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Isoelectrical Points and Solubility of 6-Aminopenicillanic Acid in Water + 1-Butanol + Butyl Acetate[†]

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The dissociation constants of 6-aminopenicillanic acid (6-APA) in water + 1-butanol + butyl acetate at (278.15 to 293.15) K were measured by potentiometric titration, and the isoelectric point (pI) was determined accordingly. Solubilities at different pH values in the above system were also determined by the synthetic method. Results showed a remarkable decrease in dissociation constant, isoelectrical points, and solubility in water + 1-butanol + butyl acetate than in pure water. Solubility models based on the dissociation constant have a good correlation with the experimental data.

Introduction

6-Aminopenicillanic acid (CAS NO. 551-16-6), 6-amino-3,3-dimethyl-7-oxo-4-thio-1-azabicyclo[3,2,0]heptane-2-carboxylic acid, is the precursor of all semisynthetic penicillins and cephalosporin β-lactam antibiotics. As can be seen from Figure 1, 6-aminopenicillanic acid is an ampholyte with an amino group and a carboxyl group in its structure, ionizing by two steps in solution

$$AA^{+} \overset{K_1}{\rightleftharpoons} AA^{\pm} \overset{K_2}{\rightleftharpoons} AA^{-} \qquad (1)$$

Its existing form and solubility have considerable relation with solution pH.

As referred to before,[1] the industrial production of 6-aminopenicillanic acid includes enzymatic hydrolysis of penicillin G, extraction, and crystallization. Some solvents will be interlaced into the mother liquid of crystallization which comes from the extraction step.

The isoelectric point (pI) and solubility of an ampholyte determines its operational end point and yield and plays a critical importance in crystallization. However, the pI is greatly influenced by the solvents in the crystallization system.[2] To design an optimized crystallization process pertinent in industrial production, it is necessary to determine the pI and solubility values in various solvent systems.[3]

In this work, the dissociation constant and solubility in water + 1-butanol + butyl acetate at (278.15 to 293.15) K was determined. A solubility model based on the dissociation constant achieved a good correlation with the experimental data.

Experimental Section

Materials. 6-Aminopenicillanic acid was prepared by recrystallizing the white crystalline powder obtained from the North China Pharmaceutical Co. Ltd. from pure water. Its mass fraction purity, determined by HPLC according to the literature,[4] is higher than 99.5 %. Distilled deionized water of HPLC grade, 1-butanol, butyl acetate, and ammonia (25 %) of analytical research grade reagent obtained from the Tianjin University KeWei Company were used as received. Dilute sulfuric acid (40 %) was prepared by diluting concentrated sulfuric acid with distilled water. Analytical sodium hydroxide was used to prepare the sodium hydroxide solution of 5 mol·L^{-1}.

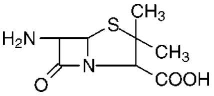

Figure 1. Chemical structure of 6-aminopenicillanic acid.

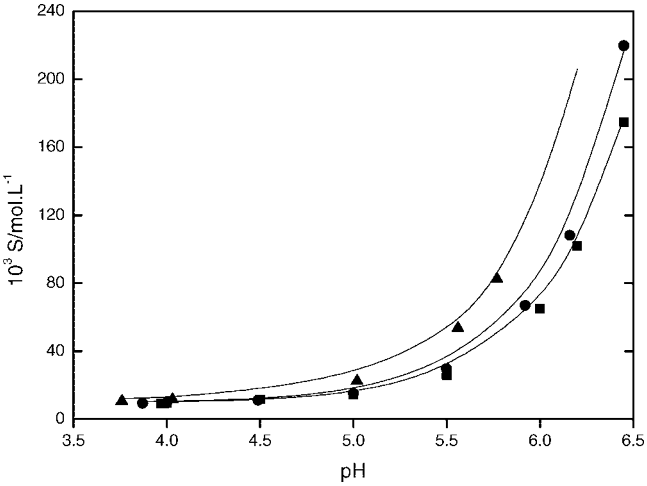

Figure 2. Experimental and calculated solubility S of 6-aminopenicillanic acid from T = (278.15 to 293.15) K in water + 1-butanol + butyl acetate as a function of pH: ■, 278.15 K; ●, 283.15 K; ▲, 293.15 K; points, experimental data; line, calculated values by eq 4.

Apparatus and Procedures. a. Dissociation Constant. The dissociation constant was measured by the potentiometric titration method.[2] A cylindrical-jacketed glass vessel (100 mL) maintained at a desired temperature by water circulated from a water bath with a thermoelectric controller (type 501, China) was placed on a magnetic stirrer. The jacket temperature could be maintained within ± 0.05 K of the required temperature. The cell has a perforated rubber cover plate to prevent the solvent from evaporating, through which a mercury-in-glass thermometer with an uncertainty of ± 0.05 K and a pH meter (Mettler Toledo SevenEasy S20) with an uncertainty of ± 0.01 pH were inserted. The pH meter was calibrated before use. A

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microsyringe was prepared to introduce sodium hydroxide solution into the system. During the whole process, continuous stirring was performed with a magnetic stir bar. The masses of the samples were determined using an analytical balance (Mettler Toledo AB204-N, Switzerland) with an uncertainty of ± 0.0001 g.

A predetermined volume of solvent was added to the inner chamber and then stirred continuously at the required temperature for 1 h to ensure that the temperature was constant. A known amount of 6-APA crystals was added into the inner chamber of the vessel, and a clear 6-APA solution was prepared by adding dilute sulfuric acid until pH = 1.2. Then, a known amount of sodium hydroxide solution with a microsyringe was added, and the cumulant mass consumed and the stable pH were recorded. This process was repeated until pH = 8. At the half-equivalence point, the apparent p*K* values were recorded along with the mass of titrant, and the isoelectric point was determined by the following eq 2

$$\mathrm{pI} = \frac{\mathrm{p}K_1 + \mathrm{p}K_2}{2} \tag{2}$$

The uncertainty of the experimental dissociation constant and corresponding pI values is about 2 %.

b. Solubility. Solubilities were measured by a synthetic method.[5] The apparatus for determining solubility was the same as that used for determining dissociation constant.

After the solvent reaching the stable required temperature, the pH value was adjusted to the required data, and a known amount of solute below its solubility was placed in the inner chamber of the vessel. When the solute dissolved completely, additional solid of known mass (about 1 mg to 5 mg) was introduced into the vessel. This procedure was repeated until the solute could not dissolve after equilibrium for 1 h. Samples were taken of the supernatant liquid phase using a plastic syringe and filtered through a 0.45 μm HPLC disposable filter. The composition measurements were made by HPLC (Agilent Technologies 1200, USA) with an SB C18 reverse phase column, and the method was given in ref 4. The uncertainty of the experimental solubility values is about 2 %.

Results and Discussion

The mass of titrant and pH in dissociation constant measurements at different temperatures were listed in Table 1. The relation of titrant mass and pH at each temperature was simulated with eq 3, and its parameters were listed in Table 2.

$$y = A_0 + A_1x + A_2x^2 + A_3x^3 + A_4x^4 \tag{3}$$

where *y* is the accumulated mass of titrant added; *x* is the pH; and A_0, A_1, A_2, A_3, and A_4 were the parameters of eq 3.

Table 1. Mass of Sodium Hydroxide Solution Added *m* and the Corresponding Solution pH in Dissociation Constant Measurements

m/g	pH	*m*/g	pH	*m*/g	pH
T/K = 278.15		*T*/K = 283.15		*T*/K = 293.15	
0.33690	1.514	2.3677	1.893	1.5773	2.078
1.0642	1.663	3.1949	2.050	2.0955	2.320
1.6583	1.810	3.6735	2.370	2.6900	2.456
2.0958	1.998	4.0247	2.942	3.2721	2.875
2.3328	2.310	4.2614	3.351	3.5092	3.707
3.1546	2.616	4.5648	4.174	4.2179	4.853
3.4118	2.666	5.0912	4.734	4.4478	5.065
3.5018	2.800	5.3306	5.245	4.7057	5.245
3.6488	2.939	5.5403	5.506	5.1871	5.431
3.8852	3.695	6.4352	5.873	5.6473	5.620
4.2991	4.577	6.7039	6.914	6.1206	8.901
4.5382	5.130	6.8152	10.11		
4.7150	5.423				
4.9472	5.540				
5.2000	5.708				
5.6920	5.968				
6.0504	7.332				

The secondary derivatives method[6] was applied to obtain the inflection points of sodium hydroxide solution mass versus solution pH curves, and the pH of the inflection points was the negative logarithm of the dissociation constant. The pI value of each system at a temperature was the arithmetic mean of the two dissociation constants (eq 2). The values of dissociation constants and isoelectric points were listed in Table 3.

The solubilities of 6-APA in the water + 1-butanol + butyl acetate system at different pH and temperatures were listed in Table 4 and shown in Figure 2. They were simulated with the following eq 4

$$S = S_0\left(\frac{\alpha_{\mathrm{H}^+}}{k_1} + 1 + \frac{k_2}{\alpha_{\mathrm{H}^+}}\right) \tag{4}$$

where *S* is the total solubility which is a function of the solution pH; S_0 is the solubility of the electrical zwitterions at the isoelectric point (pI) which was estimated by eq 2; α_{H^+} is the

Table 2. Parameters of Equation 3 for Correlations of Mass of Sodium Hydroxide Solution Added and the Corresponding Solution pH from *T* = (278.15 to 293.15) K

T/K		A_0	A_1	A_2	A_3	A_4	R^2
278.15	pK_1	120.25	−164.13	84.763	−19.275	1.6328	0.9975
278.15	pK_2	266.1	−246.88	84.568	−12.554	0.6863	0.9975
283.15	pK_1	−11.059	15.686	−6.8385	1.2389	−0.0731	0.9991
283.15	pK_2	1427.5	−1049.3	287.77	−34.808	1.5684	0.9998
293.15	pK_1	23.534	−40.718	28.054	−8.3087	0.9010	1.0000
293.15	pK_2	152.38	−155.21	58.334	−9.4135	0.5555	0.9918

Table 3. Dissociation Constants *p*K$_1$ and *p*K$_2$ and Isoelectric Point (pI) Values from *T* = (278.15 to 293.15) K

T/K	pK_1	pK_2	pI
278.15	2.72 ± 0.03	5.19 ± 0.05	3.96 ± 0.04
283.15	2.70 ± 0.03	5.10 ± 0.05	3.90 ± 0.04
293.15	2.66 ± 0.03	4.94 ± 0.05	3.80 ± 0.04

Table 4. Experimental and Calculated Solubility *S* of 6-Aminopenicillanic Acid from *T* = (278.15 to 293.15) K at pH = (3.76 to 6.45) in Water + 1-Butanol + Butyl Acetate

pH	$10^3S_i^{\mathrm{exptl}}$ (mol·L^{-1})	$10^3S_i^{\mathrm{calcd}}$ (mol·L^{-1})	10^2 rmsd
	T/K = 278.15		0.2028
3.97	9.15	10.18	
4.00	9.29	10.22	
4.50	11.37	11.18	
5.00	14.23	15.16	
5.50	25.65	28.01	
6.00	64.93	68.76	
6.20	101.66	103.63	
6.45	174.72	177.15	
	T/K = 283.15		0.3135
3.87	9.18	10.36	
4.00	9.52	10.39	
4.49	10.95	11.62	
5.00	15.02	16.60	
5.50	29.57	32.49	
5.92	66.87	70.56	
6.16	108.04	114.61	
6.45	219.50	216.64	
	T/K = 293.15		0.1274
3.76	10.63	12.18	
4.03	11.51	12.40	
5.02	22.37	23.56	
5.56	53.60	55.32	
5.77	82.35	83.09	

activity coefficient of H^+; $k_1$is the first dissociation constant of 6-APA; and k_2 is the second dissociation constant. The calculated solubility values of 6-aminopenicillanic acid in water + 1-butanol + butyl acetate were also given in Table 4. The root-mean-square deviation (rmsd) is defined as follows

$$\mathrm{RMSD} = \left\{ \frac{\sum_{i=1}^{N} (S_i^{\mathrm{calcd}} - S_i^{\mathrm{exptl}})^2}{N} \right\}^{1/2} \tag{5}$$

where N is the number of experimental points and S_i^{calcd} and S_i^{exptl} represent the solubilities calculated from eq 4 and the experimental values, respectively.

From the dissociation constants and solubilities in water + 1-butanol + butyl acetate obtained above, we can draw the following conclusions: (1) The negative logarithm of dissociation constant pK decreases linearly with temperature in the range (278.15 to 293.15) K. The dissociation is an endothermic process for most substances,[7] and higher temperature is beneficial to the dissociation process. As a result, the dissociation constant increases, and its negative logarithm decreases with the increase in dissociation constant. (2) The solvents 1-butanol and butyl acetate increased the pK value of 6-APA compared with pure water.[6] These solvents decrease the dielectric constant of the system and increase the association effect between ions as well, so the dissociation constants of 6-APA in this system were decreased accordingly. (3) The solubility versus solution pH graph in water + 1-butanol + butyl acetate still presents a "U" curve; however, the absolute values were lower than that in the pure water system. (4) The calculated solubilities of 6-APA with eq 4 showed good agreement with the experimental values. (5) The experimental dissociation constants, isoelectrical point, solubility, and the correlation equation applied to water + 1-butanol + butyl acetate in this work can be used as essential data and models in the practical purification process of 6-aminopenicillanic acid.

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Literature Cited

(1) Su, M.; Wang, J. K.; Hou, Y. Solubility of 6-Aminopenicillanic Acid in Aqueous Salt Solutions from 273.15 to 303.15 K. *J. Chem. Eng. Data* **2007**, *52*, 2266–2268.

(2) Ibrahim, S. A.; Rageh, N. M.; Mohamad, A. A.; Ebead, Y. H. Medium Effect on the Acid Dissociation Constants of Some Heterocyclic Azo Compounds. *J. Chem. Eng. Data* **1999**, *44*, 451–455.

(3) Butler, J. N. *Ion Balance and data processing*; Nankai University Press: Tian Jin, China, 1989.

(4) Hu, C. Q. *The Application of HPLC Method in Quality-control-analyzing of β-Lactam Antibiotics*; China Meteorological Press: Beijing, China, 2001.

(5) Hefter, G. T; Tomkins, R. P. T. *The Experimental Determination of Solubilities*; John Wiley: Chichester, 2003.

(6) Han, J. B.; Wang, J. K. The Measurement of Solubility and Isoelectric Point of 6-APA. *HeBei Chem. Eng.* **2003**, *4*, 18–21.

(7) King, E. J. *Qualitaive Analysis and Electrolytic Solutions*; Harcourt Brace: New York, 1959.

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