sc-CO₂ with

cosolvents were shown respectively. While the presence of acetone or methanol significantly enhance the solubility of the solutes, the presence of water showed no significant enhancement or decrease of the solubility of fatty acids.

Finally to see schematically the effect of cosolvent on the solubility of each solute in sc-CO₂, each solubility of a solute in mixed sc-CO₂ with 3 mol% cosolvent was divided by the solubility of the solute in pure sc-CO₂ and these results were summarized in Table 4 and in Fig. 12. As one can see in Fig. 12, the presence of cosolvent acetone and methanol (3 mol%) in sc-CO₂ tend to significantly enhance the original solubilities up to 58.1 times. However, the presence of water in sc-CO₂ usually tend to decrease the original solubility up to 10 times. As a result we found that an appropriate choice of polar cosolvent can make it possible the separation of biomolecules by sc-CO₂ and can be applied to the real supercritical extraction problems.
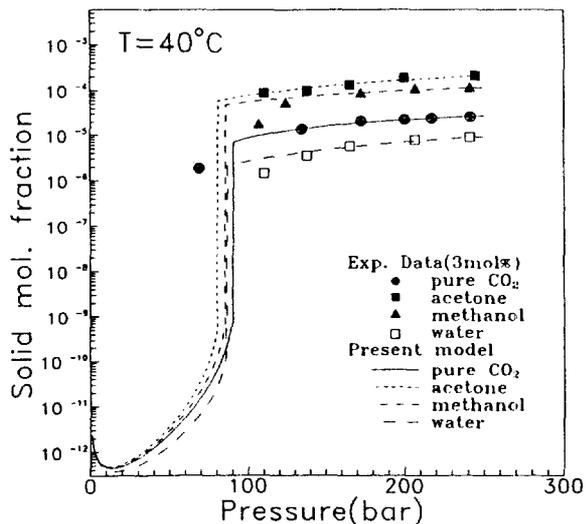
Fig. 9. Effect of cosolvents on the solubility of ergosterol in supercritical carbon dioxide at 313.15 K.
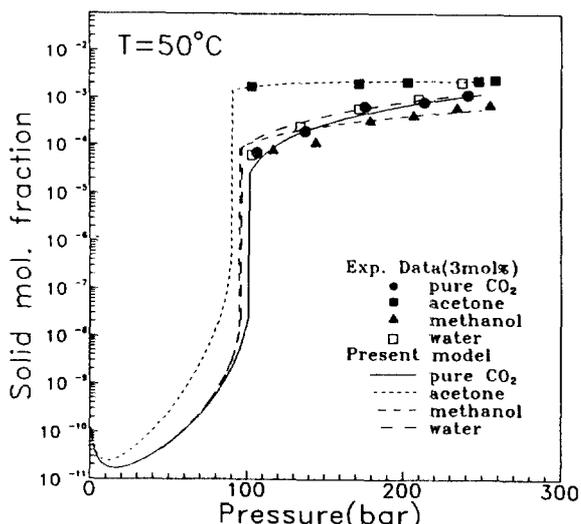

Fig. 11. Effect of cosolvents on the solubility of stearic acid in supercritical carbon dioxide at 323.15 K.
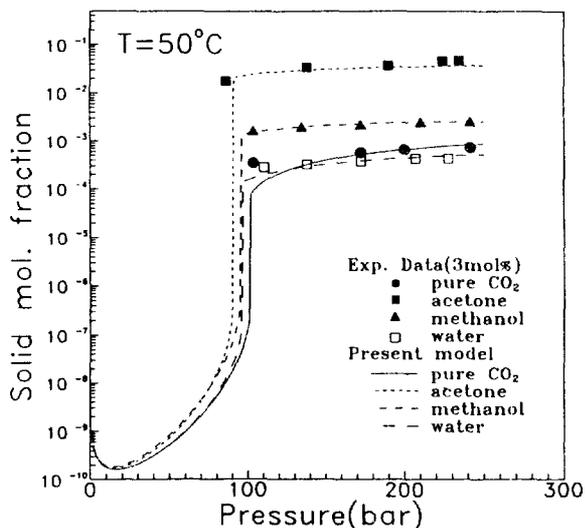

Fig. 10. Effect of cosolvents on the solubility of palmitic acid in supercritical carbon dioxide at 323.15 K.

Table 5. Normalized effect of cosolvent on the solubility of each solutes

Cosolvent		Cholesterol	Stigmasterol	Ergosterol	Palmitic acid	Stearic acid
Methanol	313.15 K	1.77	1.39	5.18	6.7	1.38
	323.15 K	2.16	2.95	5.8	30.0	0.7
Acetone	313.15 K	1.7	2.04	8.3	21.1	2.4
	323.15 K	1.46	3.3	9.8	58.1	2.7
Water	313.15 K	0.1	0.18	0.31	0.72	0.66
	323.15 K	0.33	0.12	0.26	0.7	0.91

CONCLUSION

To measure quickly and reliably the high pressure solubility data of high molecular compounds, we originally devised a new transparent microvolume circulation type equilibrium cell and ac-
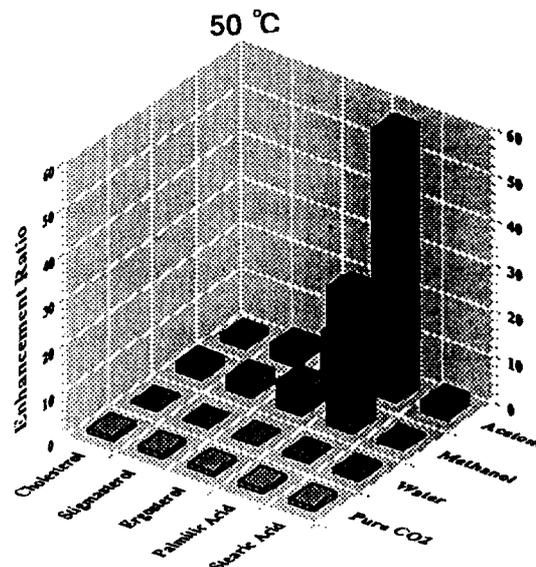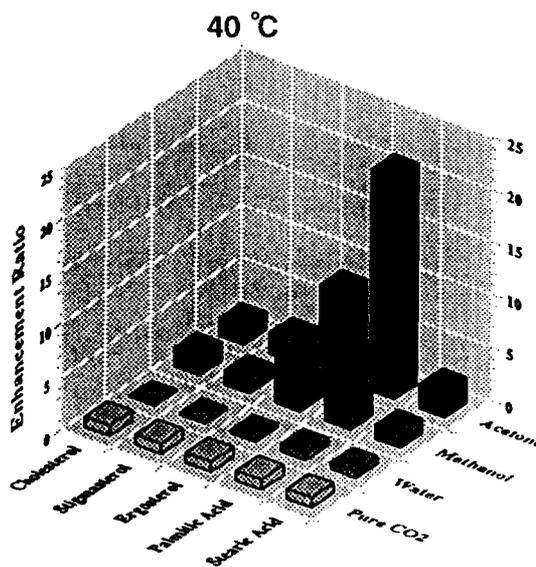

Fig. 12. Normalized 3 mol% cosolvents effects.

cordingly with this apparatus, solubilities of steroids and fatty acids in pure sc-CO_2 and in mixed sc-CO_2 with polar cosolvent were measured.

Also, to provide a new reliable tool for modeling these high pressure systems containing high and complex molecular species, an EOS based on the lattice fluid theory combined with the concept of multibody interaction was newly presented. Although the authors strongly feel that the new EOS needs further refinement, however, with two molecular parameters for pure and an additional interaction adjustable parameter for binary systems and three for ternary systems, the results obtained to date demonstrated the usability of the model to supercritical complex multicomponent systems.

Phenomenologically we found that the presence of acetone or methanol in nonpolar sc-CO_2 can significantly enhance the solubilizing power of biomolecules while the presence of water tend to decrease the solubility of such molecules in sc-CO_2.

ACKNOWLEDGEMENT

NOMENCLATURE

A : configurational Helmholtz free energy [$Jmol^{-1}$]
f_i : fugacity for component i
N_i : number of molecule of component i
N_0 : number of vacant sites or holes
N_q : total number of surface sites including holes
N_r : total number of segments including holes
P : configurational pressure
q_i : surface area parameter
R : universal gas constant [$Jmol^{-1}K^{-1}$]
r_i : number of segment per molecule
T : temperature [K]
U : lattice internal energy
V : molar volume [cm^3mol^{-1}]
V_i^* : molar hard core volume of component i [cm^3mol^{-1}]
V_H : lattice cell volume [cm^3mol^{-1}]
x_i : mole fraction of component i
z : coordination number

Greek Letters

β : reciprocal temperature [1/kT]
$δ_{ij}$: adjustable multibody interaction energy parameter
$ε^{(m)}_M$: multibody molecular interaction energy
$ε_{ij}$: molecular interaction energy
$φ_i$: fugacity coefficient for component i
θ : total surface area fraction including sites and holes

ρ : reduced density
$λ_{ij}$: adjustable interaction energy parameter
$μ_i$: chemical potential for component i

Superscripts

C : combinatorial quantity
(m) : multibody interaction
o : reference pure state property
R : residual quantity
s : solid state property
sat : saturation state property
scf : supercritical fluid state

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