Measurement and Modeling of Epigallocatechin Gallate Solubility in Supercritical Carbon Dioxide Fluid with Ethanol Cosolvent

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To extract epigallocatechin gallate (EGCG) by supercritical carbon dioxide, it is necessary to determine the solubility of EGCG in supercritical carbon dioxide fluid with cosolvents. In this study, the solubility of EGCG was determined in supercritical fluid with different mole fractions of ethanol cosolvent of 0.044 and 0.084 at temperatures ranging from (313 to 333) K and pressures from (15 to 35) MPa. EGCG solubility increased with the mole fraction of ethanol cosolvent. The maximum mole fraction solubility of EGCG under supercritical carbon dioxide is $7.34 \cdot 10^{-4}$ at a pressure of 35 MPa, temperature of 313 K, and mole fraction of ethanol of 0.084. The thermodynamic model, modified Chrastil model, and Mendez-Santiago and Teja model were applied to correlate the solubility data of EGCG in supercritical fluid containing an ethanol mole fraction of 0.084, with an average absolute relative deviation (AARD) of 0.1316, 0.1103, and 0.1109, respectively. While in supercritical CO_2 containing 0.044 ethanol, AARDs of the modified Chrastil model, Mendez-Santiago and Teja model are 0.0745 and 0.0776, respectively. The results show that the modified Chrastil model and Mendez-Santiago and Teja model have a better correlation effect than the thermodynamic model.

Introduction

Epigallocatechin gallate [EGCG, (2*R*,3*R*)-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-1(2*H*)-benzopyran-3,5,7-triol 3-(3,4,5-trihydroxybenzoate), CAS Registry No. 989-51-5, Figure 1] is the largest catechins ingredient in green tea, accounting for 0.65 mass purity of the total catechins.[1] It is widely used in many areas due to its strong antioxidative activity. EGCG also is the most important antioxidative ingredient of catechins.[2,3] Epidemiological and animal studies have demonstrated that EGCG provides protection against a variety of cancers including those of the skin, lung, prostate, and breast.[4−6] EGCG also possesses antiproteolytic, antimutagenic, and antiproliferate activities,[7] thereby regulating the growth of a cell.

Catechins are phenolic compounds found in many plant-derived food products including fruits, berries, chocolate, wine, and green tea. Generally, it is obtained from these natural plants by organic solvent extraction, metal ion precipitation, and separation of macropore adsorbents, and so forth.[8] These processes have drawbacks of multisteps, high cost, and environmental problems.

Supercritical carbon dioxide (SC-CO_2) is an inexpensive, nonflammable, and nontoxic solvent, which is considered to be an attractive alternative to extract the active components from natural material.[9] However, catechin could be hardly dissolved in nonpolar SC-CO_2 due to its strong polarity. Tena et al. extract catechin using CO_2 supercritical fluid under conditions of the extreme pressure and temperature (40 MPa and 100 °C), and only traces of product (10^{-6} mole fraction) were obtained.[10] Hence, it is necessary to add cosolvents to enhance the solubility of EGCG in SFC. Berna et al. used the semicontinuous flow method to measure its solubility in supercritical CO_2 with ethanol cosolvent at a temperature of 313 K and pressure range from (8 to 15) MPa.[11] In this work, the solubility of EGCG was measured in SC-CO_2 with and without cosolvents (ethanol and acetone). The mole fractions of ethanol cosolvent are 0.044 and 0.084, and the acetone is 0.037. The cosolvent effect curve shows the optimum cosolvent ratio and operation conditions.

Semiempirical models and the thermodynamic model were applied to correlate the solubility data. The modified Chrastil equation[12] and the Mendez-Santiago and Teja equation[13] are two common semiempirical models for correlating solubility in SC-CO_2 with cosolvent. In the thermodynamic model, the solubility of EGCG was correlated by the Peng−Robinson equation of state. The van der Waals 1 (VDW1) mixing rules were used for the calculation of the repulsive and attractive parameters. The supercritical parameters of EGCG were estimated by the group contribution method, and the binary interaction parameters k_{12} and k_{23} and the sublimation vapor pressure of EGCG p^{sub} were correlated by experimental data. Related models are useful to design supercritical fluid extraction containing cosolvents.

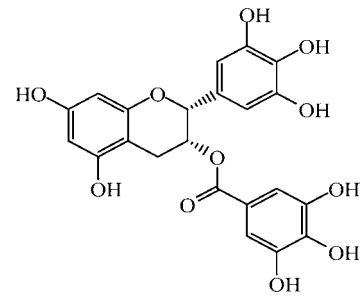

Figure 1. Chemical structure of epigallocatechin gallate (EGCG).

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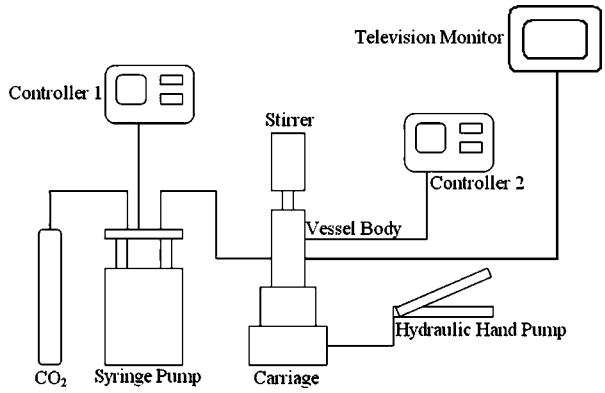

Figure 2. Schematic diagram of the phase equilibrium apparatus.

Experimental Section

Materials. EGCG (CAS Registry No. 989-51-5, 0.99 mole purity, GC grade) was purchased from Shanghai U-sea Biotech Co., Ltd. Absolute ethanol (CAS Registry No. 64-17-5, 0.998 mole purity, GC grade), supplied by a local company, was used as a cosolvent and solvent to collect the extract. High-purity CO_2 (CAS Registry No. 124-38-9, more than 0.999 vol purity, SFC grade) was supplied by Shanghai Wujing Chemical Co., Ltd. (China).

Equipment and Procedure. Super phase monitor SPM20 (Thar Technologies, Inc.) is applied to the determination of the solubility of EGCG in supercritical fluid by using the static method (Figure 2). The CO_2 cylinder used in the SPM20 set a dip tube in it to ensure the CO_2 supplied from the bottom of the cylinder in a liquid state. The syringe pump is used for CO_2 delivery, and prepressurization is controlled by controller 1. The pressure of the pump could be adjusted by the keypad of controller 1. The vessel body has an internal reaction chamber with a sapphire window and a pressure and temperature sensor. Its volume can be adjusted by a piston. The temperature in the vessel could be set on the keypad of the controller 2 and heated by four electrical heaters, while the pressure in it is controlled by utilizing a hydraulic hand pump to control the vessel piston position (also the vessel volume). The phase behavior and dissolution situation in the vessel can be observed on the television monitor via a video camera. The motor-driven stirrer is coupled with the vessel top cap. The vessel can turn in the range of 0° to 180° for the camera viewing flexibility. A sampling valve was designed with a 0.1 mL volume pipe so that samples could be taken out accurately and released into specified volume of ethanol solvents. Hence, the EGCG concentration in the samples could be measured by using high-performance liquid chromatography (HPLC).

CO_2 used in the reaction chamber is prepressurized by the syringe pump. In a typical experiment, a certain amount of cosolvent is first added into the vessel internal chamber. The cosolvent mole amount is determined by calculating the carbon dioxide mole amount at a certain preset pressure, temperature, and volume. The carbon dioxide mole amount is calculated through the following thermodynamic model. If no cosolvent is required, only solid solute is added, and the cap is covered directly. Then an adequate amount of solid solute (EGCG, accurately weighed) is loaded onto the flat, and we can observe its dissolution process though the video camera. After that, the vessel top cap mounted with a stirrer is installed, and the vessel outlet valve is closed. Then the inlet valve is opened, and the prepressurized CO_2 is pumped into the vessel body by the syringe pump until pressure equilibrium is reached. When the inlet valve is closed, the vessel becomes sealed. A record temperature value of the prepressurized liquid CO_2 and the volume value pumped into the vessel, which is shown on controller 1. They are used to calculate the amount of CO_2 pumped into the internal reaction chamber. After that, the heating button on controller 2 is turned on, and the vessel body is heated to the desired temperature set on controller 2; then the system pressure is adjusted by the hydraulic hand pump and balanced for 1 h to ensure mass transfer equilibrium. The vessel is stirred to accelerate the equilibrium. The pressure and temperature are recorded as shown on controller 2. Then the outlet valve is opened to take out a certain volume of the mixture sample in the vessel. During the process, it is important to keep the temperature and pressure stable because the change of these conditions would influence the solubility of solid solute. The pressure is increased and the process repeated, as more equilibrium data could be obtained. It is similar to the change of the temperature. In this paper, the solubility of EGCG in supercritical CO_2 with cosolvents (ethanol and acetone) was determined at temperatures from (313 to 333) K and pressures from (15 to 35) MPa.

Thermodynamic Modeling

Thermodynamic Framework. The compressed gas model was widely used as a thermodynamic model when it is involved supercritical fluid. Supercritical fluid was considered as compressed gas. In this study, we set CO_2, EGCG, and cosolvent as components 1, 2, and 3, respectively.[11−13]

For solid−gas equilibria, the molar solubility of the solid solute in the supercritical fluid can be expressed as

$$y_2 = \frac{p_2^{\mathrm{sub}}}{p\varphi_2^{\mathrm{G}}} \exp\left[\frac{v_2^{\mathrm{S}}(p - p_2^{\mathrm{sub}})}{RT}\right] \quad (1)$$

where y_2 is the solubility (mole fraction) of the solute in the supercritical fluid, p is the pressure, φ_2^{G} is the fugacity coefficient in the supercritical phase, p_2^{sub} is the sublimation vapor pressure of the pure solid, v_2^{S} is the molar volume of the solid, and T is the temperature. In most cases, the p_2^{sub} value is quite small.

φ_2^{G}, the key parameter here, can produce a very large enhancement factor and significantly influence the solubility of solid solvent in SC-CO_2. It is also important to evaluate the φ_2^{G} in thermodynamic modeling, and the accuracy of φ_2^{G} determines the practicability of the model prediction. The Peng−Robinson equation of state (PR-EoS) with the van der Waals 1 (VDW1) mixing rules was applied to calculate φ_2^{G} and correlate the solubility of EGCG in SC-CO_2 containing cosolvent systems. PR-EoS can be expressed as

$$p = \frac{RT}{v_{\mathrm{m}} - b_{\mathrm{m}}} - \frac{a_{\mathrm{m}}}{v_{\mathrm{m}}(v_{\mathrm{m}} + b_{\mathrm{m}}) + b_{\mathrm{m}}(v_{\mathrm{m}} - b_{\mathrm{m}})} \quad (2)$$

where the subscript m means the mixture (solid solvent + CO_2 + cosolvent) under supercritical conditions, p is the pressure (Pa), T is the temperature (K), v_{m} (mol·m^{-3}) is the molar volume, and a_{m} and b_{m} were the parameters of the EoS for mixture, with both of them calculated by VDW1 with one binary interaction parameter (k_{ij}).

$$a_{\mathrm{m}} = \sum_i \sum_j y_i y_j (a_i a_j)^{0.5}(1 - k_{ij}) \quad (3)$$

$$b_{\mathrm{m}} = \sum_i y_i b_i \quad (4)$$

where a_i and b_i were the parameters of EoS for component i. Both of them can be determined by the critical constants of the

Table 1. Critical Constants of Every Related Compound

compound	T_c / K	P_c / MPa	V_c / cm^3·mol^{-1}	ω
CO_2	304.15	7.376	94.18	0.225
EGCG	1450.5	2.749	942.5	–0.032
ethanol	516.25	6.379	167.1	0.635
acetone	508.2	4.66	209	0.318

pure compounds, namely, critical temperature T_{ci}, critical pressure p_{ci}, and acentric factor ω_i, and so forth.

For EGCG, the critical constants are hard to obtain by the experimental method, and there are no published data. Here, the Lydersen group contribution method was applied to estimate critical constants of the solid solute. The critical constants of CO_2 and cosolvent ethanol can be found from published data. The values obtained were shown in Table 1. The binary interaction parameter for CO_2 and ethanol, k_{12}, was accepted to be 0.086,[14] while the binary interaction parameters k_{13} and k_{23} were calculated from experimental measurements of the solubility of EGCG in SC-CO_2 with ethanol as cosolvent. Particularly, the sublimation vapor pressure of EGCG, p_2^{sub}, is also considered as a parameter, which can be obtained by experimental data simulation. All parameters were optimized by minimizing the average absolute relative deviation (AARD):

$$\mathrm{AARD} = \frac{1}{n}\sum \frac{|y_2^{cal} - y_2^{exp}|}{y_2^{exp}} \tag{5}$$

where n is the number of data points for a given temperature. The superscripts cal and exp represent the calculated and experimental data, respectively.

Empirical Models. Compared with the thermodynamic model, it is unnecessary for the empirical model to acquire information on thermodynamic property data. Particularly, it is hard to obtain thermodynamic parameters for a number of solid solutes. In this case, empirical models are widely applied. For the system of SC-CO_2 with cosolvents, the modified Chrastil model and Mendez-Santiago and Teja model are two useful empirical models, which correlate the solubility of a solute in a supercritical solvent to the density, the concentration of cosolvent, temperature, and/or pressure.

The modified Chrastil model is based on the assumption that the solute is associated with the solvent to form a solvated complex. Its formula is expressed as follows:

$$\ln S = k \ln \rho + \gamma \ln m + \alpha/T + \beta \tag{6}$$

where S is the solubility of the solute (kg·m^{-3}), ρ is the density of CO_2 and cosolvent complex (kg·m^{-3}), m is the concentration of cosolvent (kg·m^{-3}), T is the temperature (K), and k and γ are the association numbers of carbon dioxide and cosolvent, respectively. α and β are parameters; α depends on the solvation enthalpy and vaporization enthalpy of the solute, and β depends on the molecular weight and melting point of the three compounds. In this work, S and ρ are calculated by using PR-EoS with VDW1 mixing rules.

$$S = \frac{M_2 y_2}{v_m} \qquad \rho = \frac{M_1 y_1 + M_3 y_3}{v_m} \tag{7}$$

where M_i (kg·mol^{-1}) is the molar weight of component i.

All parameters of the model were optimized by minimizing the AARD of the calculated solubility and the experimental values.

The Mendez-Santiago and Teja model was derived by using a classical expansion of the Helmholtz energy around the critical point of the solvent to describe the mixture properties at infinite dilution.[15,16] The Mendez-Santiago and Teja model was expressed as

$$T \ln(y_2 p) = A + B\rho + Cm + DT \tag{8}$$

where A, B, C, and D are the parameters, which were optimized by minimizing the AARD of the calculated solubility and the experimental values.

Table 2. Mole Fraction Solubility (y_2) of EGCG in SC-CO_2 with or without Cosolvent and the System Density

		without cosolvent		0.037 acetone		0.044 ethanol		0.084 ethanol	
T / K	P / MPa	$10^4\ y_2$	ρ / kg·m^{-3}	$10^4\ y_2$	ρ / kg·m^{-3}	$10^4\ y_2$	ρ / kg·m^{-3}	$10^4\ y_2$	ρ / kg·m^{-3}
313	15.0	0.13	748.5	0.30	801.1	1.41	804.0	2.36	840.2
	20.0	0.35	830.2	0.36	856.5	2.22	863.2	3.51	885.3
	25.0	0.52	885.7	0.46	897.6	4.52	906.5	6.23	920.1
	30.0	0.77	928.2	0.52	930.4	5.28	940.9	6.92	948.6
	35.0	0.83	962.9	0.78	957.8	5.83	969.6	7.34	972.8
323	15.0					1.12	728.1	2.10	776.3
	20.0					1.93	804.7	3.07	833.7
	25.0					4.08	857.1	5.48	875.7
	30.0					4.95	897.4	6.31	909.0
	35.0					5.21	930.4	7.22	936.8
333	15.0					0.98	643.8	1.50	704.8
	20.0					1.23	742.7	2.63	778.5
	25.0					2.75	805.8	5.20	829.1
	30.0					3.46	852.6	5.91	868.0
	35.0					4.20	890.1	6.80	899.8

Results and Discussion

The solubility of EGCG in SC-CO_2 with and without cosolvent was determined at (313, 323, and 333) K. In the pressure range from (15 to 35) MPa, following the method described in the Experimental Section, results are summarized in Table 2. Each experimental data point is an average value of three experimental solubility measurements. The density data of SC-CO_2 and SC-CO_2 + ethanol binary mixture shown in Table 2 were calculated by PR EoS with the VDW1 mixing rules.

Effect of Pressure on Solubility of EGCG. The solubility of EGCG increases with the increase of pressure, while the increase of pressure raises the solvent density to result in an increase of solubility due to the stronger solute−solvent interactions. Raising the pressure at constant temperature would increase the density of SC CO_2, thereby increasing the interaction between the solute and the solvent molecules.

Effect of Temperature on Solubility of EGCG. The solute vapor pressure, solvent density, and intermolecular interactions in the fluid phase are influenced by the system temperature, and their contributions to the solubility of EGCG vary with the temperature. The crossover phenomena could be mainly attributed to the combined effects of increasing solute vapor pressure and decreasing solvent density with the increase of temperature.

Effect of Cosolvent on Solubility of EGCG in Supercritical Carbon Dioxide. As Figure 3 shows, the solubility of EGCG was very low in pure supercritical CO_2. The addition of ethanol can significantly improve its solubility. This would cause intermolecular interactions of hydrogen bonds between EGCG and cosolvent molecules.

The cosolvent effect is defined as the ratio between the solubility of EGCG in the presence of the cosolvent and in the pure CO_2 under the same conditions. The effect is presented in Figure 4. The cosolvent effect was obvious in the ethanol mole fraction of 0.084, and the maximum solubility of EGCG in the condition is 18 times higher than that in pure supercritical CO_2.

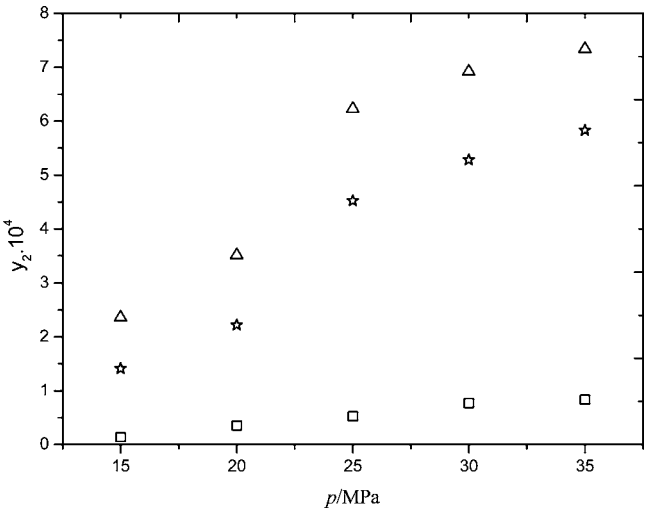

Figure 3. Effect of pure carbon dioxide and different mole fractions of ethanol cosolvent on the solubility of EGCG at a temperature of 313 K: □, 0.00; ☆, 0.044; △, 0.084.

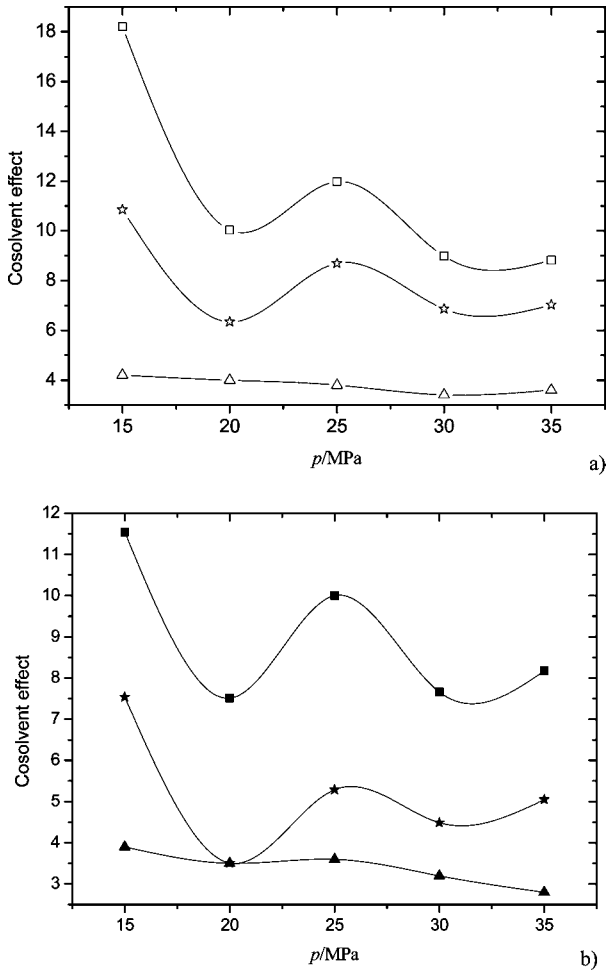

Figure 4. Cosolvent (mole fraction) effect on the solubility of EGCG at temperatures of 313 K (a): △, 0.037 acetone; ☆, 0.044 ethanol; □, 0.084 ethanol; and 333 K (b): ▲, 0.037 acetone; ★, 0.044 ethanol; ■, 0.084 ethanol.

The cosolvent effect was also obvious in the ethanol mole fraction of 0.044. In addition, whatever the cosolvent ethanol with 0.044 or 0.084 mole fraction, the cosolvent effect is higher under 313 K than that under 333 K.

The cosolvent effect is directly related to the difference between local and bulk densities, and the higher the difference

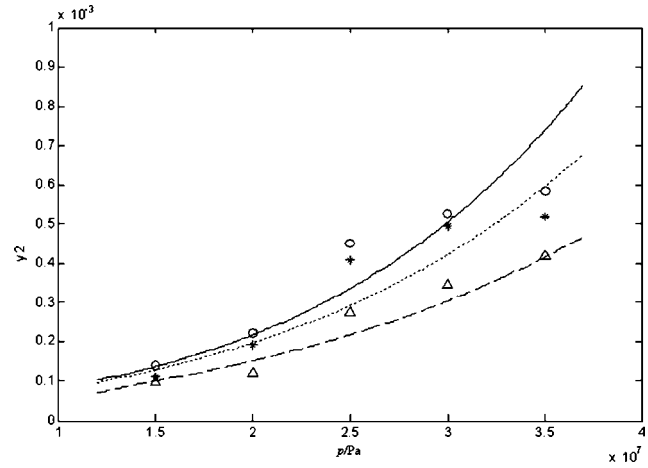

Figure 5. Correlation of the solubility of EGCG and pressure in SC-CO_2 with an ethanol mole fraction of 0.044 as cosolvent based on the PR-EoS thermodynamic model: ○, exp-313 K; ∗, exp-323 K; △, exp-333 K; —, cal-313 K; ····, cal-323 K; ---, cal-333 K.

in density, the larger the cosolvent effect.[17,18] These large local densities are induced directly and indirectly by solute−solvent interactions, and the difference between the local density and bulk density is maximized close to the critical point of the solvent.[19,20] The critical temperature with the ethanol mole fraction of 0.044 is 316 K, and for 0.084, it is 326 K, which is closer to the experimental temperature than that of pure CO_2 solvent (304 K). The cosolvent effect can be attributed to the higher local density enhancements induced by solute−solvent interactions.

The cosolvent mole fraction with 0.044 ethanol and 0.037 acetone have approximately the same critical temperature (as in Table 1), but the cosolvent effect is higher in the ethanol mole fraction of 0.044. A possible explanation is the other interactions are involved in both systems.[21,22] The CO_2-ethanol solvent contains more hydroxyl groups due to the lower molar mass of ethanol than that of acetone, so it can form more hydrogen bonds with EGCG, resulting in a higher cosolvent effect. For molar quantities, the two cosolvent mole fractions are 0.044 ethanol and 0.037 acetone, respectively; thus the number of hydroxyl groups in the former was only 0.28 higher than in the latter, but the solubility of EGCG in the former was on average 1.2 times higher than that in the latter. There is the possibility of higher effects in acetone cosolvent than ethanol cosolvent, which could make the formation of complexes more difficult in the presence of acetone than in ethanol.

As seen in Figure 4, the cosolvent effect decreases with the increase of pressure. Particularly, this situation is more obvious for the ethanol mole fraction of 0.084 as cosolvent. This can be explained by the decrease of the difference between the local and the bulk densities. With the increase of pressure, the system moves away from its critical point ((8.6 and 10.5) MPa for ethanol mole fraction of 0.044 and 0.084 mixed solvents, respectively).

Correlation of Experimental Solubility Data. The PR-EoS with the VDW1 mixing rule was used to correlate the experimental solubility data of EGCG in SC-CO_2 with ethanol as the cosolvent. Figure 5 shows a comparison between the experimental solubility of EGCG measured in this work and the PR-EoS calculation with the VDW1 mixing rule. Table 3 gives the fitted value of binary interaction parameters k_{12} and k_{23}, sublimation vapor pressure of EGCG p_2^{sub} at different temperatures, and the absolute average standard deviation between the experimental data and the calculated data of EGCG

Table 3. Fitting Parameters of the Thermodynamic Model and AARD through Figure 4 Correlation

cosolvent mole fraction	k_{12}	k_{23}	p^{sub}/Pa 313 K	323 K	333 K	AARD
0.044	–0.021	0.187	0.01474	0.01992	0.02257	0.1316

in SC-CO_2 at different contents of ethanol. It could be seen that p_2^{sub} gets larger with the increase of temperature.

In the modified Chrastil model, the logarithmic solubility−density relationship shows a linear behavior for all of the curves (Figure 6). The parameters of the model are obtained by performing a multiple linear regression on ln *S* as a function of ln ρ, ln *m*, and 1/*T*, and the correlated results are shown in Table 4. The values of association number *k* and γ are 7.933 and 2.521, respectively. That means 7.933 CO_2 molecules and 2.521 ethanol molecules associate with one molecule of solute EGCG to form a solvated complex in supercritical circumstance. *k* is larger than γ because the amount of CO_2 is larger than ethanol in the system. The value of α is negative, and this means that the associating process is an endothermal reaction. The AARDs of the fitted Chrastil equation for ethanol mole fraction of 0.044 and 0.084 were 0.1103 and 0.0745, respectively.

In the Mendez-Santiago and Teja model, $T \ln(y_2 p)$ and the density of the mixture show a linear behavior for all of the curves, as illustrated in Figure 7. The parameters of the model are obtained by performing a multiple linear regression on $T \ln(y_2 p)$ as a function of ρ, *m*, and *T*. The correlated results are shown in Table 5. The AARDs of the fitted equation for ethanol mole fractions of 0.044 and 0.084 were 0.1103 and 0.0745, respectively. Taking the thermodynamic and empirical models into consideration, we can see that the empirical models have a better accuracy than the thermodynamic model, while the thermodynamic model could predict the solubility of solutes.

Conclusions

The addition of polar cosolvent ethanol to supercritical CO_2 can improve the solubility of EGCG in the supercritical CO_2.

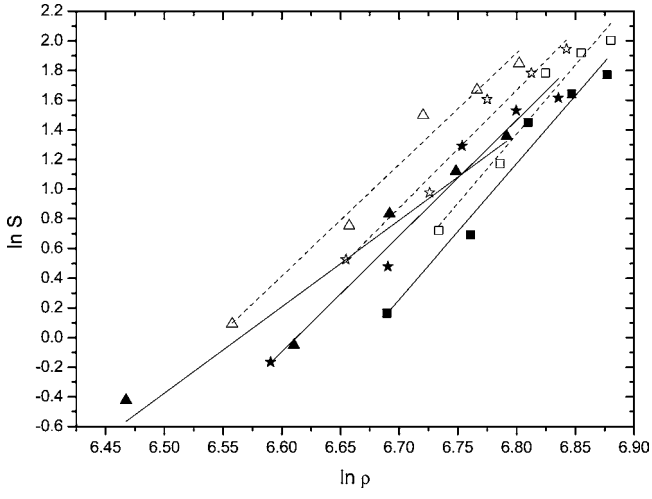

Figure 6. Relationship between the solubility of EGCG and the density of the mixture for the modified Chrastil model: △, 0.084 ethanol at 333 K; ☆, 0.084 ethanol at 323 K; □, 0.084 ethanol at 313 K; ★, 0.044 ethanol at 323 K;▲, 0.044 at 333 K; ■, 0.044 at 313 K.

Table 4. Fitting Parameters of the Modified Chrastil Model ($\ln S = k \ln \rho + \gamma \ln m + \alpha/T + \beta$) and % AARD

cosolvent mole fraction	*k*	γ	α	β	AARD
0.044	7.933	2.521	–93168	1240.3	0.1103
0.084					0.0745

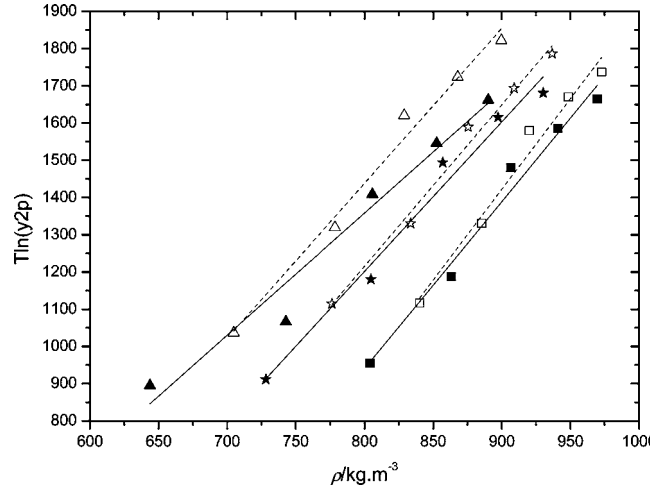

Figure 7. Relationship between $T \ln(y_2 p)$ and the density of the mixture for Mendez-Santiago and Teja model: △, 0.084 ethanol at 333 K; ☆, 0.084 ethanol at 323 K; □, 0.084 ethanol at 313 K; ★, 0.044 ethanol at 323 K; ▲, 0.044 at 333 K; ■, 0.044 at 313 K.

Table 5. Fitting Parameters of Mendez-Santiago and Teja Model ($T \ln(y_2 p) = A + B\rho + Cm + DT$) and AARD

cosolvent mole fraction	*A*	*B*	*C*	*D*	AARD
0.044	–50353	4.354	7113.4	61.455	0.1109
0.084					0.0776

The solubility of EGCG in supercritical mixed solvents containing ethanol increases with the ratio of ethanol in supercritical CO_2. Cosolvent effects were maximized in supercritical fluid containing the 0.9 CO_2 and 0.084 ethanol mixed solvent, and operating conditions were closer to the critical point of the solvent. The thermodynamic model and two empirical models including the modified Chrastil model and Mendez-Santiago and Teja model were applied to correlate the solubility data of EGCG. The results show that the two empirical models have better correlation effects than the thermodynamic model. On the other hand, the thermodynamic model can predict the solubility of EGCG, while empirical models cannot. This solubility data can be useful for EGCG extraction from green tea by supercritical fluid conditions.

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