J. Chem. Eng. Data **2006,** *51,* 2038−2040

Measurement and Correlation of Solubilities of Luteolin in Organic Solvents at Different Temperatures

Bin Peng,[†] Junqing Zi,[‡] and Weidong Yan*,[†]

Department of Chemistry, Zhejiang University, Hangzhou 310027, People's Republic of China, and Department of Chemistry, Dali University, Dali 671000, People's Republic of China

The solubilities of luteolin in methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, acetone, hexane, and dimethyl sulfoxide (DMSO) at T = (273.15, 283.15, 298.15, 313.15, and 333.15) K were measured. The solubility of luteolin increases with increasing temperature, but there was no correlation with the number of carbon in the solvents. The nonideal solution model and an empirical equation were used to correlate the solid−liquid equilibrium data.

Introduction

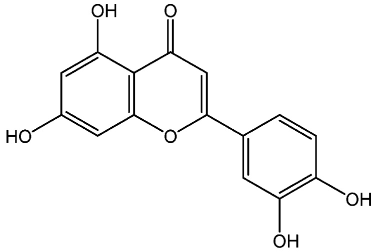
Luteolin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-chromen-4-one; CASRN 491-70-3) usually occurs as glycosylated forms in celery, green pepper, perilla leaf, and chamomile tea.[1] Figure 1 shows the chemical structure of luteolin. Luteolin has various advantageous properties; for example, it is an excellent antioxidant and a very good free-radical scavenger. In addition, it inhibits both enzymatic, nonenzymatic, and CCl_4-induced lipid peroxidations. Luteolin has a favorable influence on the card ovascular system and can prevent the development of arteriosclerosis.[2] The anticancer effect of luteolin is evident inter-alia from the considerable antiproliferative activity against various human tumor cell lines.[3] As an inhibitor of the aldose reductase, luteolin can also have a preventative effect against the development of diabetic cataracts.[2]

Luteolin was synthesized as described in the literature[2] and recrystallized from organic solvent. In order to select the proper solvent and to design an optimized separation process, it is necessary to know its solubility in different solvents. Solubility data of luteolin in a range of organic solvents have not been found in the literature.

In this work, the solubilities of luteolin in methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, acetone, hexane, and DMSO (dimethyl sulfoxide) at T = (273.15, 283.15, 298.15, 313.15, and 333.15) K were measured by a saturation method. Two equations were used to correlate the experimental data.

Experimental Section

Materials. A light yellow powder of luteolin (> 0.98 mass fraction) was supplied by Skyherb Natural Product Co, Ltd. It was recrystallized twice from ethanol, dried in a vacuum oven at T = 393.15 K for 24 h, and stored in a desiccator to avoid absorbing water. The purity is higher than 0.995 mass fraction, determined by HPLC (Shimadzu LC-10AD) according to the United States Patent.[2] The melting point of luteolin is T = 601 ± 0.4 K (literature:[4] 601 to 603 K), determined by a thermal analyzer (Netzsch STA409 PG/PC). All of the organic solvents (analytical grade) were dried with molecular sieves (3 to 4) Å. The mass fraction purities determined by gas chromatograph were > 0.999.



Luteolin ($C_{15}H_{10}O_6$)

Figure 1. Molecular structure of luteolin.

Sample Preparation. Glass-stoppered test tubes (10 mL) with a Teflon-coated magnetic stirrer were used to prepare saturated solutions (about 5.0 mL) of luteolin with excess solid solute (about 0.5 g) in organic solvents. The test tubes were stoppered and sealed up with parafilms to prevent evaporation of solvents. Then the test tubes were directly placed in a constant-temperature thermostatic bath (PolyScience circulators 9501, USA) with temperature accuracy of ± 0.25 K and stability of ± 0.01 K. The uncertainty of equilibrium temperature was ± 0.02 K. Undissolved solid and solution were allowed to settle about (24 to 36) h to ensure equilibrium before sampling. For each test tube, three samples of approximately (0.1 to 0.3) mL were withdrawn from the clear saturated solution using preheated glass syringes. The glass syringe with saturated solution was weighted using a Sartorius type 1712 analytical balance with an uncertainty of ± 0.01 mg. In order to prevent evaporation of solvents during the weighing procedure, the needle was closed with silicon rubber. The saturated solution was injected into the volumetric flask (10 mL) immediately to prevent precipitating. After that, the mass of glass syringes with the remaining solution was weighed. The mass of saturated solutions that were put into volumetric flasks can be found. The solutions of samples used to analyze were diluted to mark with methanol. The reference standard solution of luteolin containing 23.6 $\mu g \cdot mL^{-1}$ of luteolin was prepared in methanol.

Chromatographic Conditions. The solubility was determined using HPLC (LC-10AD, Shimadzu, Japan). The analysis was performed on Diamonsil ODS C_{18} column (250 mm × 4.6 mm,

---

* Corresponding author. E-mail: yanwd@hzcnc.com. Tel: +86 571 87951430. Fax: +86 571 87951895.
[†] Zhejiang University.
[‡] Dali University.

10.1021/je060133l CCC: $33.50

Table 1. Mole Fraction of Luteolin x in Different Solvents at T= (273.15, 283.15, 298.15, 313.15, and 333.15) K

	$10^3\,x^a$				
solvent	T = 273.15 K	T = 283.15 K	T = 298.15 K	T = 313.15 K	T = 333.15 K
methanol	0.46 ± 0.01	0.48 ± 0.01	0.54 ± 0.04	0.71 ± 0.01	0.86 ± 0.04
ethanol	0.76 ± 0.05	1.14 ± 0.01	1.85 ± 0.08	2.68 ± 0.28	3.78 ± 0.20
1-propanol	1.57 ± 0.25	2.03 ± 0.01	2.45 ± 0.06	2.71 ± 0.11	3.22 ± 0.17
2-propanol	1.07 ± 0.09	1.30 ± 0.001	1.94 ± 0.14	2.17 ± 0.02	2.67 ± 0.04
1-butanol	1.38 ± 0.02	1.45 ± 0.16	1.79 ± 0.20	2.19 ± 0.03	2.90 ± 0.12
acetone	0.53 ± 0.02	0.91 ± 0.06	1.56 ± 0.02	2.34 ± 0.07	3.16 ± 0.07
hexane	1.10 ± 0.11	1.23 ± 0.01	1.54 ± 0.03	1.73 ± 0.04	2.01 ± 0.13
DMSO[b]			51.3 ± 1.50	66.25 ± 1.96	84.98 ± 0.96

[a] Expanded uncertainty (±) were calculated using standard diviation SD × coverage factor k. $k = 2$. [b] DMSO, dimethyl sulfoxide.

5 μm), The optimum separation of HPLC was carried out with a mobile phase composed of acetonitrile and an aqueous solution having a volume fraction of 0.1 % of phosphoric acid in a volume ratio of 35:65 (pH = 2.6) at a flow rate of 1.0 mL·min^{-1}. The injected volumes of sample and reference standard solutions were 20 μL. The detective wavelength was set at 254 nm.[3] All chromatograph procedures were performed at room temperature.

Results and Discussion

Before the sample of luteolin was used to determine the solubility, it must be dried because luteolin is easily hydrated. The temperature of dehydrates for luteolin was determined by using a thermal analyzer Netzsch STA409 PG/PC in a nitrogen atmosphere from T = (303 to 573) K. Water in the hydrated luteolin (mass fraction 7.31 %) was driven off at T = (333 to 377) K. The sample was not decomposed or melted below 573 K. According to the results of thermal analysis, T = 393 K was chosen to dry the sample of luteolin.

The solubilities of luteolin in these solvents are too small to be measured by using gravimetrical method.[5] As a result, high-performance liquid chromatograph was chosen to determine the concentration of saturated solution of luteolin in the organic solvents at a given temperature. To prove the reliability of the experimental method, known amounts of luteolin were completely dissolved in methanol at T = 298.15 K. The concentration of solution was measured by HPLC. The average relative uncertainty was 3.1 %.

The solubility data of luteolin in different solvents at T = (273.15, 283.15, 298.15, 313.15, and 333.15) K are given in Table 1. Mole fraction values in Table 1 are the average values taken from two test tubes and three samples for each tube at the same solvent. Expanded uncertainty (±) for each data point is given in Table 1. The experimental data of luteolin in DMSO at T = (273.15 and 283.15) K are not given in Table 1 because DMSO is a solid at temperatures below 293 K.

To describe the solubility behavior in this work, the experimental data were correlated using the solubility formula for nonideal solution, which can be described as[6]

$$\ln\left(\frac{1}{\gamma_x x}\right) = \frac{\Delta_{\text{fus}}H}{RT_{\text{t}}}\left(\frac{T_{\text{t}}}{T} - 1\right) - \frac{\Delta C_p}{R}\ln\left(\frac{T_{\text{t}}}{T} - 1\right) + \frac{\Delta C_p}{R}\ln\frac{T_{\text{t}}}{T} \qquad (1)$$

Table 2. Parameters of Equation 4 Correlated from Experimental Data

	parameters		
solvent	A	B	10^3 RMSD
methanol	−1015.4	−4.0329	0.02
ethanol	−2433.9	1.8012	0.10
1-propanol	−1031.6	−2.6091	0.04
2-propanol	−1405.0	−1.6532	0.09
1-butanol	−1167.2	− 2.3727	0.02
acetone	−2699.1	2.4730	0.15
hexane	−1037.1	− 3.0178	0.06
DMSO[a]	−1428.0	1.8287	0.33

[a] DMSO, dimethyl sulfoxide.

where γ_x is the activity coefficient of luteolin on a mole fraction basis, x is the mole fraction solubility of luteolin, $\Delta_{\text{fus}}H$ is the fusion enthalpy of luteolin, ΔC_p is the heat capacity difference between the solid and the liquid forms of luteolin, T is the equilibrium temperature (K), T_{t} is the triple-point temperature, and R is the gas constant. The value of ΔC_p is so small, compared to $\Delta_{\text{fus}}H$, that the second and third terms can be neglected. It is permissible to substitute normal melting temperature, T_{m}, for triple-point temperature T_{t}. Equation 1 is rewritten as[7]

$$\ln(\gamma_x x) = \frac{\Delta_{\text{fus}}H}{RT_{\text{m}}}\left(1 - \frac{T_{\text{m}}}{T}\right) \qquad (2)$$

The activity coefficient of the solute in an organic solvent is represented by the simple empirical relation:[6]

$$\ln \gamma_x = \frac{C}{RT}(1 - x)^2 \qquad (3)$$

where C is a parameter. If the solubility of luteolin in an organic solvent is very low, eq 4 can be obtained from eqs 2 and 3:

$$\ln x = \frac{A}{T} + B \qquad (4)$$

where A and B are parameters. The data for solubility of luteolin in different solvents were correlated with eq 4. The results are given in Table 2.

The values of the root-mean-square deviations (rmsd) are also listed in Table 2. The rmsd are defined as

$$\text{RMSD} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_{\text{c}i} - x_i)^2\right]^{1/2} \qquad (5)$$

where x_{c} is the solubility calculated by solution equation or empirical equation, respectively, and n is the number of experiment points. The data for solubility of luteolin in ethanol was plotted as shown in Figure 2.

The solubility data were correlated with the empirical equation[8]

$$x = a(T/\text{K})^2 + b(T/\text{K}) + c \qquad (6)$$

where x represents the solubility of luteolin in different solvents; T is the absolute temperature; and a, b, and c are empirical parameters. These parameters were fitted using the experimental data in Table 1. The parameters and rmsd are listed in Table 3.

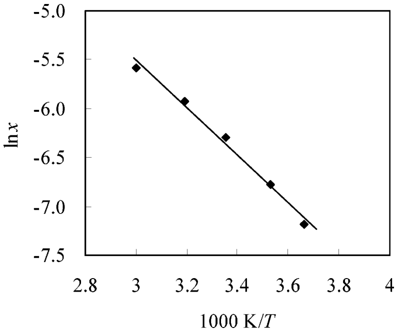


Figure 2. Solubility of luteolin in ethanol at different temperatures: ◆, experimental data; line calculated by eq 4 using parameters in Table 2.

Table 3. Parameters of the Solution (eq 6) Correlated from Experimental Data

	parameters			
solvent	$10^7 a$	$10^5 b$	$10^3 c$	10^3 RMSD
methanol	0.6889	−3.4650	4.7724	0.01
ethanol	1.6382	−4.8446	1.7496	0.01
1-propanol	−1.5719	12.147	−19.831	0.03
2-propanol	−1.4504	11.483	−19.505	0.04
1-butanol	2.5999	−13.188	17.984	0.01
acetone	0.1719	3.4227	−10.130	0.02
hexane	−1.0876	8.2524	−13.420	0.01
DMSO[a]	0[b]	96.089	−234.99	0.10

[a] DMSO, dimethyl sulfoxide. [b] Parameter *a* in eq 6 is set to be equal to zero for the luteolin + DMSO system.

The solubilities of luteolin in pure organic solvents, which are usually used as useful solvents in laboratory and industries, were measured by using a saturation method at T = (273.15, 283.15, 298.15, 313.15, and 333.15) K. The solubilities of luteolin in organic solvents increase with rising temperature. Two equations are used to correlate the experimental data of the solubility of luteolin in eight solvents at different temperatures respectively. Equation 4 is a simplified thermodynamic equation. Equation 6 is an empirical polynomial equation with three parameters. Comparing the fitted results, it can be seen that the correlated result of eq 6 is a little better. But its parameters have no any physical meaning.

Literature Cited

(1) Shimoi, K.; Okada, H.; Furugori, M.; Goda, T.; Takase, S.; Suzuki, M.; Hara, Y.; Yamamoto, H.; Kinae, N. Intestinal absorption of luteolin and luteolin 7-*O*-*β*-glucoside in rats and humans. *FEBS Lett.* **1998**, *438*, 220−224.
(2) Herwig, B.; Ralf, R.; Alice, L.; Christine, K. Method for producing luteolin and luteolin derivatives. U.S. Patent No. 6,538,021, 2003**.**
(3) Chang, J. S.; Hsu, Y. L.; Kuo, P. L.; Kuo, Y. C. Chiang, L. C.; Lin, C. C. Increases of Bax/Bcl-X_L ratio and arrest of cell cycle by luteolin in immortalized human hepatoma cell line. *Life Sci.* **2005**, *76*, 1883−1893.
(4) Jia, L. Y.; Sun, Q. S.; Huang, S. W. Isolation and identification of flavonoids from *Chrysanthemum morifolium* Ramat. *Chin. J. Med. Chem.* **2003**, *13*, 159−164.
(5) Fu, R. R.; Yan, W. D.; Zhu, M. Solubility and density of the disodium salt hemiheptahydrate of ceftriaxone in water + methanol mixtures. *J. Chem. Eng. Data* **2004**, *49*, 262−263.
(6) Prausnitz, J. M.; Lichtenthaler, R. N.; Gomes de Azevedo, E. *Molecular Thermodynamics of Fluid-Phase Equilibria*, 3rd ed.; Prentice Hall: New York, 1999; p 641.
(7) Dong, H; Fu, R. R.; Yan, W. D.; Zhu, M. Solubility and density of the salt hemiheptahydrate of ceftriaxone in (acetone + water) at T = (298.15, 308.15, and 318.15) K. *J. Chem. Thermodyn.* **2004**, *36*, 155−159.
(8) Li, D. Q.; Liu, D. Z.; Wang, F. A. Solubilities of terephthalaldehydic, *p*-toluic, benzoic, terephthalic, and isophthalic acids in *N*-methyl-2-pyrrolidone from 295.65 K to371.35 K. *J. Chem. Eng. Data* **2001**, *46*, 172−173.

Received for review March 23, 2006. Accepted August 8, 2006.

JE060133L