Articles

Solubility of Ferulic Acid and Tetramethylpyrazine in Supercritical Carbon Dioxide

Yongyue Sun,[†,‡] Shufen Li,[*,†] and Can Quan[†]

Key Laboratory for Green Chemical Technology of the State Education Ministry, School of Chemical Engineering & Technology, Tianjin University, Tianjin 300072, PR China, and Tianjin Municipal Institute for Drug Control, Tianjin 300070, PR China

The solubility of ferulic acid (3-(4-hydroxy-3-methoxyphenyl)-2-propenoic acid) and tetramethylpyrazine (2,3,5,6,-tetramethylpyrazine) in supercritical carbon dioxide (SC-CO_2) was determined at temperatures from (308 to 338) K and pressures from (10 to 35) MPa. The data presented in this work will help to assess possibility of extraction from *Ligusticum chuanxiong* with SC-CO_2. Experimental data of the solubility of ferulic acid and tetramethylpyrazine were correlated by a Chrastil equation, and the values of average absolute relative deviation (AARD) were 12.92 % and 4.23 %.

Introduction

One of the modern mild methods used for obtaining natural products is supercritical fluid extraction (SCFE) because it possesses several advantages over traditional liquid-solvent-based extraction methods including improved selectivity, expeditiousness, automation, and environmental safety.[1,2] However, the solubility of the compounds to be extracted needs to be known if an efficient SCFE process is to be designed.

Ferulic acid and tetramethylpyrazine are the most important medical components of *Ligusticum chuanxiong*. Ferulic acid possesses antioxidative properties by virtue of the phenolic hydroxyl group in its structure. Studies have shown that ferulic acid could inhibit malondialdehyde (MDA) production from platelets,[3] inhibit erythrocyte lysis induced by MDA and hydroxyl radicals, and inhibit lipid peroxidation induced by H_2O_2 and $O_2^{\bullet-}$.[4] Furthermore, studies have suggested that the antioxidative activity of ferulic acid might be the result of the direct capture of free radicals by the phenolic hydroxyl group.[5,6] Tetramethylpyrazine was known to increase cerebral blood flow and ischemic attacks.[7] It was reported that tetramethylpyrazine might ameliorate learning deficits induced by permanent occlusion of the bilateral common carotid arteries in rats,[8] and its effectiveness as a cognitive enhancer had also been demonstrated.[9]

To extract ferulic acid and tetramethylpyrazine from *Ligusticum chuanxiong* with SCFE effectively, the solubility of ferulic acid and tetramethylpyrazine in SC-CO_2 needs to be known. But the solubility of tetramethylpyrazine in SC-CO_2 has not been reported. Murga[10] and Sovová[11] had previously reported the solubility of ferulic acid. But the former only determined the solubility under the temperatures of 313K, 323K and 333K, and the latter determined the solubility under the temperatures of 301K, 313K, 323K and 333K. Furthermore, some of their solubility data differed greatly. The present investigation was undertaken to determine the solubility of ferulic acid and tetramethylpyrazine in SC-CO_2, under the conditions of temperatures from (308 to 338) K and pressures from (10 to 35) MPa.

* Corresponding author. E-mail: shfli@tju.edu.cn. Tel: +86-22-2740-8578. Fax: +86-22-2740-7388.
† Tianjin University.
‡ Tianjin Municipal Institute for Drug Control.

Experimental Section

Materials and Chemicals. The carbon dioxide (purity 99.9 %) was used as a supercritical fluid. Ferulic acid (purity 99 %) was purchased from Aldrich. Tetramethylpyrazine(purity 98 %) was purchased from Acros Organics (USA). Ethanol (HPLC grade) was supplied by Tianjin Chemical Reagent Factory (Tianjin, China). All chemicals were used without further purification.

Experimental Procedure. Solubility determination was performed through a Spe-ed SFE instrument (Applied Separations Inc., Allenton, PA), shown schematically in Figure 1. Liquid CO_2 was pressurized with a gas booster pump and then charged into the extraction column to desired pressure. A digital pressure gauge (OMEGA DP-41, uncertainty 0.1MPa) measured and indicated the operating pressure to an accuracy of about 1 % over the measuring rang. The extraction column is 10 mL with 11.1 mm inner diameter and 103.5 mm long, being packed with alternative layers of the test solid (about 500 mg) and glass beads (30−60) mesh, with glass wool at either end. The extraction column was heated through an oven and its temperature was indicated and controlled by the thermocouple to within ±0.1K. The supercritical CO_2 with dissolved solutes passed from the extraction column through a heated micrometer valve and expanded to ambient pressure. The solid solute was absorbed by ethanol in the vial. The gas flowed through the vial further to the wet test meter to measure its volume. The flow rate was controlled by the micrometer valve, varying from 0.10 to 0.15 standard L min^{-1}; within this range, the flow rate did not affect the equilibrium solubility. As small part of the

10.1021/je049715y CCC: $30.25
Published on Web 06/03/2005

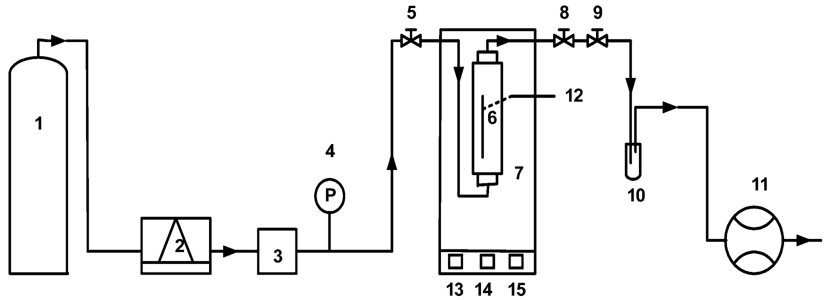

Figure 1. Schematic diagram of the experimental apparatus. 1, CO_2 cylinder; 2, liquid-cooled bath; 3, gas booster pump; 4, pressure gauge; 5, inlet valve; 6, extraction column; 7, constant-temperature oven; 8, outlet valve; 9, micrometer valve; 10, vial; 11, wet-test meter; 12, thermocouple; 13, oven temperature indicator; 14, column temperature indicator; 15, micrometer valve temperature indicator.

Table 1. Ferulic Acid Solubility in SC-CO_2: Pressure *P*, Temperature *T*, SC-CO_2 Density *ρ*, Mass Fraction Solubility *S*, and Mole Fraction Solubility *y*

T/K	P/MPa	ρ/g·L^{-1}	10^6S	10^7y	P/MPa	ρ/g·L^{-1}	10^6S	10^7y
308	10	707.9	3.061	6.936	25	901.7	4.537	10.279
	15	815.0	3.663	8.299	30	929.6	5.204	11.791
	20	866.1	4.101	9.292	35	952.8	6.010	13.617
318	10	493.3	2.019	4.574	25	857.7	6.083	13.781
	15	741.8	4.080	9.245	30	890.9	7.325	16.596
	20	813.1	5.022	11.378	35	917.8	8.076	18.299
328	10	336.2	1.126	2.551	25	811.2	7.068	16.014
	15	653.7	4.399	9.966	30	850.8	8.316	18.841
	20	755.0	5.570	12.619	35	881.9	9.411	21.323
338	10	268.2	0.969	2.195	25	762.4	7.965	18.046
	15	555.5	4.197	9.509	30	809.5	10.062	22.799
	20	692.3	5.947	13.475	35	845.2	11.708	26.527

Table 2. Tetramethylpyrazine Solubility in SC-CO_2: Pressure *P*, Temperature *T*, SC-CO_2 Density *ρ*, Mass Fraction Solubility *S*, and Mole Fraction Solubility *y*

T/K	P/MPa	ρ/g·L^{-1}	S	y	P/MPa	ρ/g·L^{-1}	S	y
318	10	493.3	0.030	0.010	25	857.7	0.062	0.020
	15	741.8	0.046	0.015	30	890.9	0.068	0.022
	20	813.1	0.056	0.018				
328	10	336.2	0.046	0.015	25	811.2	0.144	0.046
	15	653.7	0.109	0.035	30	850.8	0.163	0.053
	20	755.0	0.135	0.044				
338	10	268.2	0.092	0.030	25	762.4	0.381	0.123
	15	555.5	0.240	0.077	30	809.5	0.404	0.131
	20	692.3	0.317	0.102				

solute settled inside the micrometer valve, ethanol was used to rinse repeatedly the micrometer valve in order to collect the solute quantitatively.

The procedure was repeated in triplicate at the identical operating conditions and the average valve was used as the solubility.

Analysis of the solute was carried out off-line by using a UV spectrophotometer (Ruili analysis instrument (China), UV1600, uncertainty 0.3 %). The wavelength was set at 320 nm for ferulic acid analysis, and 279 nm for tetramethylpyrazine.

Results and Discussion

The solubility of ferulic acid in SC-CO_2 was determined at temperatures from (308 to 338) K and pressures from (10 to 35) MPa. The solubility of tetramethylpyrazine was determined at temperatures from (318 to 338) K and pressures from (10 to 35) MPa. The experimental solubility data, at different conditions of pressure, P, and temperature, T, are listed in Tables 1 and 2 The density of CO_2 at the given pressure and temperature, obtained from the IUPAC International Thermodynamic Tables,[12] is also included in Tables 1 and 2.

Data Reproducibility. The relative standard deviation (RSD) was used to test the reproducibility of the determination. The lower the value of the RSD, the better the reproducibility. The RSD was calculated according to eq 1

$$\mathrm{RSD}/\% = \frac{\sqrt{\sum_{i=1}^{n}(S - \bar{S})^2/(n-1)}}{\bar{S}} \times 100 \qquad (1)$$

$$\bar{S} = \left(\frac{1}{n}\right)\sum_{i=1}^{n} S \qquad (2)$$

where n is the number of the determination under identical operating conditions and S is the mass fraction solubility of the solute.

The value of RSD is within 15 % for the ferulic acid determination and within 12 % for the tetramethylpyrazine determination. In some published studies, the somewhat low reproducibility also often appeared. For example, the standard error of the ferulic acid solubility determination was within 7 % and 8 % by Murga[10] and Sovová,[11] respectively; it was within 14 % for the solubility determination of *p*-coumaric acid,[10] within 16 % for protocatechuic acid,[13] within 13 % for methyl gallate,[13] and within 12 % for protoatechualdehyde.[13] The main source of the variation of the measured values was most probably an early precipitation of a small part of the extracted solid solute in front of the micrometer valve and the irregular discharge of this precipitate. Another source of the variation of the measured values was probably the measured errors, especially for the low solubility of the solute in supercritical fluid. Therefore, we can assume that the measured values fluctuate around the correct solubility value, which should be within the above given reproducibility limits.

Effect of Temperature and Pressure. From Table 1 and Figure 2, it can be seen that the solubility of ferulic acid increases with pressure at constant temperature. The effect of temperature is more complex, and retrograde solubility (crossover pressure) behavior[14-16] can be observed. The crossover pressure can be defined as the point where the slope of the plot of solubility versus temperature changes sign. It should not change with temperature. At the crossover pressure, the effects of solute vapor pressure and solvent density on solid solubility balance each other.[15,16] At pressures above the crossover pressure, the solubility of ferulic acid increases with temperature, whereas at pressures below the crossover pressure it decreases with temperature.

The solubility of tetramethylpyrazine also increases with pressure at constant temperature, as shown in Figure 3. Although tetramethylpyrazine does not show the retrograde solubility behavior in the range of pressures studied in this work, its solubility increases with temperature.

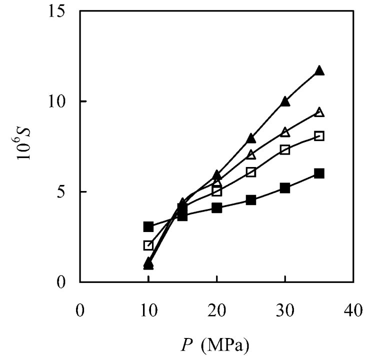

Figure 2. Effect of temperature and pressure on ferulic acid mass fraction solubility (S): ■, 308 K; □, 318 K; △, 328 K; ▲, 338 K.

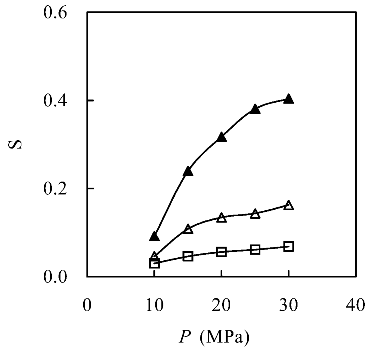

Figure 3. Effect of temperature and pressure on tetramethylpyrazine mass fraction solubility (S): □, 318 K; △, 328 K; ▲, 338 K.

Table 3. Comparison of Ferulic Acid Mole Fraction Solubility between This Work and References

T/K	P/MPa	10^7y^a	T/K	P/MPa	10^7y^b	T/K	P/MPa	10^7y^c
308	10	6.936	301	12	21.3			
	15	8.299		15	24.7			
	20	9.292		20	32.2			
	25	10.279						
	30	11.791						
	35	13.617						
318	10	4.574	313	12	19.5	313	10	8.225
	15	9.245		15	27.0		15	36.680
	20	11.378		20	42.5		20	65.480
	25	13.781		25	54.0		25	88.290
	30	16.596		28	62.1		30	112.806
	35	18.299					35	138.776
328	10	2.551	323	12	15.5	323	10	1.119
	15	9.966		15	34.9		15	40.256
	20	12.619		20	53.8		20	84.335
	25	16.014		25	85.3		25	134.271
	30	18.841		28	98.5		30	155.990
	35	21.323					35	186.018
338	10	2.195	333			333	10	0.653
	15	9.509		15	32.2		15	38.310
	20	13.475		20	67.7		20	108.507
	25	18.046		22	79.8		25	182.259
	30	22.799		25	98.1		30	244.847
	35	26.527		28	118.0		35	314.725

[a] This Work. [b] Sovová. [c] Murga.

Comparison of Ferulic Acid Data. A comparison of the solubility data of ferulic acid with existing data[10,11] is listed in Table 3. These three data sets deviate from each other, although the same method (dynamic method) has been applied for the solubility measurement. The main reason may come from the three tested ferulic acids, which were purchased from different sources with different purities (e.g., 97 % (Sigma);[10] 98 % (Fluka);[11] and 99 % (Acros Organics) in this work). The 1−3 % impurities may affect the measurement accuracy. Additionally, operation and measurement errors may also exist.

Data Correlation. The Chrastil equation,[17] which assumes the formation of a solvate complex between molecules of the SC solvent and the solute at equilibrium, was used for data correlation. This equation has the advantage of having only three parameters to fit all of the experimental data regardless of the temperature at which they are obtained. Moreover, it does not require an estimation of the properties of the pure components.

The Chrastil equation leads to the following linear relationship between the solubility of the solute, c, expressed as (g of solute)/(L of solvent), and the density of the solvent, ρ, in g/L, for a given temperature (in K):

$$\ln c = k \ln \rho + \frac{a}{T} + b \qquad (3)$$

a, b, and k are the adjustable parameters of the model.

The experimental data are correlated by the Chrastil equation. The Chrastil correlation equation for the solubility of ferulic acid in SC-CO_2 is

$$\ln(c/\text{g}\cdot\text{L}^{-1}) = 3.12 \ln(\rho/\text{g}\cdot\text{L}^{-1}) - \frac{3528.36}{T/\text{K}} - 15.40 \qquad (4)$$

The Chrastil correlation equation of tetramethylpyrazine in SC-CO_2 is

$$\ln(c/\text{g}\cdot\text{L}^{-1}) = 2.33 \ln(\rho/\text{g}\cdot\text{L}^{-1}) + \frac{10\,814.70}{T/\text{K}} + 22.15 \qquad (5)$$

AARD is used to test the correlation results. The lower the value of the AARD, the better the correlation results. It is calculated according to the following equation

$$\text{AARD}/\% = \frac{100}{n}\sum\left|\frac{c_{\text{calcd}} - c_{\text{exptl}}}{c_{\text{exptl}}}\right| \qquad (6)$$

where n is the number of solubility data used to obtain the parameters and c_{calcd} and c_{exptl} are the calculated and experimental data (g/L), respectively.

The value of AARD was 12.92 % for ferulic acid and 4.23 % for tetramethylpyrazine. It can be seen that the correlation of tetramethylpyrazine was better than that of ferulic acid.

Literature Cited

(1) McHugh, M. A.; Krukonis, V. J. *Supercritical Fluid Extraction: Principles and Practice*; Butterworths: Boston, 1986.
(2) Williams, D. F. Extraction with Supercritical Gases. *Chem. Eng. Sci.* **1981**, *11*, 1769−1788.
(3) Yin, Z. Z.; Wang, J. P.; Xu, L. N. Effect of Sodium Ferulate on Malondialdehyde from Platelets of Rats. *Acta Pharmacol. Sin.* **1986**, *7*, 336−339.
(4) Pan, H. Z.; Zhao, C. Y.; Xu, L. N. The Effect of Sodium Ferulate on Lipid Peroxidation of Eryrocyte Membrane. *Chin. J. Integr. Tradit. West. Med.* **1985**, *5*, 678−680.
(5) Ju, H. S.; Li, X. J.; Zhao, B. L.; Hou, J. W.; Han, Z. W.; Xin, W. J. Scavenging Effects of Sodium Ferulate and 18 β-Glycyrrhetic Acid on Oxygen Free Radical. *Acta Pharmacol. Sin.* **1990**, *11*, 466−470.
(6) Meng, S.; Lu, Z. J.; Zhang, Z. N.; Li, D. G. Inhibition of Respiratory Burst of Polymorphonuclear Cells by Herbal Ingredients. *Chin. Pharmacol. Bull.* **1994**, *10*, 439−441.
(7) Ho, W. K.; Wen, H. L.; Lee, C. M. Tetramethylpyrazine for Treatment of Experimentally Induced Stroke in Mongolian Gerbils. *Stroke* **1989**, *20*, 96−99.
(8) Ni, J. W. Tetramethylpyrazine Improves Spatial Cognitive Impairment Induced by Permanent Occlusion of Bilateral Common Carotid Arteries or Scopolamine in Rats. *Jpn. J. Pharmacol.* **1995**, *67*, 137−141.
(9) Watanabe, H. Candidates for Cognitive Enhancer Extracted from Medicinal Plants: Paeoniflorin and Tetramethylpyrazine. *Behav. Brain Res.* **1997**, *83*, 138−141.

(10) Murga, R.; Sanz, M. T.; Beltrnán, S.; Cabezas, J. L. Solubility of Three Hydroxycinnamic Acids in Supercritical Carbon Dioxide. *J. Supercrit. Fluids* **2003**, *27*, 239−245.
(11) Sovová, H. Solubility of Ferulic Acid in Supercritical Carbon Dioxide with Ethanol as Cosolvent. *J. Chem. Eng. Data* **2001**, *46*, 1255−1257.
(12) Angus, S.; Armstrong, B.; de Reuck, K. M. *International Thermodynamic Tables of the Fluid State, Carbon Dioxide*; Pergamon Press: Oxford, U.K., 1976.
(13) Murga, R.; Sanz, M. T.; Beltrán, S.; Cabezas, J. L. Solubility of Some Phenolic Compounds Contained in Grape Seeds, in Supercritical Carbon Dioxide. *J. Supercrit. Fluids* **2002**, *23*, 113−121.
(14) Schmitt, W. J.; Reid, R. C. The Influence of the Solvent Gas on Solubility and Selectivity in Supercritical Extraction. In *Supercritical Fluid Technology*; Penninger, J. M. L., Radosz, M., McHugh, M. A., Krukonis, V. J., Eds.; Elsevier: Amsterdam, 1985.
(15) Johnston, K. P.; Barry, S. E.; Read, N. K.; Holcomb, T. R. Separation of Isomers Using Retrograde Crystallization from Supercritical Fluids. *Ind. Eng. Chem. Res.* **1987**, *26*, 2372−2377.
(16) Foster, N. R.; Gurdial, G. S.; Yun, J. S. L.; Keat Liong, K.; Tilly, K. D.; Ting, S. S. T.; Lee, J. H. Significance of the Crossover Pressure in Solid−Supercritical Fluid Phase Equilibria. *Ind. Eng. Chem. Res.* **1991**, *30*, 1955−1964.
(17) Chrastil, J. Solubility of Solids and Liquids in Supercritical Gases. *J. Phys. Chem.* **1982**, *86*, 3016−3021.

Received for review July 31, 2004. Accepted May 9, 2005.

JE049715Y