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Solubilities of Betulinic Acid in Thirteen Organic Solvents at Different Temperatures

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Supporting Information

ABSTRACT: The solubilities of betulinic acid in methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, 2-butanol, 1-pentanol, 1-hexanol, ethyl formate, ethyl acetate, butyl acetate, acetone, and tetrahydrofuran were measured by an analytical method in the temperature range of (278.2 to 318.2) K. The experimental data were correlated with a three-parameter empirical equation and UNIQUAC model, respectively. The calculated solubilities showed good agreement with the experimental data over this temperature range. Furthermore, the relation of the solubilities and the crystal habit were discussed.

INTRODUCTION

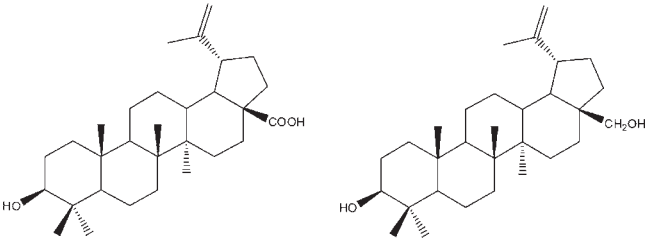
Solution crystallization is an important separation and purification technique in the production of fine chemicals and pharmaceuticals. For the selection of a suitable solvent or solvent mixture of a new active compound and design of the crystallization processes, reliable solubility data for complex molecules such as natural products and pharmaceutical drugs are essential.

Betulinic acid (3β-hydroxy-lup-20(29)-en-28-oic acid, CASRN: 472-15-1, Figure 1) is a pentacyclic triterpenoid of plant origin that is wildly distributed in many plants throughout the world. For example, this triterpenoid has been found in *Ziziphus* spp.,[1] *Syzygium* spp.,[2] *Paeonia* spp.[3] and *Doliocarpus* spp.[4] In recent years, some special biological properties of betulinic acid have drawn the attention of researchers. It has been reported to have many important biological and pharmacological activities, including antimelanoma,[5] anticancer,[6] anti-inflammatory,[7] and anti-HIV.[8]

However, there are still many difficulties, both technological and financial, in obtaining a commercial quantity (i.e., kgs) of betulinic acid.[9] In order to effectively separate the betulin and betulinic acid from a reactive mixture or plant extracts by physical or chemical method, it is necessary to determine the solubilities of betulin and betulinic acid in different solvents. The solubilities of betulin in pure solvents[10] and mixed solvents[11] have been measured. However, the solubilities of betulinic acid in organic solvents have not been found in the literature. In this work, the solubilities of betulinic acid in thirteen organic solvents were determined by an analytical method[12] at T = (278.2 to 318.2) K. A three-parameter empirical equation and UNIQUAC model were used to correlate the experimental data, respectively.

EXPERIMENTAL SECTION

Materials. The white powder of betulinic acid (0.98 mass fractions) was supplied by Skyherb Ingredients (China). A total of 100 g of betulinic acid was dissolved in 5500 L of methanol, refluxed for about 2 h, filtered, and cooled naturally to room temperature at 5 °C. About 20 g of white needle-like betulinic acid was obtained after recrystallization three times. It was dried in a vacuum oven at T = 348.2 K for 24 h and stored in a desiccator to avoid moisture absorption. The purity of betulinic acid was higher than 0.995 mass fractions, determined by HPLC (Shimadzu LC-10AT). The reference standard of betulinic acid, with a purity of higher than 0.98 mass fractions, was purchased from Sigma-Aldrich Chemical Corporation.



Betulinic acid ($C_{30}H_{48}O_3$) Betulin ($C_{30}H_{50}O_2$)

Figure 1. Molecule structure of betulinic acid and betulin.

All of the organic solvents were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (China). The solvents were dehydrated with molecular sieves (3 to 4) Å before use. The purities of the organic solvents, determined by gas chromatography, are more than >0.998 mass fraction.

Sample Preparation. The 15 cm^3 centrifuge tubes (polybutadiene styrene, PBS) with caps were used to prepare saturated solutions (about 10 cm^3) of betulinic acid with excess solid solute in organic solvents. The tube was gastight when the turncap with

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a sizable rubber band was screwed on. Then the tubes were directly placed in a constant temperature thermostatic bath (THID-0510W, China) with a temperature fluctuation of ± 0.01 K and a temperature uncertainty of ± 0.1 K. The tubes were allowed to settle for about (36 to 48) h to ensure equilibrium. For each tube, three samples of approximately (0.2 to 0.6) mL were withdrawn from the clear saturated solution using preheated glass syringes. The glass syringe with a saturated solution was weighted using a Sartorius Type CPA225D analytical balance with an uncertainty of ± 0.01 mg. The needle was closed with silicon rubber to prevent evaporation of solvents during the weighing procedure. To prevent precipitation, the saturated solution was injected into the 10 mL volumetric flask immediately. Subsequently, the mass of the glass syringe with the remaining solution was weighed. Then the mass of saturated solutions placed into volumetric flasks was calculated. The solutions of samples used for analysis were diluted to the mark with methanol.

An accurately weighed quantity of betulinic acid (RS) was dissolved in methanol to obtain a reference standard solution having the known concentration of about 0.25 $mg \cdot mL^{-1}$.

Chromatographic Conditions. The solubility was determined using HPLC (Shimadzu Corporation, Kyoto, Japan) consisting of a degasser (DGU-4A), a solvent delivery module (LC-10AT), and UV detector (SPD-10A). Data were acquired using the N2000 Chromatographic Data System (Zheda information and Technologies Ltd., Hangzhou, China).

The analysis was performed on a Diamonsil C_{18} column (250 mm × 4.6 mm, 5 μm). The optimum separation of HPLC was carried out with a mobile phase composed of acetonitrile and water in a volume ratio of 92:8 at a flow rate of 1.0 $mL \cdot min^{-1}$. The injected volumes of sample and reference standard solutions were 20 μL. The detection wavelength was set at 210 nm. All chromatograph procedures were performed at room temperature.[12]

Thermal Analysis. A differential scanning calorimeter (Q100, TA Corporation) was used to determine the fusion enthalpies and the melting temperatures for betulinic acid. About 2.5 mg betulinic acid powder was put in a closed DSC pan. For each DSC experiment, an empty DSC pan was used as a blank reference. The sample was scanned from 200 to 350 °C at a heating rate of 10 $°C \cdot min^{-1}$.

Scanning Electron Microscope. In experimental process, we observed an interesting phenomenon in that the morphology of the crystals of betulinic acid in different solvents changed. In order to explain this result, the hot saturated solutions of betulinic acid were cooled naturally to room temperature and restored at 0 °C about 48 h. Then, the precipitates of betulinic acid were dried in a vacuum oven for 24 h and observed by SEM (FEI Sirion, Holland) at room temperature. The images of samples were taken at 25.0 kV accelerating voltage.

Experimental Reliability. The solubilities of betulinic acid in most of the selected organic solvents are very low. Therefore, HPLC was employed to determine the concentration of a saturated solution of betulinic acid in organic solvent. To evaluate the reliability of the experimental method in this work, the known amounts of betulinic acid were completely dissolved in methanol. The concentrations of solutions were measured by HPLC. The average relative uncertainty was 2.28%.

Table 1. Experimental Data of Solubilities for Betulinic Acid in Different Solvents at T = (278.2, 288.2, 298.2, 308.2, and 318.2) K[a]

T/K	$10^4 x_1$			
	methanol	ethanol	1-propanol	2-propanol
278.2	1.90 ± 0.07	6.51 ± 0.08	10.31 ± 0.05	7.65 ± 0.23
288.2	2.48 ± 0.08	8.19 ± 0.06	13.71 ± 0.78	9.20 ± 0.15
298.2	3.39 ± 0.05	10.69 ± 0.29	17.78 ± 0.28	11.97 ± 0.24
308.2	4.61 ± 0.19	13.51 ± 0.02	22.10 ± 0.87	14.88 ± 0.24
318.2	7.34 ± 0.55	17.12 ± 0.40	27.99 ± 0.08	19.63 ± 0.23
	1-butanol	2-butanol	1-pentanol	1-hexanol
278.2	11.77 ± 0.25	8.21 ± 0.32	19.93 ± 0.59	22.62 ± 0.71
288.2	14.87 ± 0.02	11.28 ± 0.39	22.77 ± 0.03	28.33 ± 0.92
298.2	19.20 ± 0.60	14.92 ± 0.35	26.65 ± 0.44	32.43 ± 0.14
308.2	23.45 ± 0.71	18.90 ± 0.23	30.94 ± 0.81	37.82 ± 0.31
318.2	29.97 ± 0.13	24.47 ± 0.61	36.30 ± 0.67	45.42 ± 0.03
	ethyl formate	ethyl acetate	butyl acetate	acetone
278.2	2.00 ± 0.10	4.50 ± 0.16	8.24 ± 0.19	3.98 ± 0.14
288.2	2.72 ± 0.08	5.15 ± 0.26	10.01 ± 0.59	5.02 ± 0.86
298.2	4.28 ± 0.18	7.33 ± 0.05	11.98 ± 0.67	5.98 ± 0.09
308.2	5.48 ± 0.34	9.56 ± 0.18	15.91 ± 0.26	8.29 ± 0.12
318.2	7.86 ± 0.45	12.44 ± 0.41	19.28 ± 0.20	10.32 ± 0.34
	tetrahydrofuran			
278.2	97.50 ± 0.06			
288.2	135.60 ± 0.32			
298.2	205.61 ± 1.05			
308.2	275.73 ± 0.06			
318.2	387.54 ± 1.42			

[a] Expanded uncertainties (±) were calculated using standard deviation, SD × coverage factor k; k = 2.

MODELING SECTION

According to the solid−liquid phase equilibrium theory, the relationship between solubility and temperature is described as[13]

$$\ln\left(\frac{1}{\gamma_x x1}\right) = \frac{\Delta_{fus}H}{RT_t}\left(\frac{T_t}{T} - 1\right) - \frac{\Delta C_p}{R}\left(\frac{T_t}{T} - 1\right) + \frac{\Delta C_p}{R}\ln\frac{T_t}{T} \quad (1)$$

where γ_x is the activity coefficient of betulinic acid on a mole fraction basis, x_1 is the mole fraction solubility of the solute, $\Delta_{fus}H$ is the enthalpy of fusion of betulinic acid, ΔC_p is the change of the heat capacity, T is the absolute temperature, T_t is the tripe-point temperature of betulinic acid, and R is the gas constant.

Three-Parameter Empirical Equation. For regular solution, the activity coefficient is given by

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

where A and B are constants. Introducing γ_x from eq 2 into eq 3 and subsequent rearrangement results in

$$\ln x_1 = \left[\frac{\Delta_{fus}H}{RT_t} + \frac{\Delta C_p}{R}(1 + \ln T_t) - A\right] - \left[B + \left(\frac{\Delta_{fus}H}{RT_t} + \frac{\Delta C_p}{R}\right)T_t\right]\frac{1}{T} - \frac{\Delta C_p}{R}\ln T \quad (3)$$

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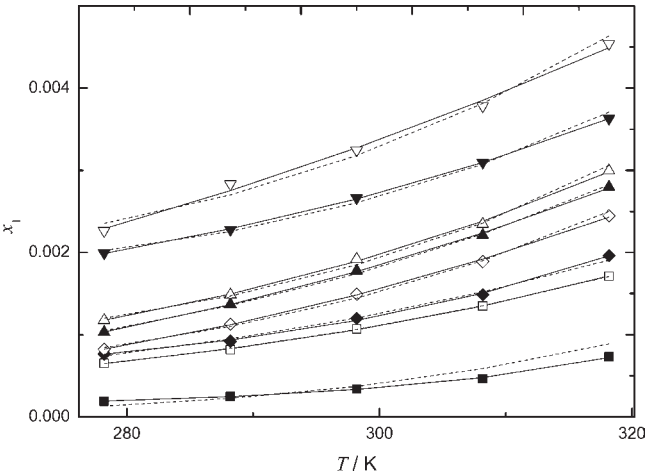

Figure 2. Solubilities of betulinic acid in alcohols: ■, methanol; □, ethanol; ▲, 1-propanol; Δ, 1-butanol; ▼, 1-pantanol; ▽, 1-hexanol; ♦, 2-propanol; ◇, 2-butanol; solid line, calculated by eq 4 using parameters in Table 2; dash line, predicted with the UNIQUAC equation using parameters in Table 3.

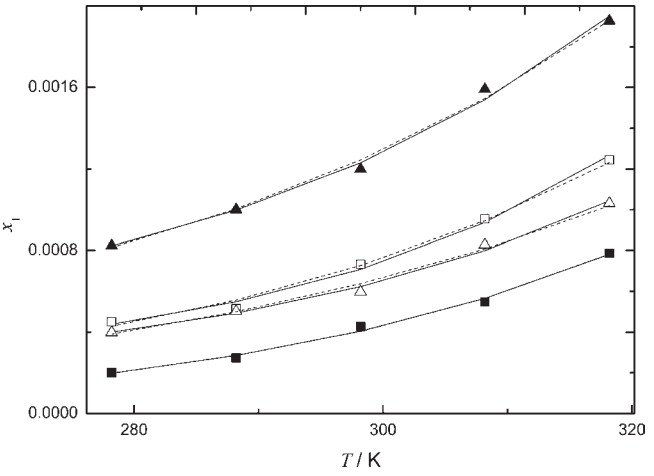

Figure 3. Solubilities of betulinic acid in esters and acetone: ■, ethyl formate; □, ethyl acetate; ▲, butyl acetate; Δ, acetone; solid line, calculated by eq 4 using parameters in Table 2; dash line, predicted with the UNIQUAC equation using parameters in Table 3.

Equation 3 could be expressed as

$$\ln x_1 = a + \frac{b}{T/K} + c \ln \frac{T}{K} \tag{4}$$

Where a, b, and c are empirical parameters. The values of the three parameters a, b, and c together with the root-mean-square deviation (rmsd) are also listed in Table 2. The rsmd are defined as

$$\text{rmsd} = \left[\frac{1}{n}\sum_{i=1}^{n}(x_{i,\text{calcd}} - x_{i,\text{exptl}})^2\right]^{1/2} \tag{5}$$

where $x_{i,\text{calcd}}$ is mole fraction solubility calculated by eq 4 using the parameters in Table 2, $x_{i,\text{exptl}}$ is the experimental values of mole fraction solubility of betulinic acid, and n is the number of experimental points.

Table 2. Parameters of eq 4 for Betulinic Acid in Different Organic Solvents

solvent	a	b/K	c	10^4 rmsd
methanol	−460.777	17609.797	69.100	0.10
ethanol	−83.347	1564.493	12.505	0.07
1-propanol	23.006	−3169.758	−3.285	0.15
2-propanol	−212.325	7349.299	31.755	0.14
1-butanol	−62.912	764.367	9.491	0.22
2-butanol	34.416	−3853.555	−4.915	0.16
1-pentanol	−100.226	3054.047	14.752	0.07
1-hexanol	−7.022	−1210.887	0.941	0.56
ethyl formate	−53.151	−575.555	8.296	0.14
ethyl acetate	−258.364	9154.994	38.683	0.23
butyl acetate	−172.523	5735.778	25.727	0.28
acetone	−206.736	7044.206	30.842	0.18
tetrahydrofuran	−47.714	−669.958	8.081	0.38

The solubilities of betulinic acid in Table 1 were plotted as shown in Figure 2 and Figure 3. The solid lines were calculated by eq 4 using parameters in Table 2.

UNIQUAC Equation. In chemical literature, there are assumptions commonly employed: the first one is that ΔC_p at T_f is equal to zero. For most substances there is a little difference between the triple point temperature and the normal melting point temperature, and also there is a negligible difference between the enthalpies of fusion at these two temperatures. Therefore, the normal melting temperature (T_m) can be substituted for the triple point (T_t). Therefore, a simplified form of eq 1 can be stated

$$\ln\left(\frac{1}{\gamma_1 x_1}\right) = \frac{\Delta_{\text{fus}}H}{RT_m}\left(\frac{T_m}{T} - 1\right) \tag{6}$$

The activity coefficient of betulinic acid in eq 6 can be calculated by the UNIQUAC equation

$$\ln \gamma_i = \ln\frac{\phi_i}{x_i} + \left(\frac{z}{2}\right)q_i \ln\frac{\theta_i}{\phi_i} + l_i - \frac{\phi_i}{x_i}\sum_{j=1}^{m} x_j l_j - q_i \ln\left(\sum_{j=1}^{m}\theta_j\tau_{ji}\right) + q_i - q_i\sum_{j=1}^{m}\frac{\theta_j\tau_{ij}}{\sum_{k=1}^{m}\theta_k\tau_{kj}} \tag{7}$$

where

$$\phi_i = \frac{r_i x_i}{\sum_{j=1}^{m} r_j x_j}, \quad \theta_i = \frac{q_i x_i}{\sum_{j=1}^{m} q_j x_j}$$

$$l_j = \frac{z}{2}(r_j - q_j) - (r_j - 1)$$

The coordination number z is set equal to 10. r_i and q_i are the structural parameters of pure solvent i. The structural parameters of betulinic acid were calculated by the functional group approach.[14]

$$r = \sum_{i=1}^{m} n_i R_i, \quad q = \sum_{i=1}^{m} n_i Q_i \tag{8}$$

where m is the number of functional groups in the molecule and n is the repeating number of each function group. The structural parameters R_i and Q_i of function group i were taken from the

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Table 3. Parameters of UNIQUAC Equation for Betulinic Acid in Different Organic Solvents

	$\Delta\mu_{12}$	$\Delta\mu_{21}$	
solvent	J·mol^{-1}	J·mol^{-1}	10^3 rmsd
methanol	−301.48	51490.07	0.09
ethanol	−1848.51	5286.81	0.01
1-propanol	−1967.17	4247.27	0.02
2-propanol	−2012.43	4394.32	0.03
1-butanol	2880.26	−1937.91	0.04
2-butanol	1973.25	−1626.411	0.03
1-pentanol	2935.42	−2025.03	0.04
1-hexanol	2593.684	−1916.656	0.08
ethyl formate	−1724.55	3470.28	0.01
ethyl acetate	1690.17	−1544.73	0.02
butyl acetate	1736.76	−1614.97	0.03
acetone	−1917.11	5048.06	0.02
tetrahydrofuran	1541.23	−1016.75	2.13

literature. The values calculated for r and q of betulinic acid are 20.8562 and 19.323, respectively. Two adjustable parameters, τ_{ij} and τ_{ij}, are expressed by

$$\tau_{ij} = \exp\left(-\frac{\Delta\mu_{ij}}{RT}\right), \quad \tau_{ji} = \exp\left(-\frac{\Delta\mu_{ji}}{RT}\right) \tag{9}$$

where $\Delta\mu_{ij}$ and $\Delta\mu_{ij}$ are interaction parameters and can be fitted to the experimental data by a nonlinear least-squares method. The solvent−solute interaction parameters are given in Tables 3 together with the root-mean-square deviations (rmsd).

RESULTS AND DISCUSSION

Thermodynamic Properties of Betulinic Acid. The melting temperature was determined to be 588.45 K for betulinic acid and the result agrees with that in literature.[15] The DSC curve of betulinic acid is given in Figure S1 in Supporting Information. However, due to the decomposition of betulinic acid close to the melting point, it is difficult to accurately determine $\Delta_{fus}H$ of betulinic acid. Thus, $\Delta_{fus}H$ was estimated by using the method[16]

$$\Delta_{fus}H = T_m \times (50 - R\ln\sigma + R\ln(2.85^{\tau})) \tag{10}$$

where R is the universal gas constant, σ is the rotational symmetry of the molecule, and τ is the number of effective torsional angle which is calculated for any compound by using a following equation:

$$\tau = \mathrm{SP3} + 0.5\mathrm{SP2} + 0.5\mathrm{RING} - 1 \tag{11}$$

where SP3 is the number of sp^3 chain atoms, SP2 is the number of sp^2 chain atoms, and RING is the number of fused-ring systems (these do not include end group). For betulinic acid, σ is 1 and τ is 2.5 according the literature. So the enthalpy of fusion of betulinic acid was estimated to be 42.23 kJ·mol^{-1}.

Solubility and Crystals of Betulinic Acid. As can be seen from Tables 2 and 3 and Figure 2 and 3, the results correlated by empirical and UNIQUAC equations are satisfactory. However, the results of the empirical model were better than the UNIQUAC model. This means that the temperature may play a more important role on interaction parameter in eq 9. Those interaction parameters listed in Tables 3 could be used to predict the solubilities of betulinic acid at different temperatures.

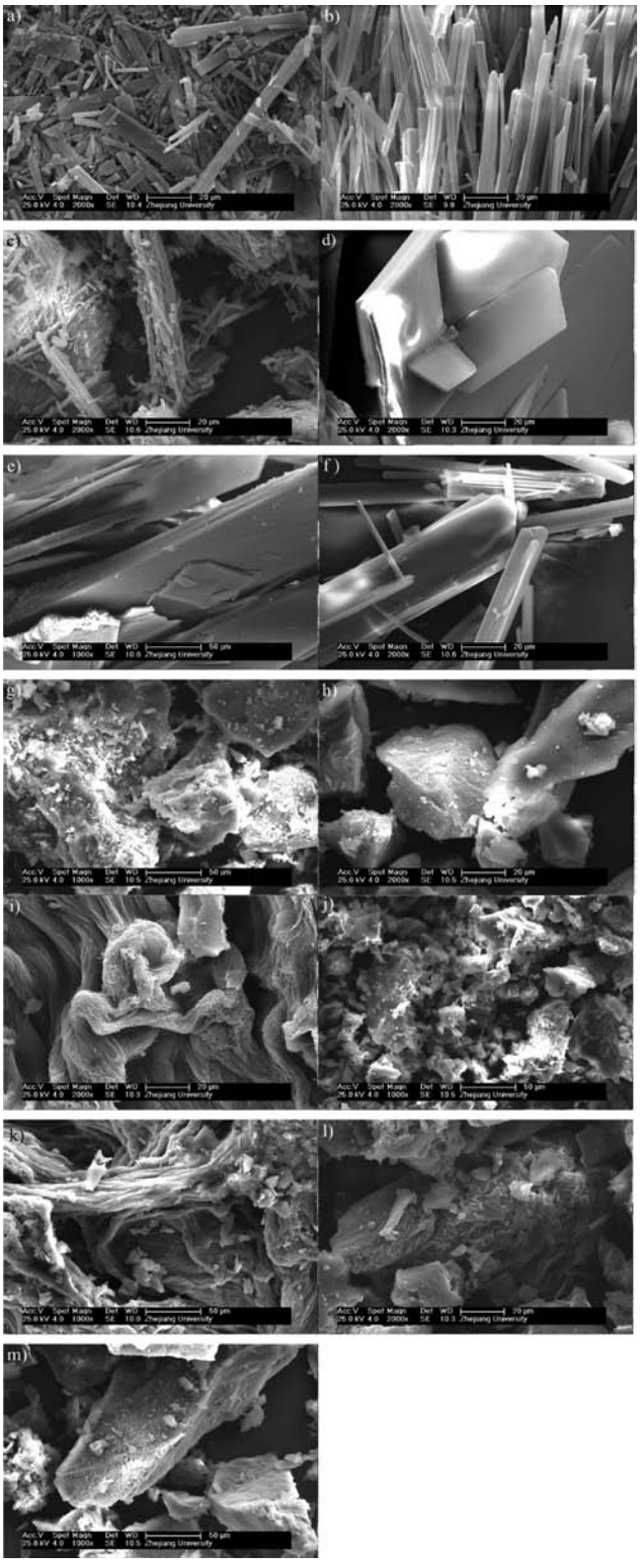

Figure 4. SEM images of the BEA particles crystallized in different solvents: (a) methanol, (b) ethanol, (c) 1-propanol, (d) 2-propanol, (e) 1-butanol, (f) 2-butanol, (g) 1-pantanol, (h) 1-hexanol, (i) ethyl formate, (j) ethyl acetate, (k) butyl acetate, (l) acetone, and (m) THF.

It is apparent from Figure 4 that crystal habit of betulinic acid changes in morphology at different solvents. Betulinic acid crystals obtained in most of alcohols show regular shape (rod-like in methanol, ethanol, 1-propanol, and 2-butanol and plate-like in 2-propanol and 1-butanol). However, the crystals obtained in 1-pantanol, 1-hexanol, esters, acetone, and THF show a glassy appearance.

CONCLUSIONS

The solubilities of betulinic acid in 13 organic solvents were measured and correlated by empirical equation and UNIQUAC equation. As illustrated from Figures 2 and 3 and Table 1, the solubility of betulinic acid in pure organic solvents increases with increasing temperature; the solubility of betulinic acid in the studied alcohols increases with the increase of molecular weight of alcohol. Compared with low solubilities in esters and acetone, THF is an excellent solvent for betulinic acid. The nature of solvents and stronger solvating interactions could have a large influence on this phenomenon, by which is a complicated matter that we should study furthermore.

Overall observation of morphology of the betulinic acid in different solvents indicates that crystals in alcohol have better appearances with regular shape and clean surfaces when compared with the crystal formed in esters, acetone, and THF. According to the literature, dissolution is heavily conditioned by the amount of exposed crystal surface; different morphology is likely to affect solubility.[17] However, it is difficult to draw a clear conclusion between solubilities and morphology in current experiment.

Theoretical yields of recrystallization for betulinic acid in different solvents have been calculated and listed in Table S1 in the Supporting Information. In brief, these experimental solubilities and correlation equations in this work can be used as necessary data and a model in the production and purification process of betulinic acid in industry.

ASSOCIATED CONTENT

Supporting Information. Theoretical yield of recrystallization for betulinic acid in different solvents (Table S1) and DSC curve of betulinic acid (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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