Solubility of Genistein in Water, Methanol, Ethanol, Propan-2-ol, 1-Butanol, and Ethyl Acetate from (280 to 333) K

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The solubility of genistein in water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate was measured at temperatures ranging from (280 to 333) K under atmospheric pressure using high-performance liquid chromatography (HPLC). Ethanol could be the most suitable solvent for purification and crystallization of geinstein. The experimental data were well-correlated with the Apelblat equation, which could be used as a useful model in the purification process of genistein.

Introduction

Genistein (4′,5,7-trihydroxyisoflavone, CAS RN: 446-72-0) is an important member of the isoflavone family which derived from the legumes, lupine, fava bean, soybeans, kudzu, and psoralea, and they are all excellent food sources.[1] The molecular structure of genistein is illustrated in Figure 1. Genistein has many medical effects such as antioxidant,[2] antitumorigenic,[3] antiatherosclerosis,[4] and antiangiogenic,[5] so genistein is very useful.

Some researchers had reported that genistein could be extracted directly from natural products, such as soybeans,[5] the *Ginkgo biloba* leaf,[6] and so forth. Although many extracting technologies have been developed to increase the production of genistein, its application is limited because of the low content in plants. Recently, much attention has been focused on the biotransformation of genistein from genistin.[7,8] No matter which process was adopted for the preparation of genistein, the crude product would not be applied in clinic or as a food additive before purification. High-purity genistein could be obtained by crystallization. Crystallization processes were the key steps that determined the quality of the final product. The solubility of solid compounds in solvents played a crucial role in the determination of proper solvents and the development and operation of crystallization processes. Therefore, knowing the solubility of the product was necessary. In fact, the solubility of genistein in common solvents was scarcely reported.

In this work, the solubility of genistein in water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate was measured from (280 to 333) K using high-performance liquid chromatography (HPLC).[9,10]

Experimental Section

Reagents and Apparatus. Genistein was supplied by Shanghai Tauto Biotech Co., Ltd., China, and the mass fraction of the genistein was higher than 0.99 determined by high-performance liquid chromatography (model: LC-10AT vp plus, Shimadzu, Japan). Methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate which were all HPLC grade were purchased from Tianjin Kermel Chemical Reagent Factory, China, and purified water was purchased from Hangzhou Wahaha Group Co, Ltd. A DSHZ-300A water bath shaker was supplied by Taicang Laboratorial Equipment Factory, China.

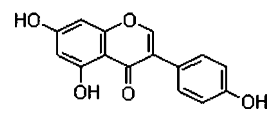

Figure 1. Molecular structure of genistein.

Sample Preparation. An excess amount of genistein was added to 50 mL of the six solvents with their temperatures ranging from (280 to 333) K. The temperature was controlled by a thermostat (uncertainty of ± 0.1 K) in the shaker or in the low-temperature incubator. The suspended solution was kept shaken with 100 rotations per minute for 24 h. After attaining equilibrium, the supernatant liquid was held still for 2 h and filtered through a 0.22 μm membrane which was the same temperature with the samples. The filtered solution was diluted to an appropriate concentration for HPLC analysis. Each measurement was repeated five times. The measuring relative uncertainty of the experimental solubility value was ± 1 %.

Sample Analysis. The HPLC system (model: LC-10AT vp plus, Shimadzu, Japan) was used, equipped with a 20 μL injector loop, UV detector (SPD-10A VP Plus) set at a wavelength of 260 nm, a ODS C18 reverse phase column (250 mm × 4.6 mm, 5 μm), two liquid chromatographs (LC-10AT), and a communications bus module (CBM-10A). A linear HPLC binary gradient was used as follows: solvent A was pure methanol (HPLC grade), and solvent B was 0.1 % phosphoric acid in triple-distilled water. Following the injection of 10 μL of a sample, solvent B was decreased from (80 to 55) % over 10 min and held at 55 % for 5 min. The flow rate was held at 1.0 $mL \cdot min^{-1}$ throughout the running time, which was a total of 15 min.

Results and Discussion

The solubility data of water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate at different temperatures are listed

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Table 1. Solubility (c) of Genistein in Water, Methanol, Ethanol, Propan-2-ol, 1-Butanol, and Ethyl Acetate from (280 to 333) K

T/K	$10^3\,c$/mol·L^{-1}	$10^2(c - c^{calc})/c^a$	T/K	$10^3\,c$/mol·L^{-1}	$10^2(c - c^{calc})/c$
	Water			Methanol	
280	0.0027	6.86	280	23.54	2.96
290	0.0039	6.60	290	27.78	0.26
298	0.0053	7.11	298	32.54	0.00
303	0.0058	−2.64	303	35.93	−0.38
308	0.0069	−4.45	308	41.99	4.64
313	0.0082	−6.50	313	45.54	2.23
323	0.0131	1.91	323	57.03	3.09
333	0.0189	−0.25	333	69.02	0.16
	Ethanol			Propan-2-ol	
280	28.18	−2.75	280	13.62	−4.21
290	32.16	−2.30	290	16.66	−7.72
298	37.40	1.54	298	20.90	−3.49
303	40.12	1.10	303	24.46	0.67
308	43.44	1.29	308	27.94	2.47
313	47.11	1.37	313	31.25	2.04
323	56.07	1.98	323	38.62	0.32
333	64.51	−1.64	333	46.89	−3.01
	1-Butanol			Ethyl Acetate	
280	8.579	−1.55	280	27.38	0.69
290	10.90	−5.54	290	28.47	0.00
298	13.83	−3.57	298	29.62	0.00
303	16.87	2.78	303	30.70	0.01
308	19.89	5.68	308	31.39	0.00
313	22.29	3.84	313	32.43	0.00
323	28.05	0.58	323	35.06	0.01
333	36.14	0.06	333	37.69	0.00

[a] c^{calc}: calculated value based on eq 1 correlated from the experimental solubility of genistein in the six solvents.

Table 2. Parameters of Equation 1 Correlated from the Experimental Solubility of Genistein in the Six Solvents

solvent	A	B	C	10^2 rrmsd
water	−175.92	4730.19	27.16	5.17
methanol	−115.74	3763.70	18.71	2.37
ethanol	−126.18	4670.66	20.03	1.82
propan-2-ol	−73.50	1648.34	12.47	3.70
1-butanol	−71.00	1221.91	12.21	3.55
ethyl acetate	−72.54	2965.78	11.58	0.24

in Table 1. The solubility of genistein in different solvents was in the following order: methanol ≈ ethanol > propan-2-ol >1-butanol > water. The solubility in ethyl acetate was higher than the one in propan-2-ol at lower temperatures but contrary to it at higher temperatures. From the results, it was found that the solubility of genistein increased with increasing polarity of the solvents to some extent, but the solubility of genistein in water was an exception because the polarity of water was highest but the solubility of genistein in water was lowest.

The experimental solubility of genistein increased with the temperature increase. Thus, the solubility of genistein as a function of temperature could be computed by the Apelblat equation[11] deduced from the solid−liquid phase equilibrium as follows

$$\ln(10^3\,c/\mathrm{mol\cdot L^{-1}}) = A + \frac{B}{T/\mathrm{K}} + C\ln(T/\mathrm{K}) \qquad (1)$$

where A, B, and C were the parameters, T was the absolute temperature, and c was the molar solubility of genistein. The correlated values of A, B, and C of the six solvents and the relative root-mean-square deviations (rrmsd) are listed in Table 2. The rrmsd was defined as

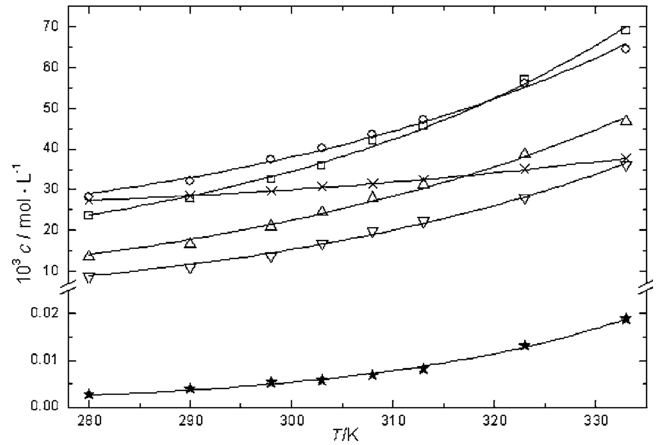

Figure 2. Solubility of genistein in the six solvents from (280 to 333) K: ★, water; □, methanol; ○, ethanol; △, propan-2-ol; ▽, 1-butanol; ×, ethyl acetate. The corresponding lines were from the calculated values based on eq 1.

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

where n was the number of experimental points and C_i^{cal} and C_i^{exp} represented the calculated and the experimental solubility values, respectively. The calculated solubility of genistein at different temperatures in six solvents (water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate) accorded with the experimental data, which are also shown in Figure 2.

Conclusions

(1) The solubility of genistein in water, methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate was a function of temperature and increased with an increase of temperature.

(2) Methanol, ethanol, propan-2-ol, 1-butanol, and ethyl acetate all presented a good ability of dissolving genistein, and the molar solubility of genistein in methanol and ethanol was higher than in the other four solvents. In view of low toxicity and low price, ethanol could be the most suitable solvent for purification and crystallization.

(3) The solubility data were well-correlated with the modified Apelblat equation, which could be used as a useful model in the purification process of genistein.

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