Solubility of Rutin in Ethanol + Water at (273.15 to 323.15) K

Bin Peng,[†] Ruopeng Li,[‡] and Weidong Yan*,[†]

Department of Chemistry, Zhejiang University, Hangzhou 310027, China, and Hangzhou Skyherb Technologies Co., Ltd., Hangzhou 310013, PR China

The solubilities of rutin in ethanol + water were measured by using high-performance liquid chromatography (HPLC) in the temperature range from (273.15 to 323.15) K. The experimental data were correlated with a three-parameter equation. Four crystal forms of rutin obtained in different compositions of ethanol + water were characterized by TG, DSC, and scanning electron microscopy (SEM).

Introduction

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rutinoside, CAS No. 153-18-4, Figure 1), also called rutoside,[1] quercetin-3-rutinoside, or sophorin, is a citrus flavonoid glucoside derived from buckwheat leaves, the petioles of the pheum species, and the fruit of the Fava D'Anta tree (from Brazil), as well as other sources. The therapeutic value of rutin has been recognized for more than a century, and it has drawn substantial research interest in the recent years. Rutin is known for its antioxidant activity and ability to scavenge free radicals. Furthermore, it has various special biomedical values, as described in our previous work.[2]

Usually, rutin is isolated from the plant material by solvent extraction.[1,3] In this process, rutin can be rapidly degraded by hydrolysis to the corresponding aglucon, e.g., quercetin. In that case, the crude product of rutin which was prepared according to the usual method always contains a relatively large amount of quercetin.[4] As rutin is increasingly in demand in the food, pharmaceutical, and cosmetic industries, crude rutin has to be purified by crystallization. To improve the purity of rutin and yield, the solubility data of rutin in different solvents are required.

In our former research, solubilities of rutin in several pure solvents had been measured.[2] According to the experimental data, some volatile solvents and their mixtures are efficient to separate rutin. However, binary mixtures of ethanol and water provide an environmentally friendly and cost-effective alternative to toxic organic solvents for purification of rutin by crystallization. In this work, the solubilities of rutin in different concentrations of ethanol−water solution 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 semiempirical equation was adopted to correlate the experimental data. Four types of rutin crystals obtained at different compositions of ethanol + water were characterized by scanning electron microscopy (SEM).

Experimental

Materials. The yellow powder of rutin (mass fraction 0.98) was supplied by Skyherb Natural Product Co., Ltd. (China) and recrystallized twice in boiling water. The crystals of rutin trihydrate were dried in a vacuum oven at T = 408.2 K for 12 h to eliminate the water of crystallization and then stored in a desiccator to avoid absorbing water.[5] The mass fraction purity was higher than 0.99, determined by high-performance liquid chromatography (Shimadzu LC-10AD).

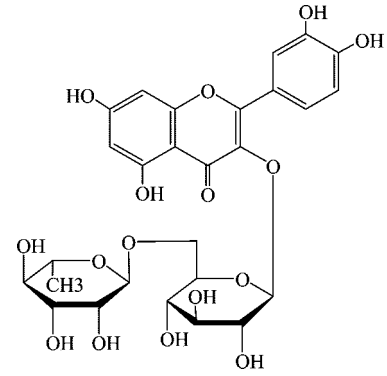

Figure 1. Chemical structure of rutin.

Ethanol, which was purchased from Sinopharm Chemical Reagent Co., Ltd. (China), was of analytical grade and dehydrated with molecular sieves (3 to 4) Å before use. The purities (mass fraction) of the solvents, determined by gas chromatography, were > 0.99. Deionized water was distilled by using a quartz sub-boiling purifier. The pH value of pure water was 5.96, determined by an ORION pH meter 811 (Orion, USA).

Solubility Measurement. Binary solvent mixtures were prepared by mass using a Sartorius CP225D analytical balance with an accuracy ± 0.01 mg. The uncertainty of the mole fraction of mixed solvents was 0.0003. The 15 cm^3 centrifuge tubes (PBS) with caps were used to prepare saturated solutions (about 10 cm^3) of rutin with excess solid solute in mixed solvents. The tube was gastight when the turncap with a sizable rubber band was screwed on. Then the tubes were directly placed in a constant-temperature thermostatic bath (THJD-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 (24 to 36) h to ensure solid−liquid equilibrium and stabilization of the configuration of rutin before sampling. For each tube, two samples of approximately (0.1 to 1) mL were withdrawn from the clear saturated solution using preheated glass syringes. For the saturated solution of rutin in water, the sample was

* Corresponding author. Tel.: 0086 571 87951430. Fax: 0086 571 87951895. E-mail: yanweidong@zju.edu.cn.
[†] Zhejiang University.
[‡] Hangzhou Skyherb Technologies Co., Ltd.

10.1021/je800816f CCC: $40.75
Published on Web 03/03/2009

Table 1. Solubilities of Rutin (1) in Different Compositions of Ethanol (2) + Water (3) Mixtures at T = (273.2, 283.2, 293.3, 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 = 0.1000	
273.2	(1.65 ± 0.04)·10^{-1}	(2.97 ± 0.08)·10^{-1}	(1.63 ± 0.09)·10^{-1}	(3.39 ± 0.20)·10^{-1}
283.2	(2.94 ± 0.07)·10^{-1}	(5.29 ± 0.13)·10^{-1}	(2.24 ± 0.09)·10^{-1}	(4.67 ± 0.19)·10^{-1}
293.2	(3.26 ± 0.14)·10^{-1}	(5.87 ± 0.25)·10^{-1}	(4.32 ± 0.05)·10^{-1}	(8.99 ± 0.10)·10^{-1}
303.2	(4.56 ± 0.09)·10^{-1}	(8.22 ± 0.16)·10^{-1}	(6.44 ± 0.16)·10^{-1}	1.34 ± 0.03
313.2	(5.65 ± 0.12)·10^{-1}	1.02 ± 0.02	1.26 ± 0.05	2.63 ± 0.11
323.2	(7.87 ± 0.22)·10^{-1}	1.42 ± 0.04	2.32 ± 0.03	4.84 ± 0.06
	x_2 = 0.2000		x_2 = 0.3000	
273.2	1.29 ± 0.03	3.04 ± 0.07	3.26 ± 0.07	8.63 ± 0.19
283.2	1.98 ± 0.10	4.69 ± 0.23	4.52 ± 0.18	11.94 ± 0.48
293.2	2.90 ± 0.02	6.85 ± 0.06	6.06 ± 0.13	16.01 ± 0.34
303.2	3.22 ± 0.08	7.62 ± 0.20	6.80 ± 0.08	17.97 ± 0.20
313.2	5.00 ± 0.16	11.81 ± 0.39	9.95 ± 0.13	26.29 ± 0.33
323.2	8.71 ± 0.34	20.57 ± 0.80	12.50 ± 0.37	33.04 ± 0.97
	x_2 = 0.4000		x_2 = 0.5000	
273.2	4.62 ± 0.07	13.50 ± 0.19	4.23 ± 0.04	13.56 ± 0.12
283.2	4.95 ± 0.19	14.48 ± 0.54	4.90 ± 0.05	15.70 ± 0.16
293.2	6.79 ± 0.18	19.84 ± 0.52	6.08 ± 0.07	19.49 ± 0.23
303.2	7.72 ± 0.02	22.57 ± 0.07	7.15 ± 0.06	22.90 ± 0.19
313.2	11.03 ± 0.17	32.24 ± 0.49	9.69 ± 0.06	31.04 ± 0.20
323.2	12.54 ± 0.05	36.66 ± 0.15	10.91 ± 0.10	34.94 ± 0.34
	x_2 = 0.6000		x_2 = 0.7000	
273.2	3.71 ± 0.07	12.94 ± 0.26	3.01 ± 0.07	11.35 ± 0.25
283.2	4.09 ± 0.05	14.26 ± 0.17	3.44 ± 0.11	12.97 ± 0.41
293.2	5.20 ± 0.08	18.13 ± 0.27	4.50 ± 0.05	16.93 ± 0.20
303.2	5.71 ± 0.08	19.90 ± 0.28	4.80 ± 0.04	18.08 ± 0.13
313.2	8.02 ± 0.04	27.95 ± 0.14	5.96 ± 0.06	22.44 ± 0.23
323.2	8.92 ± 0.05	31.06 ± 0.19	7.31 ± 0.01	27.51 ± 0.05
	x_2 = 0.8000		x_2 = 0.9000	
273.2	3.36 ± 0.03	13.57 ± 0.11	5.06 ± 0.07	21.90 ± 0.30
283.2	3.54 ± 0.08	14.32 ± 0.31	5.31 ± 0.06	22.97 ± 0.28
293.2	4.62 ± 0.09	18.71 ± 0.36	8.38 ± 0.05	36.22 ± 0.20
303.2	5.02 ± 0.11	20.32 ± 0.44	9.66 ± 0.17	41.79 ± 0.71
313.2	6.92 ± 0.04	27.97 ± 0.16	12.09 ± 0.13	52.28 ± 0.29
323.2	7.87 ± 0.10	31.83 ± 0.40	14.65 ± 0.09	63.35 ± 0.40
	x_2 = 1.0000			
273.2	75.73 ± 1.76	347.68 ± 8.09		
283.2	81.14 ± 0.64	372.44 ± 2.91		
293.2	93.14 ± 1.00	427.30 ± 4.59		
303.2	112.38 ± 2.97	515.07 ± 13.54		
313.2	129.22 ± 2.82	591.78 ± 6.42		
323.2	142.71 ± 1.71	653.19 ± 7.76		

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

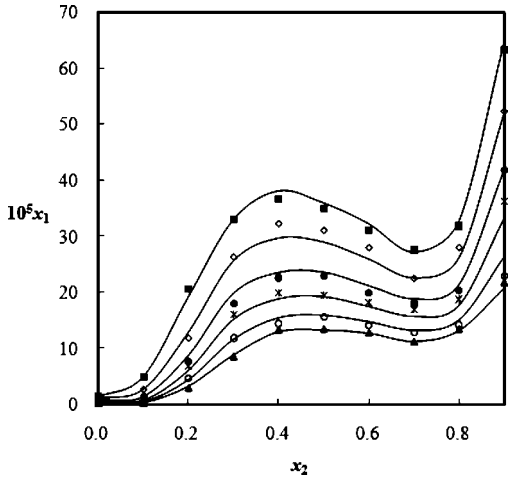

Figure 2. Solubilities of rutin vs the mole fraction of ethanol (0.0 to 0.9) on a solute-free basis in ethanol + water at different temperatures. ▲, 273.2 K; ○, 283.2 K; *, 293.2 K; ●, 303.2 K; ◇, 313.2 K; ■, 323.2 K; line, correlated with eq 3 using the parameters in Table 2.

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

x_2[a]	a	b/K	c	10^5 rmsd
0.0000	62.673	−5480.652	−9.851	4.93·10^{-2}
0.1000	−535.055	19175.810	80.612	5.41·10^{-2}
0.2000	296.191	10046.511	44.398	9.18·10^{-1}
0.3000	−71.206	798.211	10.508	8.74·10^{-1}
0.4000	−192.968	6568.406	28.516	1.38
0.5000	−131.778	3980.693	19.302	9.72·10^{-1}
0.6000	−176.706	6065.833	25.942	1.13
0.7000	−102.596	2847.576	14.810	5.97·10^{-2}
0.8000	−205.078	7354.960	30.164	1.05
0.9000	−57.741	508.733	8.449	1.96
1.0000	−94.722	2948.087	13.948	12.57

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

withdrawn with the help of syringes with attached 0.45 μm filters to avoid entrainment of the solids. The glass syringe with saturated solution was weighted. More details of the experimental setup have already been described before.[2]

The solubility of rutin 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 mm × 4.6 mm, 5 μm), with mobile phase composed of

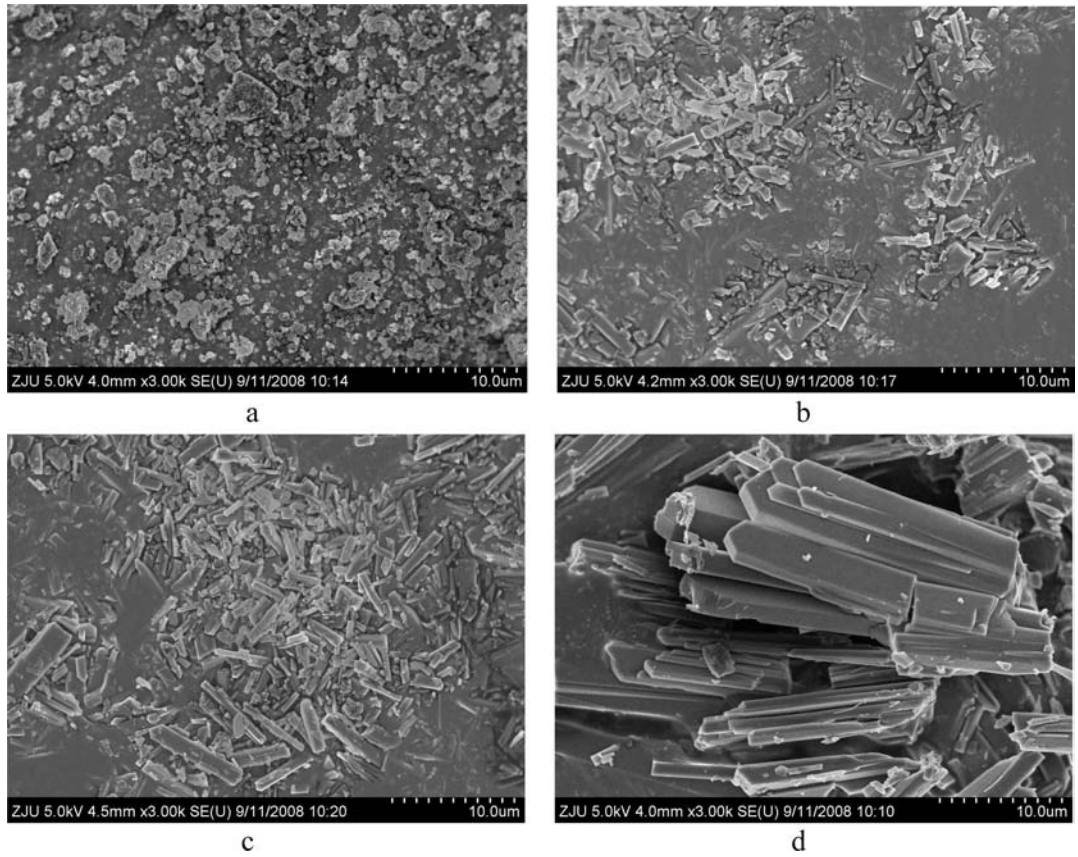

Figure 3. Scanning electron micrograph of rutin crystal in pure water, ethanol + water saturated solution, and pure ethanol. (a) Pure water; (b) $x_2 = 0.4$; (c) $x_2 = 0.8$; (d) pure ethanol.

acetonitrile and 0.1 % phosphoric acid aqueous solution in a volume ratio of 25 to 75 at a flow rate of 1.0 mL·min^{-1} and a detection wavelength of 350 nm. The reference standard solution containing about 0.20 mg·mL^{-1} of rutin was prepared in methanol.

Results and Discussion

HPLC was used to determine the mole fraction of a saturated solution of rutin in ethanol + water solutions. To check the reliability of the HPLC analysis method, known masses of rutin were completely dissolved in methanol, and the mole fraction of the solution was measured by HPLC. The average relative deviation was 0.33 %.

The solubilities of rutin in ethanol + water are listed in Table 1. Molalities, m_1, and mole fraction, x_1, values are the average values taken from four measurements with 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 rutin in different compositions of ethanol + water mixtures (0.0 to 0.9) were plotted in Figure 2.

According to the solid−liquid phase equilibrium, the experimental data of rutin could be expressed for a nonideal solution[6]

$$\ln\left(\frac{1}{\gamma_1 x_1}\right) = \frac{\Delta_{\mathrm{fus}}H}{RT_{\mathrm{t}}}\left(\frac{T_{\mathrm{t}}}{T} - 1\right) - \frac{\Delta C_P}{R}\left(\frac{T_{\mathrm{t}}}{T} - 1\right) + \frac{\Delta C_P}{R}\ln\frac{T_{\mathrm{t}}}{T} \quad (1)$$

where γ_1 is the activity coefficient of rutin in a mixed solvent referring to the subcooled liquid; x_1 is the mole fraction solubility of rutin; $\Delta_{\mathrm{fus}}H$ is the enthalpy of fusion of rutin; ΔC_p is the change of the heat capacity; T is the absolute temperature; T_{t} is the triple-point temperature of rutin; and R is the gas constant. Given a temperature, the solubility of rutin is only a function of its activity coefficient in solution. The activity coefficient of rutin in solution determines its solubility as the solvent composition changes. The experimental data of solubility were correlated by a three-parameter empirical equation.

The activity coefficient of rutin is given by[7]

$$\ln \gamma_1 = A + \frac{B}{T/\mathrm{K}} \quad (2)$$

where A and B are parameters. Introducing γ_1 from eq 2 into eq 1 and subsequent rearrangement results in

$$\ln x_1 = a + \frac{b}{T/\mathrm{K}} + c \ln T/\mathrm{K} \quad (3)$$

where a, b, and c are empirical parameters. They 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

$$\mathrm{rmsd} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_{1,i}^{\mathrm{cal}} - x_{1,i})^2\right]^{1/2} \quad (4)$$

where $x_{1,i}^{\mathrm{cal}}$ is the solubility calculated by eq 3 using the parameters in Table 2; $x_{1,i}$ is the experimental value of mole fraction solubility of rutin; and n is the number of experimental points.

As can be seen from Table 2, the results correlated by this empirical equation are satisfactory. Table 1 and Figure 2 showed some regular conclusions: (1) the solubilities of rutin in ethanol + water increase slightly with increasing temperature, (2) at the same temperature, the solubility of rutin in pure ethanol is much larger than in any other ethanol + water mixed solvent, (3) the experimental data indicate that the solubilities increase from $x_2 = 0.0$ to 0.4 and slowly decrease until $x_2 = 0.7$. From $x_2 = 0.7$ to 1.0, the solubility curve of rutin climbs rapidly with an increase of molar ratio of ethanol + water.

To explain the interesting phenomenon above, four saturated solutions of rutin ($x_2 = 0.0$, 0.4, 0.8, 1.0) were taken from the tubes at $T = 323.2$ K and crystallized at room temperature, simultaneously. The crystals obtained at these different mixed solvents were dried in a vacuum oven at $T = 293.15$ K and characterized by TG and DSC under a nitrogen atmosphere from (313.2 to 873.2) K.

The TG curves illustrate that dehydration of rutin takes place in the range about $T =$ (378 to 423) K, and the anhydrous solid starts to decompose at about 523 K. The ratios of mass loss between (393 and 423) K at $x_2 =$ (0.0, 0.4, 0.8, 1.0) to the whole mass are 3.88 %, 4.91 %, 4.95 %, and 5.16 %, respectively (see Figure S1 in the Supporting Information). The melting temperatures of four crystal forms of rutin are not the same (see Figure S2 in the Supporting Information). At the same time, four types of rutin crystals were analyzed by SEM at 3000 magnification. The granule shape, size, and morphology of crystals are presented in Figure 3. SEM examinations indicate that crystal forms of rutin have changed in morphology at different compositions of ethanol + water. The crystals of rutin obtained in pure water show fragmentary shape and irregular form. The two types of the crystals obtained at $x_2 = 0.4$ and 0.8, which are small in size, both exhibit stick and sheet shape. However, the crystals growing in pure ethanol display the regular crystal in form and size. The irregularities in solubilities of rutin in ethanol + water mixtures may be due to these complicated interaction forces including van der Waals forces, hydrogen bond, salvation, and also the polymorphic forms of crystals.

There are significant differences in solubilities of rutin in ethanol, water, and ethanol + water at different temperatures. The appropriate method for the purification of rutin is that the crude rutin (mass fraction 0.9) is dissolved in warm ethanol, filtered, and crystallized by adding water to the solution at room temperature.

Supporting Information Available:

Figures S1 and S2. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Samo, K.; Martina, K.; Ivan, K. Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis. *J. Agric. Food Chem.* **1999**, *47*, 4649–4652.
(2) Zi, J. Q.; Peng, B.; Yan, W. D. Solubilities of rutin in eight solvents at $T =$ 283.15, 298.15, 313.15, 323.15, and 333.15 K. *Fluid Phase Equilib.* **2007**, *261*, 111–114.
(3) Paniwnyk, J.; Beaufoy, E.; Lorimer, J. P.; Mason, T. J. The extraction of rutin from flower buds of *Sophora japonica*. *Ultrason. Sonochem.* **2001**, *8*, 299–301.
(4) James, F. C.; Joseph, N.; William, L. P.; Philadelphia, P. Method of purifying rutin. US Patent No. 2,520,127, 1950.
(5) Vyaznikova, M. Y.; Nikolaeva, S. S.; Smirnova, L. P.; Rebrov, L. B.; Bykov, V. A. Study of the state of water in a rutin standard. *Pharm. Chem. J.* **1997**, *31*, 36–38.
(6) Prausnitz, J. M.; Lichtenthaler, R. N.; Azevedo, E. G. *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed.; Prentice Hall: New York, 1999.
(7) Kondepudi, D.; Prigogine, I. *Modern Thermodynamics: From Heat Engines to Dissipative Structures*; John Wiley & Sons Ltd.: Chichester, England, 1998.

Received for review November 2, 2008. Accepted January 26, 2009.

JE800816F