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SHORT ARTICLE

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Measurement and Correlation of Solubilities of Asiaticoside in Water, Methanol, Ethanol, *n*-Propanol, *n*-Butanol, and a Methanol + Water Mixture from (278.15 to 343.15) K

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ABSTRACT: The solubilities of asiaticoside in water, methanol, ethanol, *n*-propanol, *n*-butanol, and a methanol + water mixture were determined over the temperature range from (278.15 to 343.15) K by a static analytical method. The concentrations of asiaticoside in the saturated solutions were analyzed by reverse-phase high-performance liquid chromatography. An "*N*"-type curve was found for the solubilities of asiaticoside in the methanol + water mixture. Two empirical equations were proposed to correlate the experimental data, which fit the data well.

1. INTRODUCTION

Asiaticoside[1,2] (2,3,23-trihydroxy-*O*-6-deoxy-α-L-mannopyranosyl-(1→4)-*O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, (2α,3β,4α)-Urs-12-en-28-oic acid, CAS No. 16830-15-2, Figure 1), a triterpene glycoside from *Centella asiatica*, has been claimed to possess various physiological effects, such as wound healing[3,4] and antidepressant-like effects.[5,6]

In our laboratory, a process combining crystallization[7] with macroporous resin column chromatography[8,9] for preparing high-purity asiaticoside from *C. asiatica* has been developed, and research on the induction period of asiaticoside has been undertaken, which is necessary for optimization of the crystallization process and product quality control. Solubility information on asiaticoside is crucial for this research. To the best of our knowledge, however, no experimental solubilities of asiaticoside in solvents are available in the literature.

In this study, the solubilities of asiaticoside in water, methanol, ethanol, *n*-propanol, *n*-butanol, and a methanol + water mixture were determined over the temperature range from (278.15 to 343.15) K. The measured solubilities indicated the effects of solvent and temperature on the solubility of asiaticoside, providing useful information for research of the induction period of asiaticoside in mixed solvents.

2. EXPERIMENTAL SECTION

2.1. Chemicals. At first, the raw material asiaticoside (~90 % purity), which was purchased from the Guangxi Changzhou Natural Products Development Co., Ltd. (Guangxi, China), was recrystallized four times with methanol. It was then dried for 24 h in a vacuum drying oven at a temperature of 323.15 K. Through quantitatively analyzing by high-performance liquid chromatography (HPLC) with asiaticoside standards (> 98.5 % purity) from Sigma, it is confirmed that the purity of asiaticoside reagent used for the solubility determinations was over 98.0 %. Analytical reagent (AR) grade methanol, ethanol, and *n*-butanol were obtained from the Sinopharm Chemical Reagent Co., Ltd. AR grade *n*-propanol was obtained from the Shanghai Lingfeng Chemical Reagent Co., Ltd. Distilled water was purchased from the Hangzhou Wahaha Group Co., Ltd. HPLC grade methanol and acetonitrile were obtained from Merck.

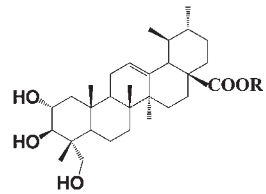

Figure 1. Chemical structure of asiaticoside (R = Glu-Glu-Rha, where Glu and Rha represent glucose and rhamnose, respectively).

2.2. Apparatus and Procedure. The solubility determination in this study was carried out by a static analytical method that was described in our previous work.[10−12] All experiments were carried out in a precision water bath, and the uncertainty of temperature was ± 0.05 K of the desired value. A total of 12 h of stirring and 6 h of settling was employed to ensure equilibrium was reached, and the reproducibility of the data was within 0.5 %. A condenser with a balloon on top was connected to the vessel to prevent the solvent from evaporating and keep a constant composition of the binary solvent.

2.3. Analysis. About 1 g of saturated solution was quickly taken out, put into the bottom of a volumetric flask with a stopper, weighed, and diluted with 1:1 (volume ratio) methanol—water solvent to a certain volume. Then the composition of the pretreated samples was directly determined by HPLC (Agilent 1100). The analysis was carried out by a Synergi 4 μ Hydro-RP 80A reverse-phase column (4.6 mm × 250 mm, 4 μm, Phenomenex). The samples were detected at a wavelength of 205 nm. The flow rate was 0.5 $mL\cdot min^{-1}$, and the column temperature was 298.15 K. The mobile phase was methanol—water (55:45, v/v) except for *n*-butanol, which was analyzed with acetonitrile—water (27:73, v/v). The linear range was (0.0237 to 0.3796) $mg\cdot mL^{-1}$ for *n*-butanol, and the others were (0.0197 to 0.9550) $mg\cdot mL^{-1}$.

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Table 1. Solubilities of Asiaticoside at Different Temperatures in Five Solvents, Together with Error Limits Using the 95 % Confidence Level

T/K	$10^3 x$				
	water	methanol	ethanol	*n*-propanol	*n*-butanol
278.15		0.9023 ± 0.004			
283.15		0.9037 ± 0.0019			
288.15		0.9728 ± 0.002			
293.15		1.0254 ± 0.0026			
298.15	0.0019 ± 0.00001	1.1264 ± 0.0027	0.1404 ± 0.0003	0.052 ± 0.0002	0.0244 ± 0.0002
303.15	0.0021 ± 0.00001	1.2209 ± 0.0017	0.1425 ± 0.0004	0.0606 ± 0.0003	0.0293 ± 0.0003
308.15	0.0025 ± 0.00001	1.3869 ± 0.0025	0.1458 ± 0.0002	0.0678 ± 0.0001	0.0345 ± 0.0003
313.15	0.0031 ± 0.00001	1.6025 ± 0.0025	0.1516 ± 0.0003	0.0806 ± 0.0003	0.0422 ± 0.0004
318.15	0.0042 ± 0.00002	1.8242 ± 0.0084	0.1653 ± 0.0011	0.0961 ± 0.0002	0.051 ± 0.0002
323.15	0.0054 ± 0.00003	2.1595 ± 0.0062	0.1758 ± 0.0009	0.1193 ± 0.0003	0.0636 ± 0.0001
328.15	0.0071 ± 0.00002	2.5251 ± 0.0377	0.1952 ± 0.0004	0.1443 ± 0.0006	0.0803 ± 0.0001
333.15	0.0102 ± 0.00006		0.2172 ± 0.0006	0.1806 ± 0.0003	0.1009 ± 0.0004
338.15				0.2297 ± 0.0005	0.1319 ± 0.0002
343.15				0.3042 ± 0.001	0.175 ± 0.0006

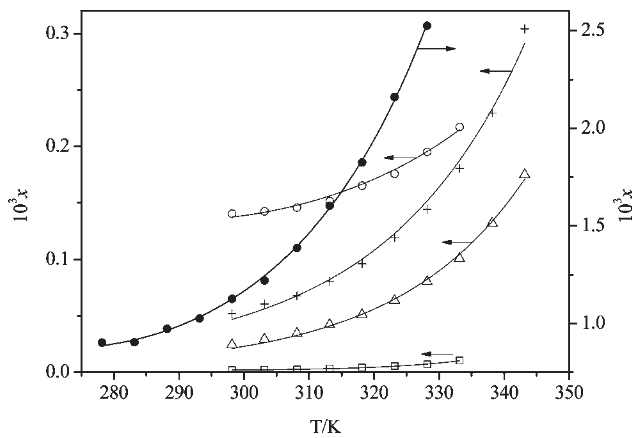

Figure 2. Solubilities of asiaticoside in five pure solvents: □, water; ●, methanol; ○, ethanol; +, *n*-propanol; △, *n*-butanol; —, calculated by eq 1.

3. RESULTS AND DISCUSSION

At each temperature, six samples were taken and analyzed. The experimental data for the solubilities of asiaticoside in water, methanol, ethanol, *n*-propanol, and *n*-butanol at different temperatures are listed in Table 1, with error limits using the 95 % confidence level. The results show that the solubilities of asiaticoside in all five solvents increases with temperature. The solubility of asiaticoside in methanol is the highest and is more dependent on temperature than in other solvents. The solubility of asiaticoside in water is the lowest and is less sensitive to temperature.

A nonlinear equation was proposed to correlate the solubility data of asiaticoside in five pure solvents as a function of temperature as follows.

$$\ln x = a + bT + c/T \qquad (1)$$

where x refers to the mole fraction solubility, T is the absolute temperature, and a, b, and c are parameters. The calculated curves from eq 1 are shown in Figure 2, which give very good agreement.

Table 2. Parameters of Equation 1 for Asiaticoside in Pure Solvents

solvent	a	$10^3 b$	c	10^3 rmsd
water	−156.498	252.607	20270.1	0.00009
methanol	−67.6114	111.303	8239.95	0.0129
ethanol	−54.8553	79.5552	6631.67	0.0026
n-propanol	−43.1184	73.4135	3358.07	0.0060
n-butanol	−41.1354	72.1962	2638.19	0.0025

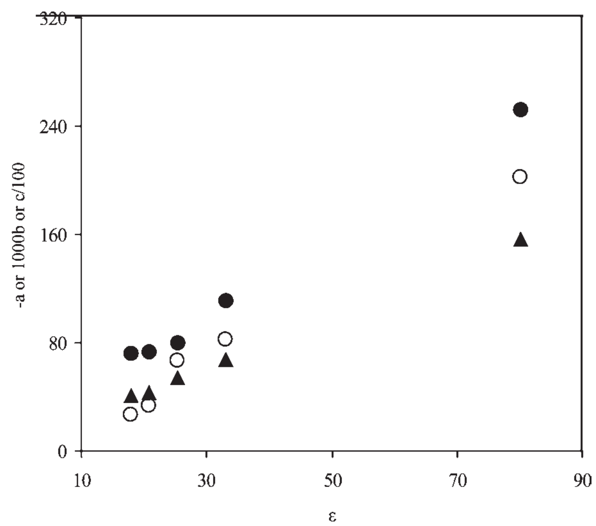

Figure 3. Relationship between the parameters of eq 1 with the dielectric constant of the solvents: ▲, $-a$; ●, $1000b$; ○, $c/100$.

The parameters a, b, and c in eq 1 are presented in Table 2, with the root-mean-square deviations (rmsd's) defined by

$$\text{rmsd} = \sqrt{\sum_{i}^{n} (x_{c,i} - x_i)^2 / n} \qquad (2)$$

Table 3. Solubilities of Asiaticoside in a Methanol + Water Mixture at Different Temperatures, Together with Error Limits Using the 95 % Confidence Level

x_m	$10^3 x$						
	298.15	303.15	308.15	313.15	318.15	323.15	328.15
0.05882	0.0059 ± 0.00002	0.0069 ± 0.00002	0.0081 ± 0.00002	0.0098 ± 0.00002	0.0124 ± 0.00004	0.0155 ± 0.00005	0.0188 ± 0.00026
0.1233	0.0108 ± 0.00007	0.0144 ± 0.00007	0.0181 ± 0.00003	0.024 ± 0.00012	0.0317 ± 0.00014	0.0407 ± 0.0001	0.0564 ± 0.00005
0.1942	0.0277 ± 0.0001	0.0371 ± 0.0003	0.0455 ± 0.0001	0.0655 ± 0.0003	0.0835 ± 0.0004	0.11 ± 0.0004	0.1518 ± 0.0002
0.2727	0.0562 ± 0.0003	0.0744 ± 0.0001	0.1012 ± 0.0002	0.1414 ± 0.0005	0.1891 ± 0.001	0.2478 ± 0.0015	0.3292 ± 0.0017
0.3600	0.11 ± 0.0003	0.1427 ± 0.0007	0.1905 ± 0.0004	0.2656 ± 0.0007	0.3428 ± 0.0018	0.4742 ± 0.0012	0.6357 ± 0.0012
0.4576	0.1728 ± 0.0003	0.2277 ± 0.0003	0.3046 ± 0.0008	0.4056 ± 0.0015	0.536 ± 0.0032	0.7193 ± 0.0012	0.9557 ± 0.0018
0.5676	0.2181 ± 0.0002	0.2782 ± 0.0005	0.3794 ± 0.001	0.4952 ± 0.0015	0.645 ± 0.0049	0.8392 ± 0.0016	1.1219 ± 0.0022
0.6923	0.2148 ± 0.0004	0.2757 ± 0.0005	0.3718 ± 0.0011	0.4775 ± 0.0003	0.6325 ± 0.0032	0.8018 ± 0.0015	1.0829 ± 0.0017
0.8351	0.1976 ± 0.0003	0.2524 ± 0.0006	0.3251 ± 0.0003	0.4265 ± 0.0003	0.5851 ± 0.0012	0.7816 ± 0.0012	1.018 ± 0.0017
0.8740	0.2055 ± 0.0005	0.259 ± 0.0011	0.3347 ± 0.0008	0.4505 ± 0.0014	0.5899 ± 0.0006	0.7851 ± 0.0012	1.0169 ± 0.0027
0.9144	0.2444 ± 0.0008	0.3074 ± 0.0006	0.3933 ± 0.0012	0.5146 ± 0.002	0.6768 ± 0.0021	0.8829 ± 0.0022	1.1095 ± 0.0037
0.9564	0.3724 ± 0.0012	0.4588 ± 0.0013	0.5777 ± 0.0015	0.746 ± 0.0042	0.9524 ± 0.0017	1.2111 ± 0.0028	1.5552 ± 0.0077
0.9780	0.5638 ± 0.0011	0.6749 ± 0.008	0.8153 ± 0.0015	1.0394 ± 0.0014	1.2297 ± 0.0018	1.4573 ± 0.0255	1.9807 ± 0.0112

Table 4. Parameters of Equation 3 for Asiaticoside in Different Compositions of a Methanol + Water Mixture from (298.15 to 328.15) K

T/K	A_0	A_1	A_2	A_3	A_4	A_5	10^3 rmsd
298.15	−13.3246	22.2468	−60.79607	139.321	−178.211	83.8529	0.0379
303.15	−13.0924	21.6715	−52.854	112.993	−145.221	69.6936	0.0343
308.15	−12.8954	21.1916	−45.6375	90.9672	−119.161	58.8705	0.0333
313.15	−12.6403	20.6428	−38.1998	66.9627	−89.7726	46.521	0.0262
318.15	−12.3826	20.1204	−31.3088	43.2809	−58.7079	32.638	0.0321
323.15	−12.1449	19.642	−24.50417	20.7351	−30.4081	20.4846	0.0457
328.15	−11.8892	19.106	−16.2318	−6.25452	2.28549	6.97227	0.0452

where x_i is experimental solubility, $x_{c,i}$ is the calculated solubility, and n is the number of experimental points. Figure 3 illustrated the relationship between the parameters of eq 1 with the dielectric constant of the solvents at 293.15 K, indicating that a, b, and c in eq 1 have approximately a linear relation with the dielectric constant.

The solubility data of asiaticoside in a binary methanol + water mixture are listed in Table 3 together with error limits using the 95 % confidence level and also shown in Figure 4. The plots at each temperature exhibit an "N"-type curve. This phenomenon may arise from the effect of methanol—water interactions in the mixed solvent and the effect of specific interactions between the mixed solvent and the asiaticoside which has a highly polar sugar chain unit.[13]

A fifth-order polynomial equation was proposed to correlate the solubility data of asiaticoside in the binary methanol + water mixture as a function of x_m as follows:

$$\ln x = A_0 + A_1 x_m + A_2 x_m^2 + A_3 x_m^3 + A_4 x_m^4 + A_5 x_m^5 \quad (3)$$

where x refers to mole fraction solubility, x_m is the methanol mole fraction of the binary methanol + water solvent mixture, and A_0, A_1, A_2, A_3, A_4, and A_5 are parameters.

The values A_0, A_1, A_2, A_3, A_4, and A_5 in eq 3 are listed in Table 4, together with the root-mean-square deviations. Furthermore, the values A_0, A_1, A_2, A_3, A_4, and A_5 were correlated as a function of temperature with the following linear equation:

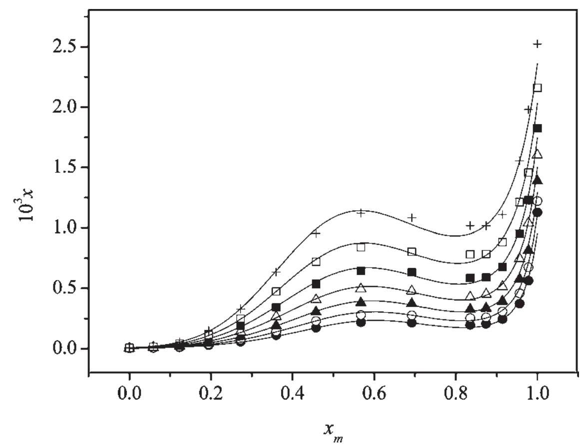

Figure 4. Solubilities of asiaticoside in the methanol + water mixture at different temperatures/K: ●, 298.15; ○, 303.15; ▲, 308.15; △, 313.15; ■, 318.15; □, 323.15; +, 328.15; —, calculated by eq 5.

$$y = e + fT \quad (4)$$

where y refers to the values of A_0, A_1, A_2, A_3, A_4, and A_5, T is the absolute temperature, and e and f are parameters. The results are presented in Table 5 with R^2 close to 1. Finally, a global

Table 5. Parameters of Equation 4

parameter	e	f	R^2
A_0	−27.642	0.047957	0.9988
A_1	53.211	−0.10395	0.9996
A_2	−496.42	1.4623	0.9994
A_3	1563.1	−4.7781	0.9995
A_4	−1948.5	5.9398	0.9994
A_5	840.29	−2.5378	0.9993

expression for the solubility of asiaticoside in the methanol + water mixture was obtained as follows:

$$\ln x = -27.642 + 53.211x_m - 496.42x_m^2 + 1563.1x_m^3 - 1948.5x_m^4 + 840.29x_m^5 + 0.047957T - 0.10395Tx_m + 1.4623Tx_m^2 - 4.7781Tx_m^3 + 5.9398Tx_m^4 - 2.5378Tx_m^5 \quad (5)$$

The calculated solubilities with eq 5 are also depicted in Figure 4, which fit the experimental data very well.

4. CONCLUSION

The solubilities of asiaticoside in water, methanol, ethanol, *n*-propanol, *n*-butanol, and a methanol + water mixture were determined over the temperature range from (278.15 to 343.15) K by a static analytical method. The data are essential for optimization of the asiaticoside crystallization process and product quality control. A nonlinear equation was proposed to correlate the solubility data of asiaticoside in five pure solvents, and a fifth-order polynomial equation was used to correlate the solubility data of asiaticoside in a binary methanol + water mixture. Both equations fit the experimental data well.

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