Solubilities of Apigenin in Ethanol + Water at Different Temperatures

Min Xiao, Weidong Yan,* and Zizhang Zhang

Department of Chemistry, Zhejiang University, Hangzhou 310027, China

The solubilities of apigenin in ethanol + water mixtures at T = (273.2 to 323.2) K were measured by using high-performance liquid chromatography (HPLC). The solubilities of apigenin increase with increasing temperatures and go through a maximum at a specific solvent composition. The experimental data of solubilities were correlated by a three-parameter empirical equation.

Introduction

Apigenin (5,7-dihydroxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one, CAS No. 520-36-5, Figure 1) is a naturally occurring polyphenolic compound present in a variety of fruits, vegetables, and seeds. It has many biological and pharmacological activities including anti-inflammatory[1,2] and antitumor effects.[3−5] Apigenin is well-known for its antioxidant activity[6] and ability to scavenge free radicals.[7,8]

Apigenin is usually extracted from natural plants. Various volatile solvents could be used to extract apigenin, such as methanol, ethanol, acetone, chloroform, and other mixed solvents. Some common organic solvents or their mixtures are also efficient to purify apigenin by crystallization. The solubility of apigenin in binary solvent mixtures at different temperatures is necessary to be measured. Considering food and drug safety, an ethanol + water mixture could commendably be substituted for those organic solvents for crystallization. A binary mixture of ethanol and water provides an environmentally friendly and cost-effective alternative to toxic organic solvents for purification of apigenin by crystallization. The solubility data of apigenin in ethanol + water mixed solvents has not been found in the literature.

In this work, the solubilities of apigenin in ethanol + water mixtures at the mole fraction of ethanol of 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 on a solute-free basis were determined at T = (273.2, 283.2, 293.2, 303.2, 313.2, and 323.2) K. A three-parameter empirical equation was adopted to correlate the experimental data.

Experimental Section

Materials. The yellow powder of apigenin (0.95 mass fraction) was supplied by Skyherb Natural Product Co., Ltd. (China). The apigenin was dissolved in methanol and refluxed for 2.5 h and recrystallized thrice. The crystals of apigenin were dried in a vacuum oven at T = 378.2 K for 12 h and stored in a desiccator to avoid absorbing water. The purity is higher than 0.998 mass fraction, checked by HPLC (Shimadzu LC-10AD). The reference standard of apigenin, whose purity was 0.998 mass fraction, was purchased from Sigma Chemical Corporation (USA). Ethanol was of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (China), and dehydrated with molecular sieves of (0.3 to 0.4) nm before use. The purity of ethanol, determined by gas chromatography, was > 0.998 mass fraction. Deionized water was distilled by using a quartz sub-boiling purifier. The pH value of pure water was 6.6, determined by *pH* Scan 2.

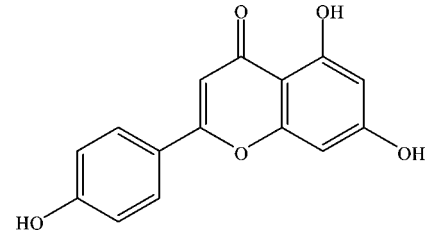

Figure 1. Structure of apigenin.

Solubility Measurement. Binary solvent mixtures were prepared by mass using a Sartorius CP225D analytical balance with an accuracy ± 0.01 mg. The uncertainty of compositions of mixed solvents was 0.0002 on a mole fraction basis. The 15 mL centrifuge tubes (PBS) with caps were used to prepare saturated solutions (about 10 mL) of apigenin with excess solid solute in mixed solvents. The tube was gastight when the turn cap with a sizable rubber band was screwed on. Then the tubes were directly placed in a constant temperature thermostatic bath (THID-0510W, China) with a temperature stability of ± 0.05 K and a temperature uncertainty of 0.1 K. The tubes were allowed to settle about 36 h in the dark to ensure solid−liquid equilibrium. For each tube, two samples of approximately (0.1 to 1) mL were withdrawn from the clear saturated solution using preheated glass syringes. The glass syringe with saturated solution was weighted with an uncertainty of ± 0.01 mg. The needle was closed with silicon rubber to prevent evaporation of solvents during the weighing procedure. The saturated solution was injected into the volumetric flask immediately to prevent precipitation. Subsequently, the mass of glass syringes with the remaining solution was weighed. The mass of saturated solutions that were put into volumetric flasks could be found. The solutions of samples used for analysis were diluted to mark with methanol. The relative uncertainty of the experimental solubility is within 0.35 %.

The solubility of apigenin was monitored by HPLC. The HPLC system (Shimadzu Corporation, Kyoto, Japan) was composed by a degasser (DGU-4A), solvent delivery module (LC-10AT), UV detector (SPD-10A), and 20 μL injector loop. The chromatographic analysis was performed on a Diamonsil C_{18} column [(150 × 4.6) mm, 5 μm] and a mobile phase

* Corresponding author. Tel.: 0086 571 87951430. Fax: 0086 571 87951895. E-mail address: yanweidong@zju.edu.cn.

10.1021/je1001208 © 2010 American Chemical Society
Published on Web 04/06/2010

Table 1. Solubilities of Apigenin (1) in Different Compositions of Ethanol (2) + Water (3) Mixtures at T = (273.2, 283.2, 293.2, 303.2, 313.2, and 323.2) K[a]

	$10^3 m_1$		$10^3 m_1$	
T/K	(mol·kg^{-1})	$10^5 x_1$	(mol·kg^{-1})	$10^5 x_1$
	$x_2'^{b} = 0.0000$		$x_2' = 0.0990$	
273.2 K	(9.27 ± 0.03)·10^{-3}	(1.67 ± 0.05)·10^{-2}	(9.49 ± 0.02)·10^{-2}	(1.97 ± 0.04)·10^{-1}
283.2 K	(2.54 ± 0.04)·10^{-2}	(4.57 ± 0.07)·10^{-2}	(1.16 ± 0.01)·10^{-1}	(2.41 ± 0.02)·10^{-1}
293.2 K	(4.99 ± 0.02)·10^{-2}	(8.99 ± 0.03)·10^{-2}	(1.46 ± 0.03)·10^{-1}	(3.03 ± 0.06)·10^{-1}
303.2 K	(7.70 ± 0.03)·10^{-2}	(1.39 ± 0.05)·10^{-1}	(1.94 ± 0.01)·10^{-1}	(4.03 ± 0.02)·10^{-1}
313.2 K	(1.06 ± 0.03)·10^{-1}	(1.91 ± 0.05)·10^{-1}	(2.58 ± 0.02)·10^{-1}	(5.36 ± 0.04)·10^{-1}
323.2 K	(1.34 ± 0.01)·10^{-1}	(2.41 ± 0.02)·10^{-1}	(3.20 ± 0.01)·10^{-1}	(6.66 ± 0.02)·10^{-1}
	$x_2' = 0.1995$		$x_2' = 0.2918$	
273.2 K	(3.27 ± 0.01)·10^{-1}	(7.72 ± 0.02)·10^{-1}	(7.99 ± 0.03)·10^{-1}	2.09 ± 0.08
283.2 K	(4.15 ± 0.01)·10^{-1}	(9.81 ± 0.02)·10^{-1}	1.33 ± 0.01	3.48 ± 0.03
293.2 K	(5.51 ± 0.03)·10^{-1}	1.30 ± 0.07	1.81 ± 0.03	4.74 ± 0.08
303.2 K	(8.27 ± 0.01)·10^{-1}	1.95 ± 0.02	2.42 ± 0.01	6.33 ± 0.03
313.2 K	1.17 ± 0.02	2.75 ± 0.04	3.10 ± 0.02	8.12 ± 0.05
323.2 K	1.60 ± 0.01	3.78 ± 0.02	3.98 ± 0.01	11.43 ± 0.03
	$x_2' = 0.4008$		$x_2' = 0.5026$	
273.2 K	2.21 ± 0.01	6.46 ± 0.03	3.91 ± 0.01	12.56 ± 0.02
283.2 K	3.02 ± 0.01	8.82 ± 0.03	4.91 ± 0.01	15.76 ± 0.01
293.2 K	3.71 ± 0.01	10.86 ± 0.03	6.05 ± 0.00	19.41 ± 0.01
303.2 K	4.79 ± 0.01	14.02 ± 0.03	7.46 ± 0.01	23.96 ± 0.01
313.2 K	6.10 ± 0.02	17.87 ± 0.06	8.96 ± 0.02	28.77 ± 0.04
323.2 K	7.66 ± 0.01	22.40 ± 0.03	11.27 ± 0.01	36.17 ± 0.01
	$x_2' = 0.5862$		$x_2' = 0.6862$	
273.2 K	5.47 ± 0.01	18.86 ± 0.02	7.38 ± 0.03	27.51 ± 0.05
283.2 K	6.64 ± 0.02	22.87 ± 0.02	8.52 ± 0.03	31.75 ± 0.03
293.2 K	7.88 ± 0.03	27.16 ± 0.04	9.75 ± 0.03	36.32 ± 0.04
303.2 K	9.31 ± 0.02	32.08 ± 0.05	11.08 ± 0.04	41.26 ± 0.09
313.2 K	11.11 ± 0.03	38.28 ± 0.03	13.08 ± 0.03	48.73 ± 0.03
323.2 K	13.71 ± 0.01	47.23 ± 0.02	15.84 ± 0.01	58.99 ± 0.02
	$x_2' = 0.7806$		$x_2' = 0.8988$	
273.2 K	9.10 ± 0.02	36.32 ± 0.03	8.00 ± 0.02	34.59 ± 0.03
283.2 K	10.04 ± 0.03	40.08 ± 0.05	9.04 ± 0.03	39.07 ± 0.01
293.2 K	11.09 ± 0.02	44.23 ± 0.04	10.19 ± 0.01	44.07 ± 0.03
303.2 K	12.41 ± 0.03	49.52 ± 0.05	11.51 ± 0.03	49.74 ± 0.04
313.2 K	14.55 ± 0.04	58.04 ± 0.07	13.56 ± 0.03	58.60 ± 0.05
323.2 K	17.02 ± 0.02	67.88 ± 0.04	15.99 ± 0.01	69.13 ± 0.04
	$x_2' = 1.0000$			
273.2 K	3.91 ± 0.04	18.00 ± 0.06		
283.2 K	4.73 ± 0.03	21.76 ± 0.04		
293.2 K	5.69 ± 0.04	26.23 ± 0.06		
303.2 K	6.85 ± 0.01	31.57 ± 0.03		
313.2 K	7.97 ± 0.01	36.72 ± 0.02		
323.2 K	9.52 ± 0.03	43.86 ± 0.06		

[a] Expanded uncertainties (±) were calculated using standard deviation, SD·coverage factor k; $k = 2$. [b] Mole fraction of ethanol on a solute-free basis.

composed of acetonitrile and water in a volume ratio of 35 to 65 at a flow rate of 1.0 mL·min^{-1} and detective wavelength at 256 nm. The reference standard solution containing about 60 μg·mL^{-1} of apigenin was prepared in methanol.

Results and Discussion

Before the sample of apigenin was used to determine the solubility, it had to be dried because apigenin is easily hydrated. High-performance liquid chromatography was adapted to determine the concentration of a saturated solution of apigenin in ethanol + water mixtures. To check the reliability of the HPLC analysis method, known masses of apigenin were completely dissolved in methanol, and the concentration of solution was measured by HPLC. The average relative uncertainty was 0.25 %.

The solubilities of apigenin in ethanol + water are listed in Table 1. Molalities, m_1, and mole fraction, x_1, values are the average values taken from three measurements at the same composition of an ethanol + water mixture. The expanded uncertainty (±) for each data point is given in Table 1. The experimental data of solubility of apigenin in different compositions of ethanol + water mixtures (0.0 to 1.0) on a solute-free basis were plotted in Figure 2 at six temperatures.

According to the solid−liquid phase equilibrium theory, the relationship between the mole fraction and solubility and temperature could be expressed by the modified Apelblat equation[9]

$$\ln x_1 = a + \frac{b}{T/\mathrm{K}} + c \ln T/\mathrm{K} \qquad (1)$$

where x_1 is the mole fraction solubility of apigenin and T is an absolute temperature (K). a, b, and c are empirical parameters. These parameters were obtained by nonlinear least-squares fit and listed in Table 2 together with the root-mean-square deviations (rmsd) for the mixed solvent system. The rmsd's are defined as

$$\mathrm{rmsd} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_{1,i}^{\mathrm{cal}} - x_{1,i}^{\mathrm{exp}})^2\right]^{1/2} \qquad (2)$$

where $x_{1,i}^{\mathrm{cal}}$, the mole fraction solubility, is calculated by eq 1 using the parameters in Table 2. $x_{1,i}^{\mathrm{exp}}$ is the experimental value

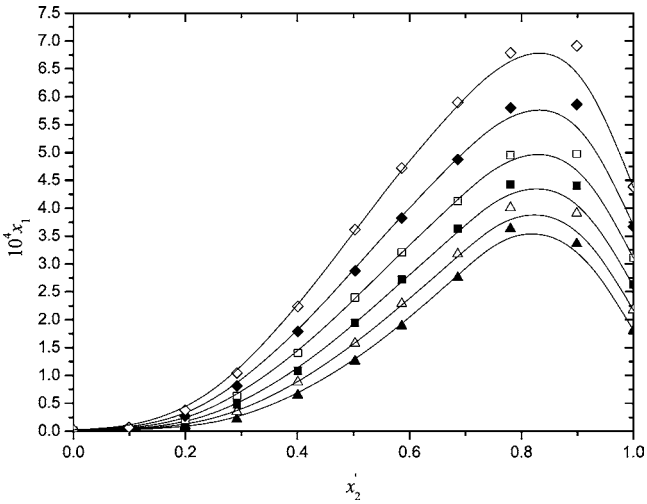

Figure 2. Solubilities of apigenin (1) vs the mole fraction of ethanol (0.0 to 1.0) on a solute-free basis in ethanol (2) + water (3) at different temperatures. ▲, 273.2 K; △, 283.2 K; ■, 293.2 K; □, 303.2 K; ◆, 313.2 K; ◇, 323.2 K; line, correlated with eq 1 using the parameters in Table 2.

Table 2. Parameters of Equation [1] for Apigenin (1) in Different Solvent Compositions of the Ethanol (2) + Water (3) System

x_2^a	*a*	*b*/K	*c*	10^5rmsd
0.0000	682.36	−34557.49	−101.83	0.002
0.0990	−110.96	2466.05	15.82	0.008
0.1995	−161.46	4174.98	23.95	0.02
0.2918	114.45	−7804.72	−17.22	0.1
0.4008	−51.87	60.15	7.49	0.1
0.5026	−85.02	1832.27	12.36	0.2
0.5862	−117.57	3505.48	17.14	0.3
0.6862	−156.34	5466.33	22.84	0.4
0.7806	−169.63	6251.06	24.75	0.3
0.8988	−147.87	5189.84	21.55	0.3
1.0000	−51.31	585.29	7.23	0.2

[a] Mole fraction of ethanol on a solute-free basis.

of mole fraction solubility of apigenin, and *n* is the number of experimental points.

The solubilities of apigenin in ethanol + water mixed solvents, which are usually used as useful solvents in laboratory and industries, were measured by using a saturation method at T = (273.2, 283.2, 298.2, 303.2, 313.2, and 333.2) K. As can be seen from Table 2, the results correlated by this empirical equation are satisfactory. Table 1 and Figure 2 showed that solubilities of apigenin in ethanol + water increase with increasing temperature. The maximum solubility effect has been observed at approximately $x_2' = 0.85$ (mole fraction of ethanol on a solute-free basis). This maximum effect also exists in other systems.[10,11] The apigenin is almost insoluble in water (pH = 6.6). The fact of a markedly enhanced solubility of apigenin in ethanol + water mixtures is of great impact on the selection of the best mixed solvents in the processes of purification of apigenin by antisolvent crystallization.

There are significant differences in solubilities of apigenin in ethanol, water, and ethanol + water at different temperatures. The appropriate method for the purification of apigenin is that the crude apigenin (mass fraction 0.93) is dissolved in a warm ethanol−water mixture ($x_2' = 0.85$) at about T = 70 °C, filtered, and crystallized by adding water to the solution.

Literature Cited

(1) Matauda, H.; Morikawa, T.; Ando, S.; Toguchida, I; Yoshikawa, M. Structural requirements of flavonoids for nitric oxide production inhibitory activity and mechanism of action. *Bioorg. Med. Chem.* **2003**, *11*, 1995–2000.
(2) Raso, G. M.; Meli, R.; Carlo, G. Di.; Pacilio, M.; Carlo, R. Di. Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A. I. *Life Sci.* **2001**, *68*, 921–931.
(3) Kobayashi, T.; Nakata, T.; Kuzumaki, T. Effect of flavonoids on cell cycle progression in prostate cancer cells. *Cancer Lett.* **2002**, *176*, 17–23.
(4) Yin, F.; Giuliano, A. E; Law, R. E.; Van Herle, A. J. Apigenin inhibits growth and induces G2/M arrest by modulating cyclin-cdk regulators and ERK MAP kinase activation in breast carcinoma cells. *Anticancer Res.* **2001**, *21*, 413–420.
(5) Richter, M.; Ebermann, R.; Marian, B. Quercetin-Induced Apoptosis in Colorectal Tumor Cells: Possible Role of EGF Receptor Signaling. *Nutr. Cancer* **1999**, *34*, 88–99.
(6) Romanova, D; Vachalkova, A; Cipak, L; Ovesna, Z; Rauko, P. Study of antioxidant effect of apigenin, luteolin and quercetin by DNA protective method. *Neoplasma* **2001**, *48*, 104–111.
(7) Husain, S. R.; Cillard, J.; Cillard, P. Hydroxyl radical scavenging activity of flavonoids. *Phytochemistry* **1987**, *26*, 2489–2491.
(8) Sugihara, N; Arakawa, T; Ohnishi, M. Anti- and pro-oxidative effects of flavonoids on metal-induced lipid hydroperoxide-dependent lipid peroxidation in cultured hepatocytes loaded with alpha-linolenic acid. *Free Radical Biol. Med.* **1999**, *11/12*, 1313–1323.
(9) Prausnitz, J. M.; Lichtenthaler, R. N.; Azevedo, E. G. *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed.; Prentice Hall: New York, 1999.
(10) Zhao, G. L.; Yan, W. D. Solubilities of betulin in chloroform + methanol mixed solvents at T = (278.2, 288.2, 293.2, 298.2, 308.2 and 313.2) K. *Fluid Phase Equilib.* **2008**, *267*, 79–82.
(11) Peng, B.; Yan, W. D. Solubility of luteolin in ethanol + water mixed solvents at different temperatures. *J. Chem. Eng. Data* **2010**, *55* (1), 583–585.

Received for review February 3, 2010. Accepted March 25, 2010.

JE1001208