Solubility of Tobramycin in Binary Water−Ethanol Solvent

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The solubility of tobramycin in binary ethanol + water solvent mixtures has been measured using the static analytical method over an experimental temperature range of (288.2 to 328.2) K. The experimental solubilities were correlated with the combined nearly ideal binary solvent (CNIBS)/Redlich−Kister equation which provided an accurate mathematical representation of the experimental data.

Introduction

Tobramycin (4-*O*-(3-amino-3-deoxy-a-D-glucopyranosyl)-2-deoxy-6-*O*-(2,6-diamino-2,3,6-trideoxy-a-D-ribohexopyranosyl)-L-streptamine; Figure 1), produced by the bacterium *Streptomyces tenebrarius*, is a broad-spectrum aminoglycosidic antibiotic.[1] It is used almost exclusively in the treatment of Gram-negative bacterial infections.[2] The usage of the free base form of tobramycin as well as its sulfate salt is constantly increasing in the pharmaceutical market, and the medical benefits and its antibacterial mechanisms have been broadly discussed in the literature.[2,3] Crystallization plays an important role in the process of manufacturing high-quality tobramycin product, and the solubility of tobramycin in solvents is crucial to optimize the crystallization process.[4] However, no reports concerning the solubility of tobramycin in ethanol−water binary solvent have been previously published. In this work, solubility data of tobramycin in water−ethanol mixtures were experimentally determined using an analytical method.

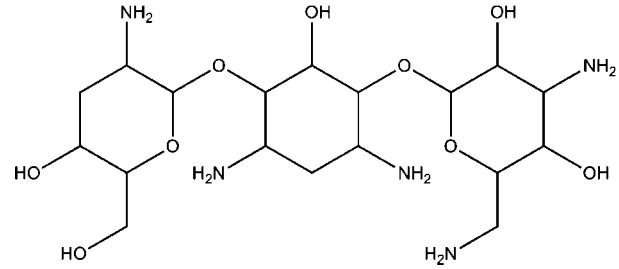

Figure 1. Chemical structure of tobramycin.

Experimental Section

Chemicals. All chemicals are of analytical grade unless specifically stated. A solid sample of tobramycin with a minimum mass fraction of 97.5 % was crystallized in the laboratory from an aqueous solution which was supplied by the Chongqing Daxin Pharmaceutical Co., Ltd. (Chongqing, China). The solid state of tobramycin sample was determined by XRD and TG analysis, depicted as Figure 2 and Figure 3. The XRD pattern of tobramycin sample includes some characteristic peaks, which proves the crystal property of the tobramycin sample. At the same time, the result of thermal gravity/thermal dilatometric analysis shows that the crystal water content of the starting material was 4.3 %, and the theoretical crystal water content of monohydrate was 3.7 %. It can be concluded that the tobramycin sample was the monohydrate, and the deviation may be due to the volatilization of inclusion water in the tobramycin sample. The tobramycin sample was dried to constant weight in vacuum at 65 °C and then stored in a desiccator. Methanol (HPLC-grade), ethanol, and trifluoroacetic acid were from the Tianjin Kewei Chemical Company (Tianjin, China). Deionized water was used throughout all experiments.

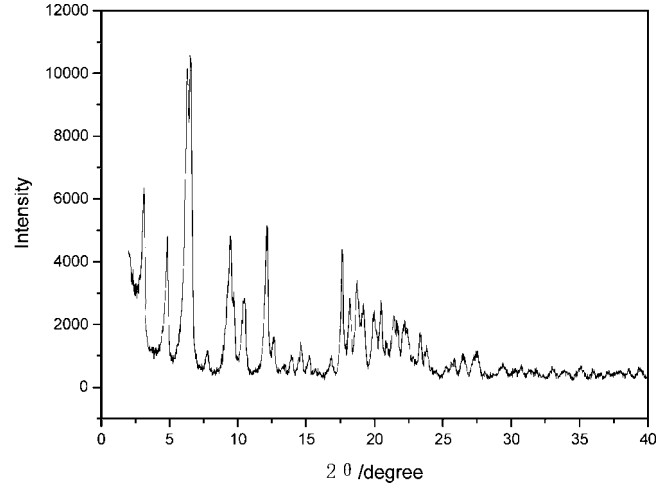

Figure 2. XRD pattern of the tobramycin sample.

Apparatus and Procedure. The experiments were carried out in a jacketed glass vessel with a working volume of 50 mL. The solution was stirred by an electric magnetic stirrer, and a microthermometer was inserted into the solvent to determine the temperature of the system. The jacketed glass vessel was kept at the desired temperature by circulating water from a constant-temperature bath (type 501A, China). The temperature of the circulated water was controlled within ± 0.2 K of the desired temperature. The concentration measurements were carried out on an HPLC (Agilent Technologies 1200, USA) with an SB C18 reverse-phase column (4.6 mm × 150 mm, 5 μm) and a refractive index detector (LabAlliance RI2001, USA). The mass of the samples and solvents was determined by an electronic analytical balance (Metler Toledo AL204, Switzerland) with a precision of 0.0001 g. Some of the solubility experiments were conducted two or three times to check the repeatability in this work, and the repeatability evaluated by mean relative deviation was less than 3 %.

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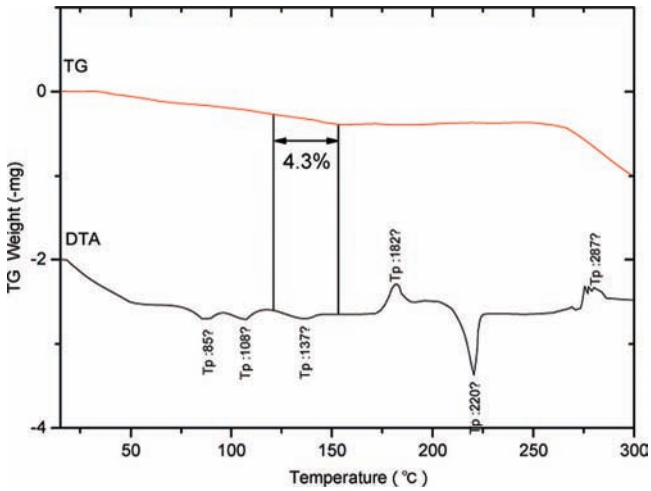

Figure 3. TG/DTA pattern of the tobramycin sample.

Table 1. Experimental Solubilities (x_A) and Calculated Solubilities (x_A^{calcd}) of Tobramycin in Binary Ethanol + Water Solvent Mixtures from T = (288.2 to 328.2) K

x_C^0	$10^3 x_A$	$10^3 x_A^{calcd}$	x_C^0	$10^3 x_A$	$10^3 x_A^{calcd}$
	T = 288.2 K			T = 296.2 K	
0.1601	0.09109	0.13953	0.1601	0.12000	0.18391
0.3202	0.69955	0.67692	0.3202	0.78346	0.70913
0.4800	2.85495	2.85902	0.4800	2.87880	2.90678
0.6399	8.54077	8.54095	0.6399	9.39686	9.39041
0.7998	18.75936	18.76192	0.7998	20.96041	20.95693
	T = 304.2 K			T = 312.2 K	
0.1601	0.15187	0.26709	0.1601	0.17240	0.23437
0.3202	1.08096	0.97007	0.3202	1.23287	1.19021
0.4800	3.40757	3.44845	0.4800	5.02377	5.03699
0.6399	11.68394	11.67608	0.6399	15.40804	15.40492
0.7998	24.08791	24.07943	0.7998	29.79806	29.79565
	T = 320.2 K			T = 328.2 K	
0.1601	0.20430	0.24722	0.1601	0.2431	0.16934
0.3202	1.60227	1.57887	0.3202	2.01789	2.03933
0.4800	6.58380	6.59107	0.4800	9.062777	9.05642
0.6399	17.90186	17.89967	0.6399	21.88315	21.88445
0.7998	34.67695	34.67438	0.7998	40.81464	40.81287

Solubility Measurements. The solubility was measured by the static analytical method. For each experiment, an excess amount of tobramycin was added into the solvent. Then the equilibrium bottle was heated to a constant temperature. A 5 mL syringe was used to withdraw about 2 mL of suspension each time, and the solution was filtered through a syringe membrane filter with a pore size of 0.45 μm. The filtered liquid was then diluted with deionized water for HPLC analysis using 100 mL volumetric flasks. The concentration of the liquid phase was measured at a 1 h interval until equilibrium was attained.

The concentration of tobramycin in the solution was determined by RP-HPLC with an RI detector. The external standard method was used in the analysis. More than 20 μL of tobramycin dilution was injected into the HPLC for quantity analysis. The mobile phase was a methanol−water mixture (1:9, v/v), containing 5.4 mL·L^{-1} of trifluoroacetic acid. The HPLC conditions were set as follows: column temperature, 303.2 K; flow rate of the mobile phase, 0.8 mL·min^{-1}; and the retention time of tobramycin was about 6 min. The calibration curve for the estimation of tobramycin was prepared by using the standard solutions in the concentration range of (0.2 to 2) mg·mL^{-1}. The uncertainty in the measurement of the concentration of tobramycin was less than 2 %.

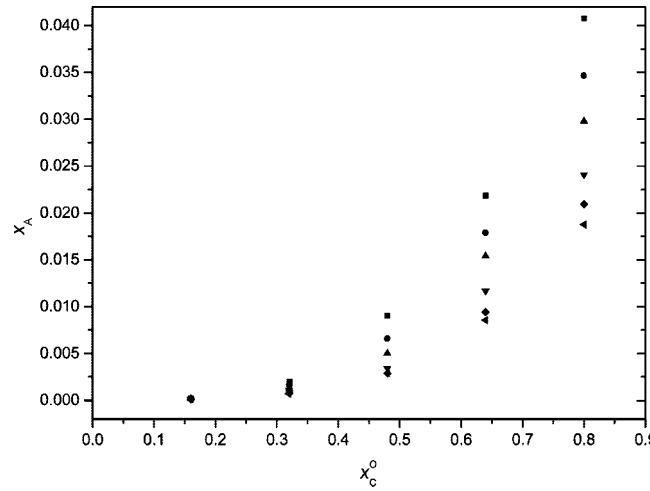

Figure 4. Solubilities of tobramycin (A) in binary ethanol (B) + water (C) solvent mixtures: ■, T = 328.2 K; ●, T = 320.2 K; ▲, T = 312.2 K; ▼, T = 304.2 K; ♦, T = 296.2 K; solid triangle pointing left, T = 288.2 K.

Table 2. Curve Fitting Parameters of Tobramycin in Binary Ethanol + Water in the Temperature Range from T = (288.2 to 328.2) K

T/K	B_0	B_1	B_2	B_3	B_4	10^3rmsd
288.2	−10.12825	5.42312	20.01016	−34.30923	16.0506	0.02401
296.2	−9.41054	2.10362	22.94792	−29.93383	10.99218	0.04571
304.2	−9.9233	13.30936	−22.89371	41.09093	−26.45741	0.07400
312.2	−10.0026	9.96619	3.05222	−7.45457	0.9276	0.03421
320.2	−10.47116	14.10571	−2.04472	−10.82627	6.53572	0.02215
328.2	−12.58705	29.71906	−36.33817	19.00062	−2.09539	0.03448

Results and Discussion

The solubility data for tobramycin (A) in ethanol (B) + water (C) mixtures at different temperatures are listed in Table 1and visually shown in Figure 4. The mole fraction solubility is defined as follows

$$x_A = \frac{m_A/M_A}{m_A/M_A + m_B/M_B + m_C/M_C} \quad (1)$$

in which x_A refers to the experimental solubility of the solute and m_A, m_B, and m_C represents the mass of solute, ethanol, and water, respectively. M_A, M_B, and M_C are molecular weights of solute, ethanol, and water, respectively.

The solubility data in binary ethanol + water solvent mixtures are described by the combined nearly ideal binary solvent (CNIBS)/Redlich−Kister model suggested by Acree and co-workers[5−7] as a possible mathematical representation for describing how the experimental isothermal solubility of a crystalline solute dissolved in a binary solvent mixture varies with binary solvent composition.

$$\ln x_A = x_B^0 \ln(x_A)_B + x_C^0 \ln(x_A)_C + x_B^0 x_C^0 \sum_{i=0}^{N} S_i (x_B^0 - x_C^0)^i \quad (2)$$

In eq 2, S_i is the model constant. N can be equal to 0, 1, 2, and 3, respectively. Depending on the values of N, four equations can be obtained from eq 2. x_B^0 and x_C^0 refer to the initial mole fraction composition of the binary solvent calculated as if solute A was not present. $(x_A)_i$ is the saturated mole fraction solubility of the solute in pure solvent i.

Substitution of $(1 - x_C^0)$ for x_B^0 in eq 2 with N = 2 and subsequent rearrangements result in eq 3

$$\ln x_A = \ln(x_A)_B + [\ln(x_A)_C - \ln(x_A)_B + S_0 + S_1 + S_2]x_C^0 + [-S_0 + 3S_1 + 5S_2]x_C^{02} + [-2S_1 - 8S_2]x_C^{03} + [-4S_2]x_C^{04} \quad (3)$$

which can be written as eq 4

$$\ln x_A = B_0 + B_1 x_C^0 + B_2 x_C^{0\,2} + B_3 x_C^{0\,3} + B_4 x_C^{04} \quad (4)$$

The experimental solubility data x_A directly correlated with eq 4 and the calculated solubilities x_A^{calcd} are listed in Table 1. The values of the five dimensionless parameters B_0, B_1, B_2, B_3, and B_4 together with rmsd are listed in Table 2.

The rmsd is defined as

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

where x_i^{calcd} is the solubility calculated from eq 4 and x_i is the experimental value of solubility. From Table 1, the following conclusions can be drawn: (1) For all selected mixed water−ethanol solvent systems, solubility is a function of temperature and solvent composition, and solubility increases with an increase in temperature and water content of the original mixed solvent. (2) The calculated solubility of eq 4 shows good agreement with the experimental values. (3) Tobramycin monohydrate can crystallize from aqueous solution by adding ethanol as antisolvent.

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