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Protonation Constants of Adenine and Adenosine in Different Aqueous Solutions of Methanol and Ethanol

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The protonation constants of adenine (K_1 and K_2) and adenosine (K_2) were determined in binary mixtures of water with methanol or ethanol containing (0, 10, 15, 20, 25, 30, 35, 40, and 45) % (v/v) using a combination of potentiometric and spectrophotometric methods at 25 °C and constant ionic strength (0.1 $\mathrm{mol \cdot dm^{-3}}$ sodium perchlorate). The protonation constants were analyzed using the normalized polarity parameter (E_T^N) and Kamlet, Abboud, and Taft (KAT) parameters. A very good linear correlation of log K versus the normalized polarity parameter was obtained. Dual-parameter correlation of log K versus π^* (dipolarity/polarizability) and α (hydrogen-bond donor acidity) as well as π^* and β (hydrogen-bond acceptor basicity) also gives good results in various aqueous organic solvent mixtures. Finally, the results are discussed in terms of the effect of the solvent on the protonation constants.

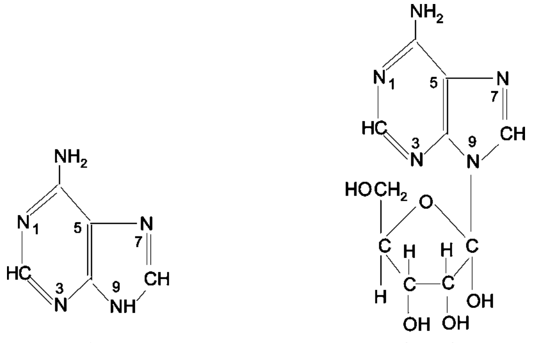
Introduction

Adenines and their nucleotides and nucleosides are very important compounds due to their vital roles within living cells and in regulation of various functions in biological systems.[1,2] These compounds are the building blocks in both DNA and RNA and are involved in a wide variety of processes like cellular metabolism, cell bioenergetics, human cancer and immunodeficiency virus (HIV) markers, etc.[3,4]

The acid−base behavior of nucleotides, nucleosides, bases, and polynucleotides is essential to deduce the speciation and the possible conformational changes with pH or the amount of organic solvent in solution. Acid dissociation constants are among the most useful physicochemical measurements describing the extent of ionization of functional groups with respect to pH. This parameter is important in research areas such as pharmaceutical drug discovery and development, where it often has a vital role in understanding the pharmacodynamic properties of new drug substances.[5−9]

Many chemical reactions of experimental and practical processes occur in solution. In a variety of chemical fields such as chemical synthesis, solvent extraction, liquid chromatography, etc., binary solutions of water and organic solvents are used. Aqueous organic solvent, mainly methanol and ethanol, mixtures have been widely used due to the sparingly or insolubility of many compounds in pure water as solvent. Further, any physicochemical property of solutions can be easily varied by changing the compositions of water or the organic solvent in the mixtures. However, chemists have usually attempted to understand solvent effects in terms of polarity, defined as the overall solution capabilities that depend on all possible (specific and nonspecific) intermolecular interactions between solute and solvent molecules. Many reports on solvent polarity scales have been published in the last few decades.[10] Previously, the solvent effect on the protonation equilibrium was believed to be guided chiefly by electrostatic interactions (Born model).[11] However, recent studies have revealed that the change in macroscopic properties such as the dielectric constant of the solvent cannot be the sole factor.[10] It is desirable to develop other empirical functions to take into account the complete picture of all intermolecular forces acting between solute and solvent molecules.

Scheme 1. Chemical Structures of Adenine and Adenosine



adenine adenosine

In continuation of our previous work,[12−16] in this study the protonation constants of adenine and adenosine have been determined in different aqueous methanol and ethanol mixtures to examine the dependence of acid−base equilibria on solvent composition.

Experimental Section

Chemicals. Adenine ($C_5H_5N_5$) and adenosine ($C_{10}H_{13}O_4N_5$) (Scheme 1) were obtained from Fluka as analytical reagent grade materials and used without further purification. Methanol and ethanol were from Merck (reagent grade) and were used as received. Sodium perchlorate was from Merck and was dried under vacuum at room temperature for at least 72 h before use. NaOH solution was prepared from a titrisol solution (Merck). Perchloric acid was from Merck and was used as supplied. All dilute solutions were prepared from double-distilled water with a specific conductance equal to (1.3 ± 0.1) $\mu\mathrm{S \cdot cm^{-1}}$.

Apparatus. The electromotive force was measured using a Metrohm model 781 pH ion-meter. A combined glass−pH

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electrode (model 6.0258.000) was modified by replacing its aqueous KCl solution with 0.01 $mol\cdot dm^{-3}$ NaCl + 0.09 $mol\cdot dm^{-3}$ $NaClO_4$ saturated with AgCl. The electrode was soaked for (15 to 20) minutes in a water−organic solvent mixture before the potentiometric measurements. All titrations were carried out in an 80 mL thermostatted double-walled glass vessel.

Spectrophotometric measurements were performed on a UV−vis Shimadzu 2100 spectrophotometer with a Pentium 4 computer and using thermostatted matched 10 mm quartz cells. The measurement cell was of the flow type. A peristaltic pump allowed circulation of the solution under study from the potentiometric cell to the spectrophotometric cell, so the absorbance and the emf of the solution could be measured simultaneously. To exclude carbon dioxide from the system, a stream of purified nitrogen was passed through a sodium hydroxide solution and then bubbled slowly through the reaction solution.

Procedure. All measurements were performed at 25 °C and a constant ionic strength of 0.1 $mol\cdot dm^{-3}$ sodium perchlorate. The protonation constants were evaluated from the measurements of absorbance versus emf by titration of 25 mL of adenine or adenosine $[(1.0 \text{ to } 3.0)\cdot 10^{-3}\ mol\cdot dm^{-3}]$ with 0.1 $mol\cdot dm^{-3}$ sodium hydroxide solution with both the same ionic strength and mole fraction of organic solvent [(0 to 45) % methanol or ethanol v/v].

In the first step, the electrode system calibration was performed by Gran's method.[17] For this purpose, a measured amount of an acidic solution, at the same condition of temperature, ionic strength, and solvent composition to be used in later experiments, was placed in the double-wall thermostatted vessel. The electrode was immersed in the solution in the vessel, and the acidic solution was titrated with a strong base (0.1 $mol\cdot dm^{-3}$ NaOH). The potential was allowed to stabilize after each addition of the titrant, and the recorded emf values were then used to obtain $E°$. The procedure was continued to pH ≅ 2.5 (lower than the pK of each base). In the second step, 25 mL of an acidic solution (0.01 $mol\cdot dm^{-3}$ $HClO_4$) of adenine or adenosine $[(1.0 \text{ to } 3.0)\cdot 10^{-3}\ mol\cdot dm^{-3}]$ at the same conditions of temperature, ionic strength, and solvent composition was titrated with a sodium hydroxide solution (0.1 $mol\cdot dm^{-3}$). The emf and the absorbance [in the interval of (250 to 310) nm] values were then determined. The procedures were repeated in different compositions of the organic solvents.

The recorded emf values were then converted to p_cH ($-\log [H^+]$) using a method described in the literature.[18] In acidic solution, the measured potential of the cell, E_{cell}, glass elec./ $HClO_4$−$NaClO_4$ in water−organic solvent // NaCl−$NaClO_4$/ Ag−AgCl, can be written as

$$E_{cell}\,(mV) = E^{\circ}_{cell} + k\cdot\log [H^+] + k\cdot\log \gamma_{H^+} + E_{LJ} \tag{1}$$

where E°_{cell} is the standard potential of the cell; E_{LJ} is the liquid junction potential; $k = 2.303RT/F$ in which R, T, and F have the usual meaning; and γ_{H^+} is the activity coefficient of the hydrogen ion. Difficulties in computing the activity coefficients of the hydrogen ion in various aqueous mixtures of organic solvents lead to measurement of emf (electromotive force) versus H^+ concentration in solution. Because the ionic strength of the solution is kept constant, the activity coefficient of the hydrogen ion is constant too. The nonideality of solutions is then included in E'_a (the specific constant of the potentiometric cell in the acidic region), so

$$E_{cell} = E'_a + k\cdot\log [H^+] \tag{2}$$

where E'_a is $E^{\circ}_{cell} + k\cdot\log \gamma_{H^+} + E_{LJ}$. The use of a glass electrode (with an aqueous inner solution) in nonaqueous media introduces a deviation from ideality, but it has been shown that the deviation is negligible and that the glass electrode is always usable in such media to measure H^+ concentrations with a linear relation of E_{cell} versus $\log [H^+]$.[19,20] In the acidic region, the hydrogen ion concentration can be expressed as

$$[H^+] = (M_{HClO_4}V_0 - M_{NaOH}V_1)/(V_0 + V_1) \tag{3}$$

where M_{HClO_4} and M_{NaOH} are the molarities of perchloric acid and sodium hydroxide, and V_0 and V_1 are the initial volume of perchloric acid and the added volume of sodium hydroxide solution, respectively. Finally

$$p_cH = (E'_a - E_{cell})/k \tag{4}$$

Results and Discussion

The protonation constants of adenine and adenosine have been determined spectrophotometrically based on the relation $A = f(pH)$.[21] The measured absorbance, A, [(270 to 310) nm in the interval of 0.5 nm] and p_cH from the spectrophotometric titration were conducted using the computer program Squad.[22,23] The data in the computer program were fitted to eq 5 by minimizing the error square sum of the diference in the experimental absorbances and the calculated ones. The program allows calculation of the protonation constants with different stoichiometries. The number of experimental points (absorbances versus p_cH) was more than 35 (maximum 50) for each titration run. During the experiments, the solutions were stable, and the absorbance values did not change with time.

The results obtained using spectrophotometric and potentiometric pH titrations for the various acidity constants of the proton donors of adenine and adenosine in different aqueous solutions of methanol and ethanol, eq 5, are listed in Table 1 together with the values reported in the literature for comparison.[24,25]

$$H_nL^{n-1} + H^+ \rightleftharpoons H_{1+n}L^n$$
$$K_{1+n} = [H_{1+n}L^n]/[H_nL^{n-1}][H^+] \tag{5}$$

where L represents adenine or adenosine and n may be 0, 1, or 2 for the different protonation equilibria of the bases. In Figure 1, the species mole fractions of both systems in different p_cH are shown in pure water.

With little differences, the protonation constant values obtained in this work are in agreement with those reported before. The differences are possibly due to the different experimental method and the different background electrolyte used. It was proposed that adenine shown in Scheme 1 may combine with its first and second protons from N9 and N1 sites, respectively.[6] Also, a third proton is combined in a very acidic pH range from the N7 site. However, adenosine may release one proton at the ribose group (in a very alkaline pH range) and N1 site in the purine moiety, respectively. It should be noted that the release of the third proton from the N7 site in adenine ($pK_3 < 1$) and the first deprotonation in adenosine ($pK_1 > 12$)

Table 1. Average Values of the Protonation Constants of Adenine and Adenosine at 25 °C and Constant Ionic Strength (0.1 mol·dm^{-3} NaClO$_4$) and Different Aqueous Alcohol Mixtures[a]

	adenine				adenosine		
alcohol % (v/v)	log K_1 (methanol)	log K_1 (ethanol)	log K_2 (methanol)	log K_2 (methanol)	log K_2 (ethanol)	log K_2 (ethanol)	ref.
0	9.83 ± 0.05	9.83 ± 0.04	4.09 ± 0.02	4.09 ± 0.03	3.64 ± 0.04	3.64 ± 0.03	this work
10	9.75 ± 0.04	9.74 ± 0.03	4.31 ± 0.02	4.32 ± 0.02	3.80 ± 0.04	3.83 ± 0.04	″
15	9.71 ± 0.04	9.68 ± 0.03	4.45 ± 0.03	4.48 ± 0.02	3.87 ± 0.04	3.93 ± 0.04	″
20	9.65 ± 0.03	9.60 ± 0.05	4.60 ± 0.02	4.70 ± 0.05	3.98 ± 0.05	4.04 ± 0.05	″
25	9.61 ± 0.05	9.55 ± 0.05	4.69 ± 0.04	4.75 ± 0.05	4.04 ± 0.03	4.08 ± 0.03	″
30	9.53 ± 0.03	9.47 ± 0.03	4.86 ± 0.03	4.93 ± 0.04	4.10 ± 0.03	4.17 ± 0.02	″
35	9.49 ± 0.04	9.43 ± 0.05	4.93 ± 0.04	5.03 ± 0.03	4.15 ± 0.04	4.21 ± 0.04	″
40	9.44 ± 0.05	9.36 ± 0.03	5.01 ± 0.05	5.11 ± 0.02	4.24 ± 0.02	4.31 ± 0.04	″
45	9.37 ± 0.05	9.27 ± 0.04	5.12 ± 0.04	5.21 ± 0.03	4.33 ± 0.03	4.39 ± 0.03	″
0	9.45		4.16		3.62		24
0	9.65		4.18		3.59		25

[a] The values reported in the literature for pure water are also listed for comparison.

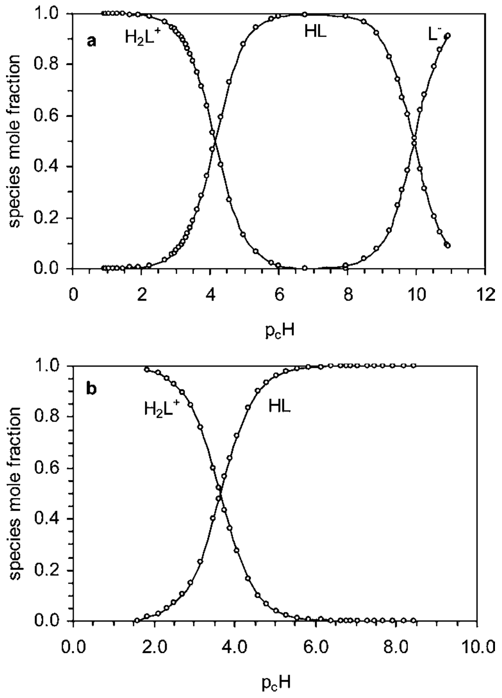

Figure 1. Distribution diagrams of the different species of (a) adenine and (b) adenosine, in water at 25 °C and ionic strength of 0.1 mol·dm^{-3} NaClO$_4$.

are not considered in this work. The assignments agree well with the previous conclusion.[26]

Solvent Effect

The protonation constants of adenine and adenosine in water−alcohol mixed solvents have different behaviors. log K_1 of adenine decreases, but log K_2 of both bases increases with increasing proportion of organic solvents in the mixtures (Table 1). In general, the standard Gibbs energy of protonation equilibria consists of two terms: an electrostatic term, which can be estimated by the Born equation, and a nonelectrostatic term, which includes specific solute−solvent interaction.[27] When the electrostatic effects predominate, then in accordance with the Born equation, eq 6, the plot of log K versus the reciprocal of dielectric constant of the media, ε, should be linear

$$\Delta\log K = (121.6n/r)(1/\varepsilon - 0.0128) \qquad (6)$$

where r is the common radius of the ions and n is the square summation of the charges involved in the protonation equilibria. For example, $n = 2$ for the charge type L$^-$ ⇌ HL, and $n = 0$ for the charge type HL ⇌ H$_2$L$^+$. Therefore, a change in polarity of the medium has a major role in log K_1 of adenine, and in this case, the correlation between log K_1 with the reciprocal of the dielectric constant of the aqueous alcohol mixtures is linear (Figure 2a). However, the linearity of the correlation between log K_2 of adenine and adenosine with the reciprocal of the dielectric constant of methanol−water and water−ethanol mixtures is poor (with correlation coefficients between 0.93 and 0.97, respectively) (Figures 2b and 2c). This indicates that the protonation constants not only depend on electrostatic forces but also strongly depend on the solute−solvent interactions of the different species in the mixtures.[28] Therefore, it is necessary to elucidate the nature of solute−solvent interactions for a better understanding of solvent effects.

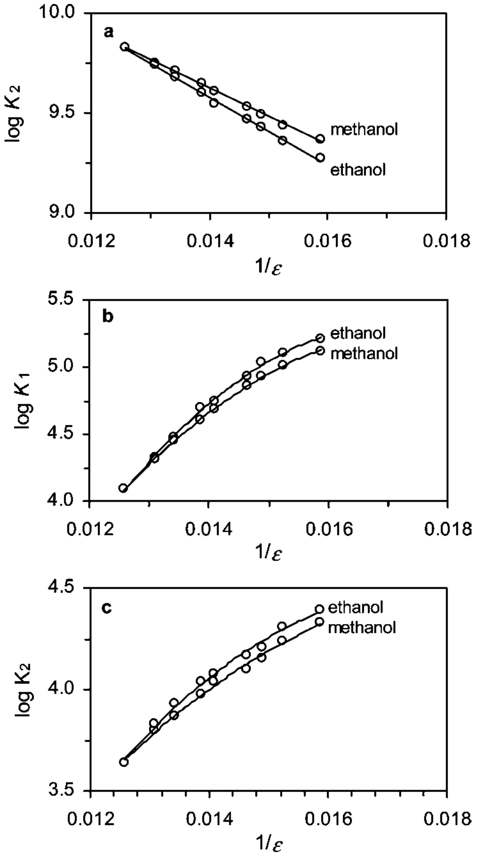

Figure 2. Plots of the experimental values of log K_2 (a), log K_1 (b) of adenine, and log K_2 (c) of adenosine versus the reciprocal of dielectric constant of the different mixed solvents at 25 °C and an ionic strength of 0.1 mol·dm^{-3} NaClO$_4$.

The deviations of log K_1 and log K_2 of both bases are larger in ethanol–water mixture solvents (Table 1). This is possibly due to the smaller dielectric constant values of ethanol–water mixtures in comparison to the same mole fraction of methanol–water mixtures. It is very difficult to interpret the variation of the protonation constant values of adenine and adenosine with respect to the percentage of methanol or ethanol in the mixtures using the dielectric constant of the solutions as a single parameter. However, it is now understood that the anion and cation species are selectively solvated by acidic or basic solvents, respectively.[29] In fact, anion solvation is closely related to the electron pair acceptability or Lewis acidity of solvents and tends to become stronger with the increase in acceptor number.[30] However, the solvent molecules approach a cation with their negative charge. Therefore, cation solvation is closely related to the electron pair donor capacity or Lewis basicity of the solvents and tends to become stronger with the increase in donor number. This behavior in the proposed aqueous organic solvents indicates why the values of log K_1 and log K_2 of adenine decrease and increase, respectively, when the organic solvent percentage increases in the mixtures. The same rule is governed for log K_2 in the case of adenosine.

To obtain a quantitative method for evaluation of the solute–solvent interaction on protonation or other equilibrium constants, during the last two decades, many empirical solvent scales have been devised.[10] Among these scales (more than 40), the most comprehensive are the solvatochromic ones, but only a few of them have found wider application in correlation analysis of solvent effects. A quantitative measurement of the solvent polarity has been introduced by Dimroth and Reichardts, E_T, based on the solvatochromic behavior of pyridinium *N*-phenoxide betaine dye.[10] This dye is the most solvatochromic compound reported to date.[10] This scale now has been revised and normalized to E_T^N, known as the normalized polarity parameter, due to the introduction of SI units. E_T^N is related to the ability of a solvent to stabilize charge separation in the dye and has the value of zero for tetramethylsilane, the least polar solvent, and 1.0 for water, the most polar solvent. According to this approach, the protonation constant values (in logarithm scale) were correlated with E_T^N as a single linear regression analysis using the computer program Microsoft Excel Solver and Linest.[31] A very good linear correlation of all log K versus E_T^N was obtained in different aqueous solutions of methanol and ethanol

$$\begin{gathered}\log K_1\ (\text{adenine, methanol}) = 6.70(\pm 0.13) + 3.16(\pm 0.14)E_T^N\\ N = 9,\ \text{rss} = 2.64\cdot 10^{-3},\ \text{ose} = 0.02,\ r^2 = 0.99,\ f = 167.79\end{gathered} \tag{7a}$$

$$\begin{gathered}\log K_1\ (\text{adenine, ethanol}) = 7.41(\pm 0.09) + 2.45(\pm 0.10)E_T^N\\ N = 9,\ \text{rss} = 2.95\cdot 10^{-3},\ \text{ose} = 0.02,\ r^2 = 0.99,\ f = 631.11\end{gathered} \tag{7b}$$

$$\begin{gathered}\log K_2\ (\text{adenine, methanol}) = 11.24(\pm 0.17) - 7.16(\pm 0.18)E_T^N\\ N = 9,\ \text{rss} = 4.32\cdot 10^{-3},\ \text{ose} = 0.02,\ r^2 = 1.00,\ f = 1516.99\end{gathered} \tag{7c}$$

$$\begin{gathered}\log K_2\ (\text{adenine, ethanol}) = 9.16(\pm 0.14) - 5.06(\pm 0.16)E_T^N\\ N = 9,\ \text{rss} = 8.06\cdot 10^{-3},\ \text{ose} = 0.03,\ r^2 = 0.99,\ f = 988.02\end{gathered} \tag{7d}$$

$$\begin{gathered}\log K_2\ (\text{adenosine, methanol}) = 7.74(\pm 0.19) - 4.06(\pm 0.21)E_T^N\\ N = 9,\ \text{rss} = 5.53\cdot 10^{-3},\ \text{ose} = 0.03,\ r^2 = 0.98,\ f = 382.36\end{gathered} \tag{7e}$$

$$\begin{gathered}\log K_2\ (\text{adenosine, ethanol}) = 6.54(\pm 0.14) - 2.85(\pm 0.16)E_T^N\\ N = 9,\ \text{rss} = 8.43\cdot 10^{-3},\ \text{ose} = 0.03,\ r^2 = 0.98,\ f = 299.80\end{gathered} \tag{7f}$$

where N, rss, ose, r^2, and f represent the number of the mixed solvents, the residual sum of squares, the overall error, regression coefficient, and *f*-test, respectively.

The normalized polarity parameters (E_T^N) for all of the water–organic solvent mixtures used in this work were obtained from the plots of each property versus the mole fraction of the organic solvent of the values that have been reported in the literature for some other percentages of aqueous solutions of the alcohols used in this study,[32] and the dielectric constant values were obtained from the literature[33] for the different mixed solvents (Table 2). The normalized polarity parameter is a blend of pure polarity (dipolarity/polarizability) and hydrogen bonding interactions. To show the magnitude of these interactions on the protonation constant, a dual-parameter correlation of log K

Table 2. Solvatochromic Parameters and the Dielectric Constants of Different Aqueous Alcohol Mixtures at 25 °C

	water–methanol					water–ethanol				
alcohol % (v/v)	α	β	π^*	E_T^N	ε	α	β	π^*	E_T^N	ε
0	1.23	0.49	1.14	1.00	79.5	1.23	0.49	1.14	1.00	79.5
10	1.19	0.51	1.13	0.96	76.4	1.13	0.52	1.14	0.95	73.7
15	1.17	0.53	1.12	0.95	74.5	1.08	0.55	1.13	0.92	70.2
20	1.14	0.54	1.10	0.93	72.1	1.03	0.57	1.12	0.90	66.0
25	1.11	0.56	1.09	0.91	71.0	0.98	0.59	1.11	0.87	61.6
30	1.08	0.57	1.07	0.90	68.3	0.95	0.61	1.09	0.84	55.0
35	1.06	0.59	1.06	0.88	67.2	0.91	0.63	1.06	0.82	47.5
40	1.04	0.60	1.04	0.87	65.6	0.89	0.66	1.04	0.80	41.1
45	1.02	0.62	1.02	0.85	63.0	0.87	0.68	1.00	0.78	35.4

versus the KAT parameters[34,35] (Kamlet, Abboud, Taft) was obtained

$$\begin{gathered}\log K_1 \text{ (adenine, methanol)} = 9.20(\pm 0.82) - \\ 2.11(\pm 0.49)\beta + 1.45(\pm 0.51)\pi^* \\ N = 9,\ \text{rss} = 6.02 \cdot 10^{-4},\ \text{ose} = 1.00 \cdot 10^{-2}, \\ r^2 = 1.00,\ f = 902.04\end{gathered} \tag{8a}$$

$$\begin{gathered}\log K_1 \text{ (adenine, ethanol)} = 10.79(\pm 0.40) - \\ 2.69(\pm 0.20)\beta + 0.31(\pm 0.06)\pi^* \\ N = 9,\ \text{rss} = 8.34 \cdot 10^{-4},\ \text{ose} = 1.18 \cdot 10^{-2}, \\ r^2 = 1.00,\ f = 965.48\end{gathered} \tag{8b}$$

$$\begin{gathered}\log K_2 \text{ (adenine, methanol)} = 9.53(\pm 0.63) - \\ 5.59(\pm 0.74)\alpha - 1.29(\pm 0.31)\pi^* \\ N = 9,\ \text{rss} = 4.33 \cdot 10^{-3},\ \text{ose} = 2.69 \cdot 10^{-2}, \\ r^2 = 1.00,\ f = 707.95\end{gathered} \tag{8c}$$

$$\begin{gathered}\log K_2 \text{ (adenine, ethanol)} = 8.80(\pm 0.35) - \\ 2.67(\pm 0.18)\alpha - 1.26(\pm 0.45)\pi^* \\ N = 9,\ \text{rss} = 5.88 \cdot 10^{-3},\ \text{ose} = 3.13 \cdot 10^{-2}, \\ r^2 = 0.99,\ f = 581.99\end{gathered} \tag{8d}$$

$$\begin{gathered}\log K^2 \text{ (adenosine, methanol)} = 7.54(\pm 0.65) - \\ 2.74(\pm 0.76)\alpha - 0.42(\pm 0.35)\pi^* \\ N = 9,\ \text{rss} = 4.60 \cdot 10^{-3},\ \text{ose} = 2.77 \cdot 10^{-2}, \\ r^2 = 0.99,\ f = 247.85\end{gathered} \tag{8e}$$

$$\begin{gathered}\log K_2 \text{ (adenosine, ethanol)} = 6.72(\pm 0.27) - \\ 1.62(\pm 0.14) - 0.93(\pm 0.35)\pi^* \\ N = 9,\ \text{rss} = 3.50 \cdot 10^{-3},\ \text{ose} = 2.42 \cdot 10^{-2}, \\ r^2 = 0.99,\ f = 384.41\end{gathered} \tag{8f}$$

The KAT equation contains nonspecific as well as specific solute–solvent interactions separately, and the latter should be subdivided into solvent Lewis-acidity interactions (hydrogen-bond acceptor, HBA solute, and hydrogen-bond donor, HBD solvent) and solvent Lewis-basicity interactions (HBD solute–HBA solvent). In general, these parameters constitute more comprehensive measures of solvent polarity than the dielectric constant alone because they reflect more reliably the complete picture of all intermolecular forces acting between solute and solvent molecules. This approach has been widely and successfully applied in the correlation analysis of all kind of solvent-dependent processes.[36] Using the solvatochromic solvent parameters, α, β, and π^*, which have been introduced in previous reports,[12−16] the multiparametric equation, eq 9, has been proposed for use in the so-called linear solvation energy relationship.

$$\log K = A_0 + a\alpha + b\beta + p\pi^* \tag{9}$$

where A_0 represents the regression value, and π^* is the index of the solvent dipolarity/polarizability, which is a measure of the ability of a solvent to stabilize a charge or a dipole by its own dielectric effects. The α coefficient represents the solvent hydrogen-bond donor (HBD) acidity; in other words, it describes the ability of a solvent to donate a proton in a solvent to a solute hydrogen bond. The β coefficient is a measure of a solvent hydrogen-bond acceptor (HBA) basicity and describes the ability of a solvent to accept a proton in a solute to solvent hydrogen bond. The regression coefficients a, b, and p measure the relative susceptibilities of the solvent dependence of log K to the indicated solvent parameters.

The procedure used in the regression analysis involves a rigorous statistical treatment to find out which parameter in eq 9 is best suited to the water–organic mixed solvents. So, a stepwise procedure and least-squares analysis were applied to select the significant solvent properties to be influenced in the model and to obtain the final expression for the protonation constants. Therefore, the KAT equation, eq 9, was reduced to single-, dual-, and multiparameters for correlation analysis of log K in various solvent mixtures. The computer program used can give the values of A_0, a, b, p, and some statistical parameters including r^2 coefficient, standard deviation of any parameter, given in brackets, and the overall standard error, ose, of log K. The KAT parameters have been taken from our previous report.[20]

Although the solvent polarity is identified as the main reason for the variation of log K values in water–organic solvent mixtures, the results show that any single-parameter correlations of log K values individually with π^* did not give good results in all cases, $r^2 \cong 0.95$ to 0.97. However, the correlation analysis of log K values with dual-parameter equations indicates significant improvement with regard to the single- or multiparameter models which is also consistent with the results obtained with normalized polarity parameter, E_T^N, regression analysis.

The coefficient of β in the correlation analysis of the dual parameter of the KAT equation in the case of log K_1 of adenine is negative and has a major role (more than 60 % in aqueous solutions of methanol or ethanol) for both systems. So, log K_1 values decrease with an increase in the hydrogen bond basicity parameter. Also, the positive sign of π^* in this case indicates that a decrease in the polarity of the mixed solvents decreases the log K_1 values. However, the coefficients of α and π^* in correlation analysis of log K_2 of both bases are negative. The negative sign of π^* values indicates that a decrease in the polarity of the mixed solvents increases the log K_2 values. Thus, an increase in the polarity increases the solvation of the species and therefore makes protonation equilibrium more likely. Moreover, the negative sign of α with a major role in dual-parameter correlation analysis of about (81 and 87) % in water–methanol as well as (68 and 64) % in water–ethanol mixtures for adenine and adenosine, respectively, shows that a decrease in the HBD acidity of the solvent also increases the solvation tendency of the produced cations. Consequently, the protonation constants increase when this parameter decreases.

Literature Cited

(1) Alberts, B.; Johnson, A.; Lewis, J.; Raff, M.; Roberts, K.; Walter, P. *Molecular biology of the cell*, 4th ed.; Garland Science: New York, 2002.

(2) Nobeli, I.; Laskowski, R. A.; Valdar, W. S. J.; Thornton, J. M. On the molecular discrimination between adenine and guanine by proteins. *Nucleic Acid Res.* **2001**, *29*, 4294–4309.

(3) Yoshimi, Y.; Watanabe, S.; Shinomiya, T.; Makino, A.; Toyoda, M.; Ikekita, M. Nucleobase adenine as a trophic factor acting on Purkinje cells. *Brain Res.* **2003**, *991*, 113–122.

(4) Helboe, T.; Hansen, S. H. Separation of nucleosides using capillary electrochromatography. *J. Chromatogr. A* **1999**, *836*, 315–324.

(5) Sigel, H. *Methods Involving metal ions and complexes in clinical chemistry: Metal ions in biological systems*; Marcel Dekker: New York, 1983; Vol. 16.

(6) Sigel, H. Interactions of metal ions with nucleotides and nucleic acids and their constituents. *Chem. Soc. Rev.* **1993**, *22*, 255–267.

(7) Casassas, E.; Tauler, R.; Marques, I. Interactions of H^+ and Cu(II) ions with poly(adenylic acid): study by factor analysis. *Macromolecules* **1994**, *27*, 1729–1737.

(8) Casassas, E.; Gargallo, R.; Izquierdo-Ridorsa, A.; Tauler, R. Application of multivariate curve resolution to voltammetric data. Part 1. Study of Zn(II) complexation with some polyelectrolytes. *React. Funct. Polym.* **1995**, *27*, 1–14.

(9) Barron, D.; Jimenez-Lozano, E.; Irles, A.; Barbosa, J. Influence of pH and pK_a values on electrophoretic behaviour of quinolones in aqueous and hydro-organic media. *J. Chromatogr. A* **2000**, *871*, 381–389.

(10) Reichardt, C. *Solvents and solvent effects in organic chemistry*, 3rd ed.; VCH: New York, 2004.

(11) Staszak, Z.; Bartecki, A. Influence of the bulk and donor-acceptor properties of solvent on ligand field spectra. *Spectrosc. Lett.* **1989**, *22*, 1193–1201.

(12) Gharib, F.; Jabbari, M.; Farajtabar, A.; Shamel, A. Solvent effects on protonation and complexation of glutamic and aspartic acids with molybdenum(VI) in different solutions of methanol. *J. Chem. Eng. Data* **2008**, *53*, 1772–1778.

(13) Gharib, F. Solvent effects on protonation and complexation of penicillamine and thallium(I) in different aqueous solutions of methanol. *J. Chem. Eng. Data* **2010**, *55*, 1547–1553.

(14) Farajtabar, A.; Gharib, F. Solvent effect on protonation constants of 5, 10, 15, 20-tetrakis(4-sulfonatophenyl)porphyrin in different aqueous solutions of methanol and ethanol. *J. Solution Chem.* **2010**, *39*, 231–244.

(15) Gharib, F.; Sadeghi, F. Solvent effects on complexation of thallium(I) with guanosine 5′-monophosphate in methanol-water mixtures. *Appl. Organomet. Chem.* **2007**, *21*, 218–225.

(16) Jabbari, M.; Gharib, F.; Mohammadpour Amini, M.; Azadmehr, A. Hydrolysis of dimethylphenyltin(IV) and triphenyltin(IV) chlorides in different aqueous ethanol solutions. *Can. J. Chem.* **2008**, *86*, 751–756.

(17) Pehrsson, L.; Ingman, F.; Johansson, A. Acid-base titrations by stepwise additions of equal volumes of titrant with special reference to automatic titrations. Theory, discussion of the Gran functions, the Hofstee method and equivalence volumes. *Talanta* **1976**, *23*, 769–780.

(18) Gameiro, P.; Reis, S.; Lima, J. L. F. C.; de Castro, B. Calibration of pH glass electrodes by direct strong acid/strong base titrations under dilute conditions. *Anal. Chim. Acta* **2000**, *405*, 167–172.

(19) Jaime Ferrer, J. S.; Couallier, E.; Rakib, M.; Durand, G. Electrochemical determination of acidity level and dissociation of formic acid/water mixtures as solvent. *Electrochim. Acta* **2007**, *52*, 5773–5780.

(20) Gharib, F.; Farajtabar, F.; Masteri Farahani, A.; Bahmani, F. Solvent effects on protonation constants of tryptophan in some aqueous aliphatic alcohol solutions. *J. Chem. Eng. Data* **2010**, *55*, 327–332.

(21) Beck, M. T.; Nagypal, I. *Chemistry of complex equilibria*; Ellis Harwood: New York, 1990.

(22) Leggett, D. J. *Computation methods for the determination of formation constants*; Plenum Press: New York, 1985.

(23) Meloun, M.; Javurek, M.; Havel, J. Multiparametric curve fitting. A structural classification of programs for analyzing multicomponent spectra and their use in equilibrium-model determination. *Talanta* **1986**, *33*, 513–524.

(24) Major, D. T.; Laxer, A.; Fischer, B. Protonation studies of modified adenine and adenine nucleotides by theoretical calculations and ^{15}N NMR. *J. Org. Chem.* **2002**, *67*, 790–802.

(25) Vojtylova, T.; Dospivova, D.; Triskova, O.; Pilarova, I.; Lubal, P.; Farkova, M.; Trnkova, L.; Taborsky, P. Spectroscopic study of protonation of oligonucleotides containing adenine and cytosine. *Chem. Pap.* **2009**, *63*, 731–737.

(26) Saenger, W. *Principles of nucleic acid structures*; Springer: New York, 1984.

(27) Barbosa, J.; Barron, D.; Beltran, J. L.; Buti, S. On the role of solvent in acid-base equilibria of diuretics in acetonitrile-water mixed solvents. *Talanta* **1998**, *45*, 817–827.

(28) Barbosa, J.; Toro, I.; Sanz-Nebot, V. Acid-base behaviour of tripeptides in solvents used in liquid chromatography. Correlation between pK values and solvatochromic parameters of acetonitrile-water mixtures. *Anal. Chim. Acta* **1997**, *347*, 295–304.

(29) Barbosa, J.; Toro, I.; Sanz-Nebot, V. Acid-base behaviour of tripeptides in solvents used in liquid chromatography. Correlation between pK values and solvatochromic parameters of acetonitrile-water mixtures. *Anal. Chim. Acta* **1997**, *347*, 295–304.

(30) Izutsu, K. *Electrochemistry in Nonaqueous Solutions*; Wiley-VCH: Weinheim, 2002.

(31) Maleki, N.; Haghighi, B.; Safavi, A. Evaluation of formation constants, molar absorptivities of metal complexes, and protonation constants of acids by nonlinear curve fitting using Microsoft Excel Solver. *Microchem. J.* **1999**, *62*, 229–236.

(32) Krygowski, T. M.; Wrona, P. K.; Zielkowska, U.; Reichardt, C. Empirical parameters of Lewis aidity and basicity for aqueous binary solvent mixtures. *Tetrahedron* **1985**, *41*, 4519–4527.

(33) Puranik, S. M.; Kumbharkhane, A. C.; Mehrotra, S. C. The static permittivity of binary mixtures using an improved bruggeman model. *J. Mol. Liq.* **1994**, *59*, 173–177.

(34) Taft, R. W.; Abboud, J. L. M.; Kamlet, M. J. Linear solvation energy relationships. 28. An analysis of Swain's solvent "acidity" and "basicity" scales. *J. Org. Chem.* **1984**, *49*, 2001–2005.

(35) Kamlet, M. J.; Abboud, J. L. M.; Abraham, M. H.; Taft, R. W. Linear solvation energy relationships. 23. A comprehensive collection of the solvatochromic parameters π^*, α, and β, and some methods for simplifying the generalized solvatochromic equation. *J. Org. Chem.* **1983**, *48*, 2877–2887.

(36) Blokzijl, W.; Engberts, B. F. N. Hydrophobic effects. Opinions and facts. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1545–1579.

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