Solubility of Folic Acid in Water at pH Values between 0 and 7 at Temperatures (298.15, 303.15, and 313.15) K

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The solubility of folic acid in water was measured at pH values between 0 and 7 at the temperatures (298.15, 303.15, and 313.15) K using a standard shake and settle method with analysis by high-performance liquid chromatography (HPLC). A temperature and dissociation constant related solubility model was built to describe the folic acid solubility performance, and the experimental data matched the model well with a square of the multiple correlation coefficient (R^2) of 0.9900.

Introduction

Folic acid (CAS registry no. 59-30-3) is the popular name of (2*S*)-2-[(4-{[(2-amino-4-hydroxypteridin-6-yl)methyl]amino}-phenyl)formamido]pentanedioic acid, which is a member of the Vitamin B family and necessary for the healthy function of a variety of bodily processes. The chemistry structure with numbered atoms of folic acid is shown in Figure 1. Folic acid participates in one-carbon metabolism and such important cellular pathways like purine, thymidylate, and methionine biosynthesis and also acts as part of the coenzyme system for DNA, amino acid, and nucleoprotein synthesis.[1−5] Folic acid deficiency can induce symptoms like megaloblastic anemia, neural tube defects, and cancer.[6] As a result, the U.S. Department of Health and Human Services announced a rule that folic acid should be added to grain products as a nutrition fortifier to ensure 400 μg intake for an adult and an additional 200 μg for pregnant women.[7,8]

The solubility of folic acid is crucial for drug delivery, absorption, transferring in the human body, and crystallization in the manufacture process. A study indicated that folic acid absorption occurred mainly in the jejunum via a saturable carrier-mediated process, although at high concentrations a diffusion-like process occurred, and the transport was maximal at pH 5 to 6 with a rather sharp pH optimum.[6] Islam et al. reported that some commercial folic acid supplements failed in the disintegration and dissolution performance on USP standards, and the pH dependency of solubility of folic acid was the ultimate reason.[3] Crystallization was a key process for the purification of folic acid in the manufacturing process, in which folic acid was crystallized and recrystallized from the aqueous solution.[9] The solubility of folic acid worked as a fundamental role in the thermodynamics and dynamics performances in the crystallization process. Although it is well-known that the solubility of folic acid is poor at neutral conditions and depends on pH in water, the solubility data are insufficient. A recordation was reported as 0.0016 mg·g^{-1} in water at 298 K.[10] A simple solubility−pH profile of folic acid at 310 K was given;[3] however, the data were deficient and deviated far from the data in the Merck Index. In this study, the experimental solubility data of folic acid are measured using a standard shake and settle with HPLC method, and a pH-dependent solubility profile at temperatures (298.15, 303.15, and 313.15) K under atmospheric pressure is determined, respectively. A dissociation constant and thermodynamic based model is built, and it matches the data well.

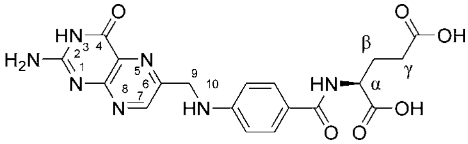

Figure 1. Structure of folic acid and the number of the atoms.

Experimental Section

Materials. The raw folic acid was purchased from Hebei Jiheng (Group) Pharmacy Co., Ltd., China, according with the BP2000 standard of stated hydrous mass fraction purity of > 0.97. This raw material was recrystallized four times from aqueous solution and dried at 318 K under vacuum for 4 h to ensure a quality of mass fraction purify > 0.995. Sodium hydroxide, hydrochloric acid, potassium hydroxide, potassium dihydrogen phosphate, dimethyl sulfoxide, ammonia, and perchloric acid used in the experiment were of analytical reagent grade purchased from Guangzhou Jingke Chemical Agent and Apparatus Co., Ltd. Potassium hydrogen phthalate was datum chemical reagent grade purchased from Xilong Chemical factory of Shantou. Chromatographic pure methanol was purchased from Honeywell Burdick & Jackon. All water used in the experiment was high purity water with a resistivity > 18.25 MΩ·cm^{-1}.

Apparatus and Procedures. Superfluous folic acid was put in a series of 50 cm^3 glass cells with different pH value adjusted by sodium hydroxide or hydrochloric acid in a shaking table (SHA-BA, Fuhua Apparatus Co., Ltd., China). The temperature of the vessel was controlled at the desired value by a thermostat within ± 0.2 K. The dissolving process would be continuous for 12 h, and the pH value of this suspension liquid was measured using a pH meter with composite Ag/AgCl-glass

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Table 1. Solubility x of Folic Acid in Water

$T = 298.15$ K		$T = 303.15$ K		$T = 313.15$ K	
	$10^6 \cdot x$		$10^6 \cdot x$		$10^6 \cdot x$
pH	$\mathrm{mol \cdot dm^{-3}}$	pH	$\mathrm{mol \cdot dm^{-3}}$	pH	$\mathrm{mol \cdot dm^{-3}}$
0.39	189.7 ± 4.1	0.39	208.2 ± 0.5	0.43	386.1 ± 2.4
0.82	34.3 ± 0.0	0.82	44.1 ± 0.7	0.85	91.1 ± 3.3
1.30	9.7 ± 0.2	1.26	12.9 ± 0.2	1.27	26.7 ± 0.3
1.78	3.2 ± 0.0	1.76	4.8 ± 0.1	1.77	11.2 ± 0.4
2.19	1.8 ± 0.0	2.20	2.5 ± 0.0	2.19	5.6 ± 0.0
2.64	1.1 ± 0.0	2.69	1.5 ± 0.0	3.04	4.0 ± 0.1
4.03	2.3 ± 0.2	4.03	3.5 ± 0.0	3.99	7.7 ± 0.1
4.51	6.3 ± 0.0	4.50	8.4 ± 0.2	4.38	16.7 ± 0.1
4.70	10.5 ± 0.1	4.67	10.9 ± 0.2	4.51	23.0 ± 0.3
5.09	30.1 ± 0.4	5.03	30.8 ± 0.8	4.76	47.5 ± 0.3
5.59	213.0 ± 1.9	5.51	221.3 ± 3.8	5.25	252.6 ± 12.0
6.03	1271.2 ± 1.7	5.87	1221.5 ± 1.4	5.68	1324.7 ± 1.0

electrode (PHS-3E and E-201-C, Shanghai Precision and Scientific Instrument Co., Ltd., China). Then, the upper portion was transferred, filtered with a 0.22 μm organic membrane filter, and diluted appropriately for HPLC (Agilent 1200 series, Agilent Technologies) subsequently. All of the measurements were repeated three times, and the average of the three measurements was considered to be the solubility.

Sample Analysis. An HPLC method reported in Chinese Pharmacopoeia was used to determine the concentration of the folic in solution.[11] The saturated folic acid solution was dissolved in superfluous ammonia and injected in the HPLC with C18 column (XDB-C18, 5 μm, 4.6 × 250 mm) at the temperature 313.15 K. The mobile phase was a methanol (80 $\mathrm{cm^3 \cdot dm^{-3}}$), potassium hydroxide (7.0 $\mathrm{mmol \cdot dm^{-3}}$), potassium dihydrogen phosphate (6.8 $\mathrm{g \cdot dm^{-3}}$) buffer solution with pH of 6.30 at a speed of 1.0 $\mathrm{cm^3 \cdot min^{-1}}$. The absorbance of standard solution and the sample was measured at 254 nm. The standard curve was obtained by using standard solution, and the calibration equation was set as $y = 1.2962x$ with $R^2 = 1.0000$ and RMSE = 5.128 (Root Mean Squared Error) in the appropriate concentration from (0 to 5.68) $\mathrm{mg \cdot cm^{-3}}$ at the same condition of the folic solubility measurement, where y was UV absorbance and x was concentration of folic acid. The dilute ratio of the folic acid solution is controlled in the range of 1.6 to 25 to make sure the concentration of the folic acid falls into the bound of the standard curve.

Results and Discussion

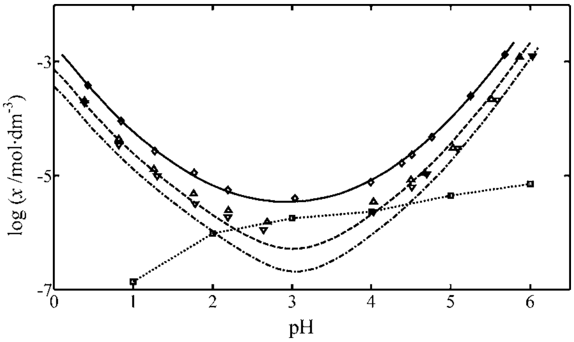

Figure 2. Solubility x of folic acid in water at different pH: ▽, average experiment data at 298.15 K; Δ, average experiment data at 303.15 K; ◊, average experiment data at 313.15 K; □ and dotted line, data at 310 K acquired from ref 3; dash-dotted line, model predicted data at 298.15 K; dashed line, model predicted data at 303.15 K; real line, model predicted data at 313.15 K.

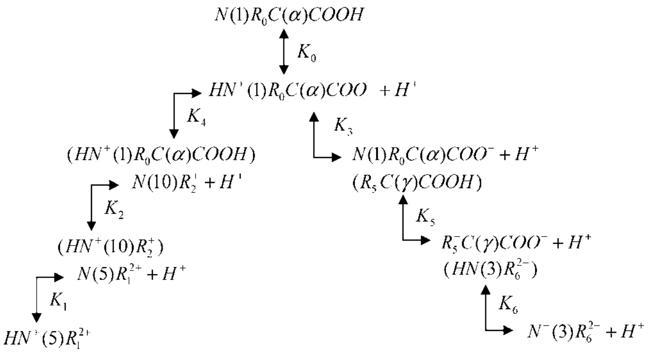

Figure 3. Solution equilibrium and dissociation reactions of folic acid in water.

Experimental Data. The experimental solubility data of folic acid at pH values between 0 and 7 at the temperatures (298.15, 303.15, and 313.15) K are listed in Table 1 and visually shown in Figure 2. The uncertainty of the results listed in Table 1 is the standard deviation from the HPLC measurement. The data display that the solubility of folic acid increases with rising temperature, and a rapid solubility higher than the solubility at weak acidic conditions appears at alkaline and strong acidic surroundings.

Solubility Model. Modeling of the experimental solubility data is beneficial to represent mathematical aspects of solubility, and the unmeasured solubility could be predicted from these models. Gupta and Heidemann established a classic solubility model for amino acids and antibiotics to present the effects of the temperature and on the solubility in water based on the fugacity and the dissociation constant.[12] However, that model discarded the effects of temperature on the dissociation constants, and just two dissociation constants were considered, which is deficient to describe the solubility performance of folic acid. Poe pointed out that there were six acidic dissociation constants for folic acid and determined four of them besides two carboxyls by potentiometer and spectrophotometric titration methods.[13,14] According to those former studies, the folic acid exhibits several ionic forms when dissolved in water as shown in Figure 3, where all the molecular formulas express the folic acid in different form except the hydrogen ion H^+. The symbol in the parentheses indicates the atom position of folic acid; K_i ($i = 0$ to 6) is the equilibrium constant of each reaction; and R_i ($i = 0$ to 2, 5, 6) is the abbreviation for part of the molecular groups. $\mathrm{N(5)}R_1^{2+}$ and $\mathrm{HN^+(10)}R_2^+$ are the same molecule with different expression to clarify the reaction. The same situation occurs between $\mathrm{N(10)}R_2^+$ and $\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COOH}$, $\mathrm{HN(3)}R_6^{2-}$ and $R_5^-\mathrm{C}(\gamma)\mathrm{COO^-}$, and $R_5^-\mathrm{C}(\gamma)\mathrm{COOH}$ and $\mathrm{N(1)}R_0\mathrm{C(\alpha)COO^-}$, respectively. K_0 is a solution equilibrium constant defined as

$$K_0 = [\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COO^-}] \qquad (1)$$

and K_i ($i = 1$ to 6) is the dissociation constant shown as

$$K_1 = \frac{[\mathrm{H^+}][\mathrm{N(5)}R_1^{2+}]}{[\mathrm{HN^+(5)}R_1^{2+}]} \qquad (2)$$

$$K_2 = \frac{[\mathrm{H^+}][\mathrm{N(10)}R_2^{+}]}{[\mathrm{HN^+(10)}R_2^{+}]} \qquad (3)$$

$$K_3 = \frac{[\mathrm{H^+}][\mathrm{N(1)}R_0\mathrm{C(\alpha)COO^-}]}{[\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COO^-}]} \qquad (4)$$

$$K_4 = \frac{[\mathrm{H^+}][\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COO^-}]}{[\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COOH}]} \qquad (5)$$

$$K_5 = \frac{[\mathrm{H^+}][R_5^-\mathrm{C}(\gamma)\mathrm{COO^-}]}{[R_5^-\mathrm{C}(\gamma)\mathrm{COOH}]} \qquad (6)$$

Table 2. Acidic Dissociation Constants pK_i of Folic Acid in Water at 298.15 K

pK_1	pK_2	pK_3	pK_4	pK_5	pK_6
-1.5^a	0.2 ± 0.1^a	2.35 ± 0.1^a	3.46 ± 0.02^b	4.56 ± 0.03^b	8.38 ± 0.03^c

[a] Ref 13. [b] Our work. [c] Ref 14.

$$K_6 = \frac{[\mathrm{H}^+][\mathrm{N}^-(3)R_6^{2-}]}{[\mathrm{HN}(3)R_6^{2-}]} \tag{7}$$

where the brackets mean the activity of the ion. The amount of the uncharged folic acid in the aqueous solution is negligible,[12] thus the apparent solubility of folic acid, x, can be expressed as

$$x = c_{\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COO^-}} + c_{\mathrm{HN^+(5)}R_1^{2+}} + c_{\mathrm{HN^+(10)}R_2^+} + c_{\mathrm{HN^+(1)}R_0\mathrm{C(\alpha)COOH}} + c_{\mathrm{N(1)}R_0\mathrm{C(\alpha)COO^-}} + c_{R_5^-\mathrm{C(\gamma)COO^-}} + c_{\mathrm{N^-(3)}R_6^{2-}} \tag{8}$$

where c is the concentration of folic acid in ionic form. The concentrations of the ions of $\mathrm{HN^+(5)}R_1^{2+}$ and $\mathrm{N^-(3)}R_6^{2-}$ are negligible at the pH range of 0 to 7 in this work as the pH range is far from $p\mathrm{K}_1$ and $p\mathrm{K}_6$ in Table 2. If a uniform activity coefficient is used simply, the solubility of folic in water can be expressed as[12,15]

$$x^T = \frac{K_0^T}{\gamma^T}\left(1 + \frac{[\mathrm{H}^+]^2}{K_2^T K_4^T} + \frac{[\mathrm{H}^+]}{K_4^T} + \frac{K_3^T}{[\mathrm{H}^+]} + \frac{K_3^T K_5^T}{[\mathrm{H}^+]^2}\right) \tag{9}$$

where x^T is the solubility of folic acid at temperature T; γ^T is the uniform activity coefficient at temperature T; $[\mathrm{H}^+]$ is the activity of the hydrogen ion; K_0^T is the solution equilibrium constant shown in eq 1 at temperature T; K_2^T, K_3^T, K_4^T, and K_5^T are the dissociation constants at temperature T shown in eqs 3 to 6. Although Kikit indicated that the activity coefficient of a solute in a mixed solvent−salt system included long-range and short-range contributions,[16,17] the physical interaction between water and solute is neglectable as the slight solubility of folic acid. Assuming all ions in folic acid solution have a uniform activity coefficient, γ^T can be expressed by the Davies equation as

$$\gamma^T = \exp\left\{-\left[D\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.3I\right)\right]\right\} \tag{10}$$

where D is a coefficient that depends on temperature and dipole moment of solvent, and I is the ion strength of solution. If the thermal capacity of the system in eq 1 is simplified as a linear relationship to temperature, according the van't Hoff Equation

$$\frac{d \ln K^\phi}{dT} = \frac{\Delta_r H_m^\phi}{RT^2} \tag{11}$$

K_0^T can be written as

$$K_0^T = \exp\left(A + \frac{B}{T} + C \ln T\right) \tag{12}$$

where K^ϕ is the standard equilibrium constant; $\Delta_r H_m^\phi$ is the molar standard enthalpy of reaction; A, B, and C are model parameters; and R is the universal gas constant. The dissociation constant K_i^T in the equations at temperature T is

$$K_i^T = K_i^{298.15} \exp\left[\frac{-\Delta_r H_i}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right] \tag{13}$$

where $K_i^{298.15}$ is the dissociation constant at 298.15 K, $\Delta_r H_i$ is the reaction enthalpy shown in eq i (i = 3 to 6). Equation 9 expresses the solubility of folic acid connecting eqs 10, 12, and 13.

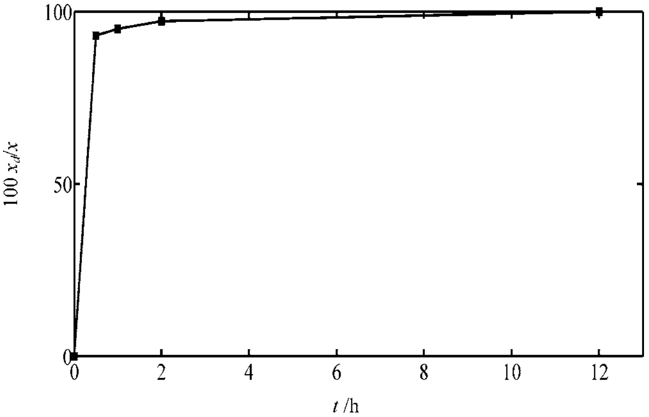

Figure 4. Dissolution performance of folic acid in water at 313.15 K.

Table 3. Parameters in Equations 12 and 13 for the Model of Folic Acid Solubility

A	B	C	$10^5\Delta_r H_2$ / J·mol^{-1}	$10^3\Delta_r H_3$ / J·mol^{-1}	$10^3\Delta_r H_4$ / J·mol^{-1}	$10^3\Delta_r H_5$ / J·mol^{-1}
−202.6	−43.59	32.34	−1.15	1.95	−2.1	2.0

A potentiometer titration method in aqueous dimethyl sulfoxide (w = 80 %) without salt added to adjust the ionic strength introduced by Fini et al.[18] was studied to determine the two carboxyls' dissociation constants at temperature (298.15 ± 0.1) K. The results are listed in Table 2.

The experimental data are fit with the model of eq 9 in the least-squares method, and the results shown in Table 3 and Figure 2, where A, B, C, $\Delta_r H_2$, $\Delta_r H_3$, $\Delta_r H_4$, and $\Delta_r H_5$ are model parameters for regression. Regression analysis indicates that the model matches the experiment data well with the R^2 of 0.9900 and the root-mean-square deviation (rmsd) of $6.32 \cdot 10^{-6}$ mol·dm^{-3}, where the rmsd is defined as

$$\mathrm{rmsd} = \left[\frac{1}{N}\sum_{i=1}^{N}(x_i^{\mathrm{cal}} - x_i^{\mathrm{exp}})^2\right]^{1/2} \tag{14}$$

N is the number of experimental points; x_i^{exp} represents the experimental solubility values; and x_i^{cal} represents the solubility calculated from eq 9. A slight error appears at high acidic conditions possibly due to: (1) a simplified uniform activity coefficient is used in the model, which is far from the real system at strong acidic conditions, and (2) the solubility under strong acidic and basic conditions is more than 1000 times the values at weak acidic condition, which leads to an ill condition for regression. Figure 2 also indicates a visible difference between the result from ref 3 and the data from this work at basic and strong acidic conditions because the active buffers are used in ref 3. A relative dissolution curve of folic acid at 313.15 K in water is checked to ensure that the solution is saturated at the end of 12 h which is shown in Figure 4, where x_d is the dynamic concentration of folic acid, and t is the time in hours. The dissolution curve indicates that folic acid solves in water rapidly for a tiny folic acid powder used in this work.

In this work, the solubility of folic acid in water is determined at pH values between 0 and 7 at the temperatures (298.15, 303.15, and 313.15) K. In general, it increases with rising temperature, and a great solubility appears in basic and high acidic conditions. A model based on the dissociation constants and thermodynamics can describe the solubility of folic acid in water well.

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