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Ionic Strength Dependence of Four Stepwise Protonation Constants for Folic Acid in Different Aqueous Solutions of Dioxane

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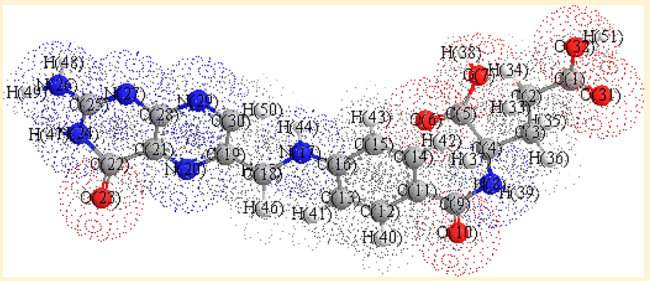
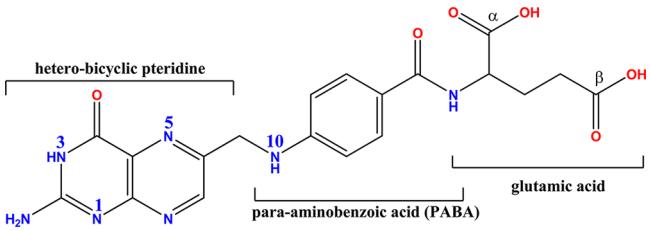
ABSTRACT: The protonation equilibria of vitamin B9 (folic acid) was studied at 298.15 K in a different water−dioxane mixtures [100 $w_{dioxane}$ = 20 %, 40 %, 60 %, and 80 %] with an ionic strength of 0.15 mol·dm^{-3} $NaNO_3$. The influence of dioxane content on the protonation processes was explained. Also, four protonation constants have been determined in 60 % dioxane with an ionic strengths of (0.15, 0.20, 0.25, and 0.30) mol·dm^{-3} $NaNO_3$ using the pH-potentiometric technique. HYPERQUAD 2008, a program based on nonlinear least-squares curve fitting was used to determine these four stepwise protonation constants of folic acid from the analysis of pH-potentiometric data. From the determined protonation constants, the representative folate species distribution diagrams were provided by HYSS 2009 program and discussed.



INTRODUCTION

Vitamin B9 ($C_{19}H_{19}N_7O_6$, folic acid, folate, or folacin) is a member of the water-soluble B-complex vitamins family. The molecular structure of folic acid showed that it is composed of three main structural units; a heterobicyclic pteridine ring linked to para-aminobenzoic acid, and one or more glutamate residues (Scheme 1). Folic acid play a crucial rule in several important biological processes in the living cell systems including DNA synthesis, repair DNA, and methylate DNA as well as to function as a cofactor in certain biological reactions.[1] Biochemically, folic acid was found to reduce homocysteitie levels and the occurrence of neural tube defects.[2] Recently, folate drugs were used to prevent colorectal cancer.[3] Folic acid also shows a promising role in the treatment of depressive disorder and the prediction of response to antidepressant treatment.[4] Many biomedical questions in research about folic acid was answered in an interesting recent article.[5] A huge number of review articles were reported about the isolation and identification of folic acid,[6] the clinical effect of folic acid supplementation on the mental performance of children;[7] fetal and infant brain development and function during pregnancy and lactation;[8] the progression of carotid intima-media thickness;[9] colorrectal adenoma recurrence;[10] cardiovascular outcomes;[11] and anemia during pregnancy.[12] Also, some studies reported that folic acid fortification prevents neural tube defects and may also reduce cancer risks.[13−15] The role of folic acid in the primary prevention of birth defects[16] and the in the health of women of reproductive age[17] were reviewed critically.

Scheme 1. Molecular Structure of Folic Acid (Vitamin B9)



The protonation, deprotonation, and solvation equilibria studies of folic acid in different water samples containing various organic media have attracted much attention recently. These properties explain the chemical structure−function relationship, from which the knowledge of the protonation constants and different ionization states of folic acid would be valuable in drug development research. An understanding of the folate pharmacokinetics and pharmacodynamics allows control of its solubility, absorption, and distribution in the human body, and how much the organic solvents influence the folate drug function and stability.[18−22] During the past few years, I was involved in a continuous research program oriented toward studying the protonation and complexation equilibria of various bimolecules with different chelating ligand properties such as amino acids, hydroxamates, phenolates, peptides, buffers and non-protein amino acids in aqueous solution and in different solvent mixtures.[18−22] Thus, the protonation equilibria of folic acid in different ionic strengths of various (water + dioxane) solutions are of particular interest in the present work.

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Table 1. Logarithms of the Protonation Constants (log β) of Folic Acid in a Water (1) + Dioxane (2) Mixture at T = 298.15 K and I = 0.15 mol·dm^{-3} $NaNO_3$

	log β			
100 w_2 (%)	log β_1	log β_2	log β_3	log β_4
20	7.2314 ± 0.0324	12.4579 ± 0.0064	16.9745 ± 0.0053	19.9647 ± 0.0032
40	7.0987 ± 0.0621	12.1547 ± 0.0076	16.3104 ± 0.0087	19.5637 ± 0.0037
60	6.8745 ± 0.0131	11.7645 ± 0.0053	15.9764 ± 0.0057	19.031 ± 0.00321
80	6.6321 ± 0.0314	11.0234 ± 0.0046	15.2319 ± 0.0061	18.6712 ± 0.0014

Table 2. Logarithms of the Protonation Constants (log β) of Folic Acid (Fa) at Temperature T = 298.15 K in a 60 % Dioxane Solutions at Different Ionic Strengths (I = 0.15, 0.20, 0.25, and 0.30 mol·dm^{-3} $NaNO_3$)

	log β			
I (mol·dm^{-3})	log β_1	log β_2	log β_3	log β_4
0.15	7.0987 ± 0.0062	12.1547 ± 0.0076	16.3104 ± 0.0087	19.5637 ± 0.0032
0.20	6.9687 ± 0.0046	11.7567 ± 0.0061	15.8124 ± 0.0047	19.0237 ± 0.0012
0.25	6.4321 ± 0.0081	11.2145 ± 0.0043	15.1729 ± 0.0057	18.4325 ± 0.0097
0.30	6.0064 ± 0.0013	10.7685 ± 0.0046	14. 7769 ± 0.0034	17.9773 ± 0.0043

EXPERIMENTAL SECTION

Chemicals and Solutions. Folic acid ($C_{19}H_{19}N_7O_6$) was supplied from Aldrich (Germany) with 99 % purity. The organic solvent, dioxane was of high purity and used without further purification (Sigma-Aldrich, Germany). Nitric acid (Panreac, Spain) solutions was prepared and standardized before use. Sodium nitrate ($NaNO_3$, 99 % purity) from Acros Organics (USA) was used. Free CO_2 sodium hydroxide solution was prepared by dissolving NaOH pellets (Acros Organics, USA) in ultrapure water, and the solution was potentiometrically standardized against potassium hydrogen phthalate with purity 99 % (Sigma-Aldrich, USA). All solutions were freshly prepared daily prior to experimental use. Chemicals were accurately weighed then dissolved in ultrapure water (18.3 MΩ·cm^{-1} resistance).

pH-Potentiometric Apparatus. The pH-potentiometric measurements of folic acid were performed using Metrohm 702 SM titrator, equipped with 664 Dosimate and a 728 magnetic stirrer, and coupled with a Dosino buret model 683. Electrode response can be read to the third decimal place in terms of pH units with a precision of ± 0.001.

Calibration of Glass Electrode Cell. A GLEE computer program (Gran's glass electrode evaluation method) was used for the daily calibration of a glass electrode by means of a strong acid−strong base titration technique as described in detail previously.[22−24]

Procedures for pH-Potentiometric Titrations. The following different appropriate water (1) + dioxane (2) mixture solutions (100 w_2 = 20, 40, 60, and 80): (a) 0.003 mol dm^{-3} HNO_3 + 0.15 mol dm^{-3} $NaNO_3$ and (b) solution a + 0.001 mol dm^{-3} folic acid, were prepared freshly (up to total volume of 50 cm^3) and titrated potentiometrically against standardized 0.1 mol dm^{-3} CO_2-free sodium hydroxide solution at 298.15° K, in pH ranges from 2.0 to 11. The total volume was adjusted to 50 cm^3 by adding ultrapure water. The temperature of the titration processes was controlled by circulation of water through an ultra-thermostatted bath through the jacket of the double-walled glass titration cell, and maintained within ± 0.1 K. Totally, four pH-potentiometric titrations were carried out under purified nitrogen atmosphere for each different appropriate water (1) + dioxane (2) mixture solutions, with reproducibility of ± 0.02. About, more than 60 pH potentiometric measurement reading points were collected for each titration run and considered in the data analysis.

Data Analysis. The four stepwise protonation constants of folic acid was calculated through a detailed data analysis as described previously using Douheret equations,[25,26] followed by ESAB,[27,28] PKPOT,[29] and HYPERQUAD 2008[30] programs estimation. The HYPERQUAD 2008 program was used to derive the different protonation constants of folic acid from the fitting of pH-potentiometric titration measurements, based on the minimization of the nonlinear least-squares sum defined by the difference between the calculated and the experimental data of the titration curves. The HYPERQUAD 2008 program permits the formation constants determination of different folic species that can be found in the aqueous and nonaqueous solutions, simultaneously.[30] Various models with possible composition of different protonated folic acid species were proposed in the program, and the model that gave the best statistical fit and was chemically sensible was chosen. Besides Hyperquad 2008, the Hyperquad Simulation and Speciation (Hyss 2009) program was also used for providing protonated folate speciation distribution diagrams that were found in different aqua-dioxane media.[31]

RESULT AND DISCUSSION

Although folic acid is well-known to be a standard chemical structure of how the different chelating ligand protonation and tautomeric ionization states force various bond necessities of folate-binding molecular geometry and the related biological activity, the protonation equilibria of folic acid in aqueous and nonaqueous solutions have not been fully studied because of its poor solubility, aggregation, and photodegradation in acidic, neutral, and alkali conditions.[32,33] Recently a pressure-assisted capillary electrophoresis technique was applied to determine preciously and accurately four pK_a values (2.38, 3.46, 4.98, and 8.08) for folic acid, in 0.16 mol·dm^{-3} $NaNO_3$ solutions.[32] In this work, by fitting the experimental data of the pH-potentiometric titrations of folic acid (FA) using the HYPERQUAD 2008 program, four protonation constants as overall equilibrium constants (log β_1, log β_2, log β_3, and log β_4) for folic acid were determined and evaluated (Tables 1 and 2). These constants were then presented as the minus logarithm of a stepwise acid dissociation constant (pK_a). The two low protonation constants values (pK_a < −1.5, 0.20) of folic acid

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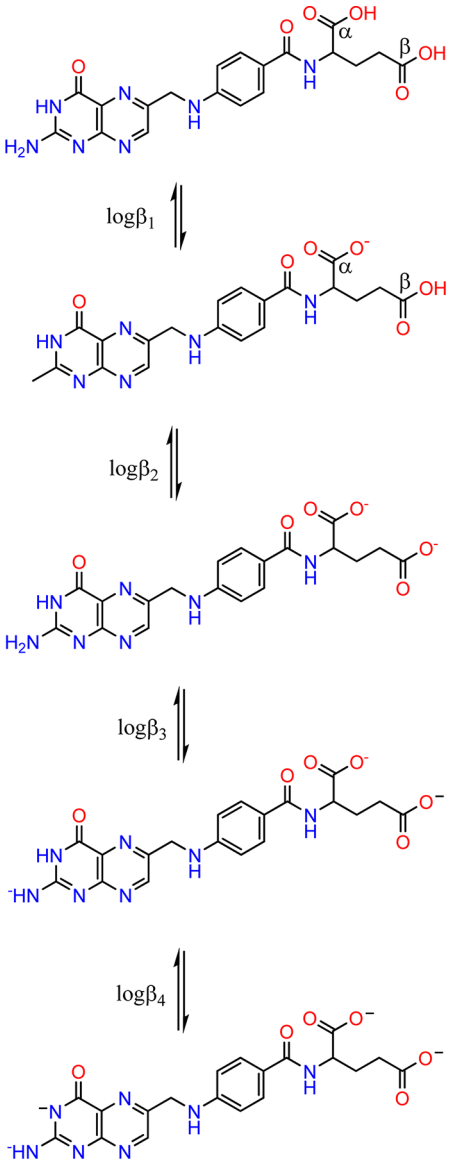
investigated can be associated with the N-5 site and N-10 site, respectively.[33,34] As shown, these two values are very low and not significant in the physiological pH region. Thus, these values are not used in the pH-potentiometric data analysis measured in the range of 2.00 ≤ pH ≤ 12.5.

As known, folic acid assigns the obtained protonation constants to some specific functional (*O, N*) donor groups. The log β_1, log β_2, and log β_3 overall protonation constants values are 7.0987 (related to α-COOH), 12.1547 (related to β-COOH), and 16.3104 (related to NH_2 amine group, site 3). The log β_4 protonation constant value of folic acid can be clearly assigned to the NH/CO (N-3 site) fragment of the pteridine ring (Scheme 2). The previous potentiometric

Scheme 2. Protonation Equilibria of Folic Acid (FA)



determination of folic acid in dimethyl sulfoxide (DMSO, 100 *w* = 80 %) showed two carboxyls' dissociation constants (pK_a values of 5.47 and 8.28) at 298.15 K.[35] More recently, the thermodynamic protonation constants model describes the solubility of folic acid in aqueous solutions discarding the property of temperature on the equilibrium constants.[36] This previous study determined only two protonation constants (pK_a values of 3.46 and 4.56), which do not explain the poor solubility and stability of a folate drug.[36]

The influence of dioxane organic solvent on the protonation equilibrium values of folic acid can be explained using the solvation chromic values of Kamlet−Taft hydrogen bond and dipolarity polarizability π^* of dioxane.[37,38] The protonation constants' values increase with an increase in dioxane content (mole fraction of dioxane) because of the decrease in the dielectric constant of bulk solvent. As the dielectric constant decreases, the ion interaction involving the proton and anionic oxygen on the acid decreases to a greater extent than the ion dipole interaction between the proton and the solvent molecule. A plot of log β values versus mole fraction of dioxane (Figure 1) shows a linear relationship. The

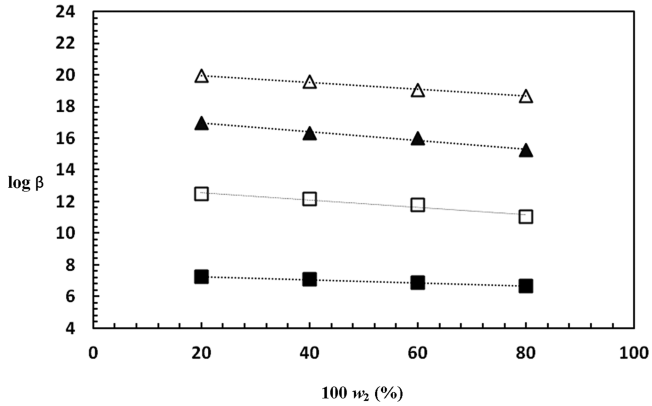


Figure 1. Plot of experimental (symbols) and correlated (dashed lines) different log β values of folic acid versus 100 w_2: ■, log β_1; □, log β_2; ▲, log β_3; Δ, log β_4.

experimental reliability in protonation constants values (log β_1, log β_2, log β_3, and log β_4) for folic acid in various solutions of water (1) + dioxane (2) mixtures can essentially be deduced as consequential from two opposing effects: (i) non-aqueous solutions of dioxane/water are more basic than water only;[37,38] (ii) low stabilization of the tautomeric ionization states for folic acid-free base by a hydrogen bond donated from dioxane solvent molecules in dioxane or a dioxane/water mixture as compared with that determined in water. The above two reasonable effects explain why higher protonation constants' values were observed in the aqueous solutions than in the non-aqueous medium as shown in Table 1.[39]

The plot of correlated and experimental four-protonation constants of folic acid at temperature *T* = 298.15 K, and different ionic strengths (*I*) versus $\sqrt{I}$ shows a full conformity with the Debye−Hückel theory when linear regression analysis is applied (Figure 2).[37] A spectrophotometric study of protonation constants of group B vitamins in different ionic strengths showed some irregular variations in reverse of the data obtained in this study for the influence of ionic strength on the protonation equilibria of folic acid, which may be attributed to experimental, handling, and instrumental errors in some cases.[40]

The bioavailability of folates and their biological effects should depend on which real folic acid species is present. The various equilibrium distributions of protonated and deprotonated folic species formed in aquo-dioxane mixtures are shown as functions of pH (Figure 3). The graphical distribution species diagrams in Figure 3 show the protonated folic acid species, which FAH_4 started to form, in very low acidic

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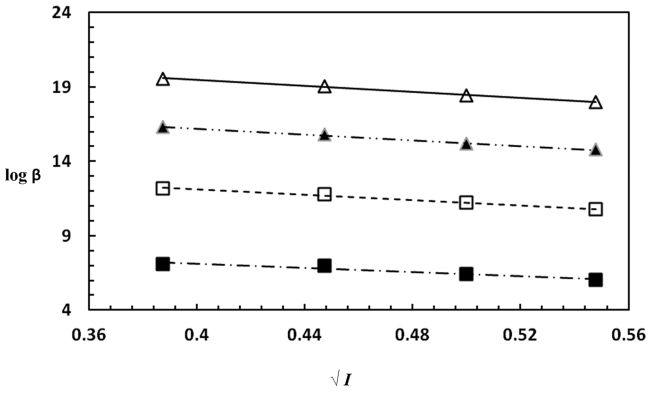

Figure 2. Plot of experimental (symbols) and correlated (dashed lines) of different log β values of folic acid versus $\sqrt{I}$/mol·dm^{-3}: ■, log β_1; □, log β_2; ▲, log β_3; △, log β_4.

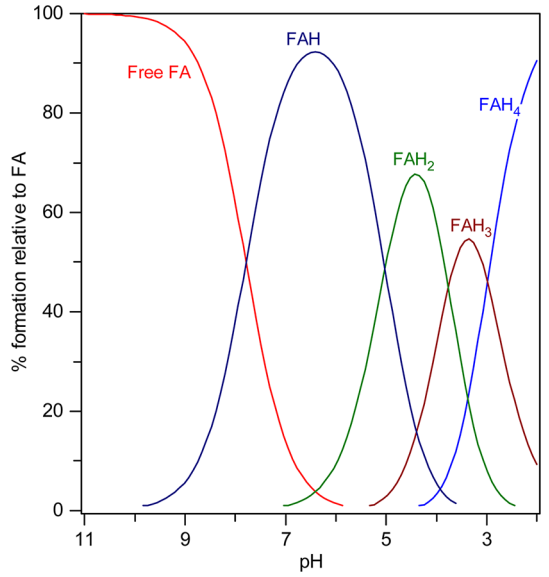

Figure 3. Species distribution curves for folic acid drug (FA) at T = 298.15 K and I = 0.15 mol·dm^{-3} $NaNO_3$. Percentages are calculated with respect to the analytical concentration of the drug (FA).

conditions (pH < 2.5), while the folic acid species FAH_3 was formed in acidic conditions (pH ≈ 2−5.5); conversely, the species FAH_2 started to form at normal acidic conditions and formed rapidly at neutral conditions (pH ≈ 3−7). The folate species FAH started to form in normal acidic conditions (pH ≈ 4) and completely formed in neutral and alkaline conditions (pH ≈ 5.5−9.5).

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Notes

The authors declare no competing financial interest.

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