J. Chem. Eng. Data **2010,** *55,* 583–585

Solubility of Luteolin in Ethanol + Water Mixed Solvents at Different Temperatures

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The solubility of luteolin in ethanol + water was measured at (273.15 to 323.15) K using high-performance liquid chromatography (HPLC). The solubility of luteolin in ethanol + water mixed solvents presents a maximum-solubility effect. The modified Apelblat equation was used to correlate the experimental data and exhibited good agreement. A relatively appropriate process was given for the purification of luteolin, with rutin as an impurity.

Introduction

Luteolin (3′,4′,5′,7′-tetrahydroxyflavone, CASRN: 491-70-3) is an important member of the flavonoid family which prosseses anti-inflammatory,[1] antioxidant,[2] and antitumorigenic properties.[3] The molecular structure of luteolin was illustrated in Figure 1. This famous bioactive constituent usually exists in glycosylated forms in celery, green peppers, perilla leaf, and chamomile tea and as an aglycone in perilla seeds.[4]

Several studies have reported that it can be extracted directly from natural herbs, such as *Reseda luteola*,[5] peanut hulls,[6] and *angelica keiskei*,[7] etc. However, although many extracting technologies (ultrasonic,[8] microwave,[9] enzyme,[10] and supercritical carbon dioxide[11]) have been developed to increase the production of luteolin, its application was limited because of the low content in plants. Recently, much attention has focused on its synthetic method.[12−16] No matter which process was adopted for the preparation of luteolin, the crude product would not be applied in clinic or as a food additive before purification.

High-purity luteolin can be obtained by crystallization. In our previous work, solubility of luteolin in pure organic solvents had been measured.[17] For food safety and separation efficiency consideration, aqueous ethanol solvent was a competent system for the purification of luteolin. In this study, the solubility of luteolin in ethanol + water was determined using HPLC at T = (273.2 to 323.2) K.

Experimental Section

Materials. Luteolin (mass fraction 0.98) was supplied by Skyherb Natural Product Co., Ltd., China. The melting point of luteolin was 610.58 K, which was measured using a differential scanning calorimeter (DSC, TA SDT Q200). After being dissolved in warm ethanol and recrystallized at least twice at room temperature, the crystal of luteolin was dried in a vacuum oven at $T = 393.2$ K for 12 h and stored in a desiccator to avoid absorbing moisture. The mass fraction of the crystal was higher than 0.99, determined by high-performance liquid chromatography (LC-10AD, Shimadzu, Japan). The anhydrous ethanol was purchased from Sinopharm Chemical Reagent Co., Ltd., China, and dehydrated with 4 Å molecular sieves before use. The purity (mass fraction) of ethanol, determined by gas chromatography, was higher than 0.99. Deionized water was distilled by using a quartz sub-boiling purifier. The pH value of pure water was 5.96, determined by pH meter 811 (Orion, USA).

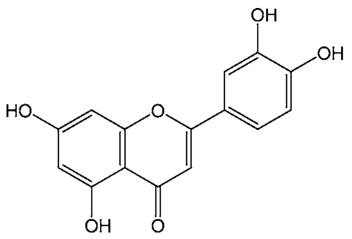

Figure 1. Molecular structure of luteolin.

Solubility Measurement. Mixed solvent with expected composition was prepared by mass using a Sartorius CP225D analytical balance with an accuracy of ± 0.01 mg. The uncertainty of compositions of mixed solvents was 0.0003 on mole fraction. The uncertainty of the temperature was 0.1 K. The experiment procedure has been described in detail elsewhere.[18]

The HPLC system (Shimadzu Corporation, Kyoto, Japan) was composed by a degasser (DGU-4A), a solvent delivery module (LC-10AT), a UV detector (SPD-10A), and a 20 μL injector loop. The chromatographic analysis was performed on a Diamonsil C_{18} column (150 mm × 4.6 mm, 5 μm), with a mobile phase composed of acetonitrile and 0.1 % phosphoric acid aqueous solution in a volume ratio of 35:65 at a flow rate of 1.0 $\text{mL}\cdot\text{min}^{-1}$ and detective wavelength at 254 nm. The reference standard solution containing about 200 $\mu\text{g}\cdot\text{mL}^{-1}$ of luteolin was prepared in methanol. The average relative uncertainty of HPLC analysis was 2.6 %.

Results and Discussion

The solubilities of luteolin were listed in Tables 1 and more visually expressed in Figure 2. Molalities, m_1 ($\text{mol}\cdot\text{kg}^{-1}$) and mole fraction, x_1 values are the average values taken from four test tubes with the ethanol + water mixtures of the same composition. Expanded uncertainty (±) for each data point is given in Tables 1. As described in Table 1 and Figure 2, the solubility of luteolin increases slightly with rising temperature. The solubility of luteolin in ethanol + water mixed solvents presents a maximum-solubility effect at $x_2' = 0.8$ (a solute-free basis).

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[19]

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

where x_1 is the mole fraction solubility of luteolin and T is an absolute temperature (K). a, b, and c are empirical parameters.

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10.1021/je900381r CCC: $40.75

Table 1. Solubilities of Luteolin (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]

T/K	m_1 (×10³)/mol·kg⁻¹	$10^5 x_1$	m_1 (×10³)/mol·kg⁻¹	$10^5 x_1$
	$x_2'^{b} = 0.00$		$x_2' = 0.10$	
273.2	$(1.15 \pm 0.06) \cdot 10^{-2}$	$(2.06 \pm 0.11) \cdot 10^{-2}$	$(1.51 \pm 0.08) \cdot 10^{-1}$	$(3.14 \pm 0.16) \cdot 10^{-1}$
283.2	$(1.51 \pm 0.05) \cdot 10^{-2}$	$(2.72 \pm 0.09) \cdot 10^{-2}$	$(1.65 \pm 0.13) \cdot 10^{-1}$	$(3.44 \pm 0.28) \cdot 10^{-1}$
293.2	$(1.93 \pm 0.07) \cdot 10^{-2}$	$(3.49 \pm 0.13) \cdot 10^{-2}$	$(2.28 \pm 0.02) \cdot 10^{-1}$	$(3.75 \pm 0.02) \cdot 10^{-1}$
303.2	$(2.60 \pm 0.08) \cdot 10^{-2}$	$(4.68 \pm 0.14) \cdot 10^{-2}$	$(3.14 \pm 0.08) \cdot 10^{-1}$	$(6.55 \pm 0.16) \cdot 10^{-1}$
313.2	$(4.05 \pm 0.19) \cdot 10^{-2}$	$(7.30 \pm 0.34) \cdot 10^{-2}$	$(5.13 \pm 0.18) \cdot 10^{-1}$	$(10.67 \pm 0.37) \cdot 10^{-1}$
323.2	$(5.26 \pm 0.14) \cdot 10^{-2}$	$(9.48 \pm 0.24) \cdot 10^{-2}$	$(7.77 \pm 0.28) \cdot 10^{-1}$	$(16.18 \pm 0.59) \cdot 10^{-1}$
	$x_2' = 0.20$		$x_2' = 0.30$	
273.2	0.93 ± 0.04	2.19 ± 0.10	3.89 ± 0.12	10.27 ± 0.33
283.2	1.40 ± 0.04	3.31 ± 0.09	6.61 ± 0.11	17.47 ± 0.29
293.2	2.33 ± 0.06	5.51 ± 0.13	8.24 ± 0.08	21.79 ± 0.22
303.2	3.82 ± 0.17	9.02 ± 0.40	14.01 ± 0.48	37.03 ± 1.26
313.2	5.14 ± 0.05	12.14 ± 0.12	18.91 ± 0.21	49.97 ± 0.56
323.2	6.92 ± 0.27	16.36 ± 0.63	26.96 ± 0.25	71.27 ± 0.66
	$x_2' = 0.40$		$x_2' = 0.50$	
273.2	10.86 ± 0.12	31.75 ± 0.34	18.58 ± 0.55	66.24 ± 1.76
283.2	14.72 ± 0.23	43.04 ± 0.66	25.06 ± 0.45	89.33 ± 1.43
293.2	19.58 ± 0.23	57.24 ± 0.68	32.95 ± 0.34	117.45 ± 1.08
303.2	27.12 ± 0.20	79.28 ± 0.58	40.65 ± 0.99	144.89 ± 3.18
313.2	35.00 ± 0.11	102.24 ± 0.31	48.13 ± 0.69	171.57 ± 2.19
323.2	47.25 ± 0.41	138.14 ± 1.19	57.48 ± 0.56	204.25 ± 1.78
	$x_2' = 0.60$		$x_2' = 0.70$	
273.2	38.15 ± 1.69	132.95 ± 5.88	56.57 ± 0.51	213.00 ± 1.91
283.2	46.14 ± 0.99	160.78 ± 3.45	67.11 ± 1.36	252.68 ± 5.11
293.2	54.91 ± 0.88	191.36 ± 3.05	77.18 ± 1.21	290.62 ± 4.55
303.2	65.56 ± 1.18	228.47 ± 4.08	84.95 ± 0.69	319.87 ± 2.58
313.2	73.62 ± 2.59	256.55 ± 8.98	95.81 ± 0.88	360.74 ± 3.27
323.2	85.32 ± 2.61	297.33 ± 9.03	108.10 ± 0.70	407.01 ± 2.62
	$x_2' = 0.80$		$x_2' = 0.90$	
273.2	75.82 ± 1.10	306.77 ± 4.41	66.14 ± 0.87	286.13 ± 3.73
283.2	82.40 ± 1.02	333.36 ± 4.10	71.66 ± 0.61	302.02 ± 2.63
293.2	94.62 ± 1.87	382.81 ± 7.49	84.39 ± 1.72	365.08 ± 7.40
303.2	103.38 ± 1.90	418.25 ± 7.63	92.94 ± 1.28	402.10 ± 5.49
313.2	115.62 ± 0.17	467.78 ± 0.67	105.74 ± 1.55	457.46 ± 6.64
323.2	126.89 ± 1.44	513.35 ± 5.77	116.33 ± 2.54	503.28 ± 10.88
	$x_2' = 1.00$			
273.2	16.40 ± 0.56	75.54 ± 2.59		
283.2	25.49 ± 0.49	117.41 ± 2.27		
293.2	37.74 ± 1.50	173.85 ± 6.89		
303.2	44.43 ± 0.90	204.67 ± 4.15		
313.2	58.77 ± 1.10	270.77 ± 5.05		
323.2	69.10 ± 2.48	318.34 ± 11.35		

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

These parameters were, respectively, 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 is 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 or eq 2 using the parameters in Table 2. $x_{1,i}^{\mathrm{exp}}$ is the experimental value of mole fraction solubility of luteolin, and n is the number of experiment points.

From Table 1 and Table 2, the calculated solubilities are in good agreement with the experimental data, which indicates that

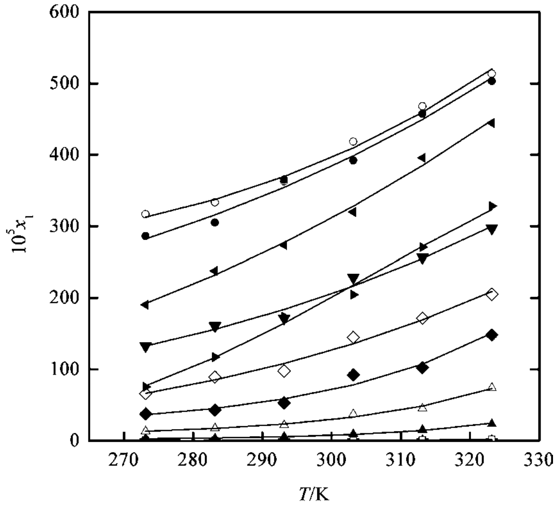

Figure 2. Solubilities of luteolin in ethanol + water. ★, $x_2' = 0.00$; □, $x_2' = 0.10$; ▲, $x_2' = 0.20$; Δ, $x_2' = 0.30$; ◆, $x_2' = 0.40$; ◇, $x_2' = 0.50$; ▼, $x_2' = 0.60$; solid triangle pointing left, $x_2' = 0.70$; ○, $x_2' = 0.80$; ●, $x_2' = 0.90$; solid triangle pointing right, $x_2' = 1.00$. Solid lines are values calculated from eq 1 with parameters from Table 2.

Table 2. Parameters of Equation 1 Correlated from Experimental Molar Fraction of Solubility of Luteolin in the Ethanol + Water Mixture

$x_2'^{a}$	a	b/K	c	10^5 rmsd
0.00	−614.713	24479.042	90.883	$5.13 \cdot 10^{-3}$
0.10	−1146.628	47801.929	170.942	$8.92 \cdot 10^{-2}$
0.20	−524.343	19540.629	78.844	0.74
0.30	−353.370	12741.397	53.083	2.30
0.40	−284.303	10145.761	42.645	6.63
0.50	−36.595	−395.113	5.477	6.26
0.60	−68.316	1527.278	10.000	7.44
0.70	−16.508	−798.858	2.349	6.45
0.80	−114.712	4075.485	16.760	6.64
0.90	−63.378	1680.943	9.154	7.86
1.00	251.657	−13596.740	−37.264	6.91

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

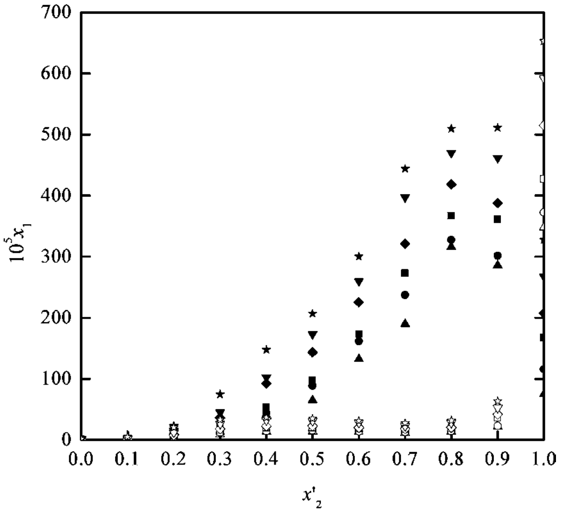

Figure 3. Solubilities of luteolin and rutin vs the mole fraction of ethanol (0.0 to 1.0) on a solute-free basis in ethanol + water at different temperatures. Luteolin: (▲, 273.2 K; ●, 283.2 K; ■, 293.2 K; ◆, 303.2 K; ▼, 313.2 K; ★, 323.2 K). Rutin:[18] (Δ, 273.2 K; ○, 283.2 K; □, 293.2 K; ◇, 303.2 K; ◇, 313.2 K; ☆, 323.2 K).

the modified Apelblat equation can be used to correlate the solubility of luteolin in ethanol + water.

As mentioned in the Introduction section of this paper, luteolin can be synthesized by many methods. However, most of them feature two or more synthesis stages or produce unsatisfactory yields of product. By far the most widely used process for the preparation of luteolin is using rutin as raw material,[16] which avoids or at least reduces the disadvantages of the prior processes and in particular permits one-step reaction and relatively high yield (> 80 %). The purity of luteolin produced in this way was approximately 95 % (mass fraction), and the main impurity was rutin. In our former research, solubility of rutin in the ethanol + water mixture was measured.[18] Its values were plotted in Figure 3, together with the experimental data of luteolin. It can be seen from Figure 3 that under the same temperature when $x'_2 = 0.8$ the solubility difference between luteolin and rutin is the biggest, while when $x'_2 = 0.3$, the solubility of luteolin is still slightly larger than that of rutin. As binary aqueous ethanol mixtures are the most often used solvents for the crystallization of luteolin, it is an optimal method that luteolin is dissolved in an ethanol + water mixture at $x'_2 = 0.8$, filtrated, and crystallized in an ethanol + water mixture at $x'_2 = 0.3$ for the antisolvent crystallization process.

Note Added after ASAP Publication: This paper was published ASAP on July 9, 2009. Changes were made to Table 1. The revised paper was reposted on July 20, 2009.

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Received for review April 25, 2009. Accepted June 25, 2009.

JE900381R