



## PROTONATION CONSTANTS OF INOSINE 5'-MONOPHOSPHATE IN VARIOUS AQUEOUS SOLUTION OF ETHANOL

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### ABSTRACT

The protonation constants of Inosine 5'-monophosphate (K1 to K2) were determined in binary mixtures of water with ethanol containing (0, 10, 20, 30, 40, 50 and 60) %(v/v) using a combination of potentiometric and spectrophotometric methods at  $(25 \pm 0.1^\circ\text{C})$  and constant ionic strength (0.1 mol dm<sup>-3</sup> sodium perchlorate). The protonation constants were analyzed using the normalized polarity parameter (ETN) 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 logK versus  $\pi^*$  (dipolarity/polarizability) and  $\beta$  (hydrogen-bond donor acidity) as well as  $\pi^*$  and (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. As percent of Ethanol increases in solvent mixtures the pKa constants also increased. There is linear relationship between acidity constants and the mole fraction of ethanol in the solvent mixtures. Effect of solvent composition on acidity constants and pure spectrum of each component are also discussed.

**KEYWORDS** aqueous solution of ethanol, cation, dissociation constants, inosine 5'-monophosphate, spectroscopic study,

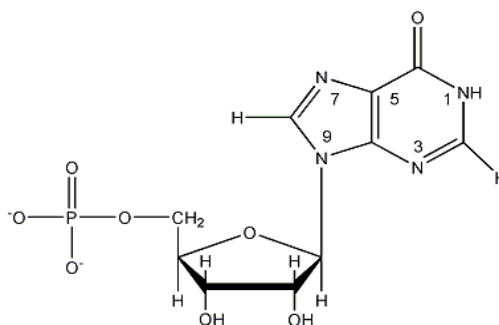
### INTRODUCTION

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. But in determination of acidity constants of these molecules we are faced with several drawbacks, such as low solubility in aqueous solutions and the low values of acidity constants. Therefore, in order to enhance the acidity constants on one hand and to increase the solubility on the other, we forced to choose mixed solvents. 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 (Shamel *et al.*, 2010).

Alcohol solutions have many applications in various fields of industry such as medicine, food and chemistry (Koohyar *et al.*, 2012). Also mixed solvents are interesting, because two solvents mixed together produce a solvent with quite different properties, both, physically (dielectric, density and viscosity) and chemically (acid-base and donor-acceptor properties). As far as the acid-base properties are concerned, an important feature is that the nature of the solvent is crucial for the strength of acids and bases. In particular, important is the proton affinity, in other words, the proton-donating and proton-accepting properties of solvent, as well as its polarity. In addition, the ionization degree of solute depends on the dielectric constant of solvent. Media of high dielectric constant are strongly ionizing, whereas those of low dielectric constants ionize to a lesser extent. By mixing solvents of different polarity in proper ratio, dielectric constant of the medium can be varied and, at the same time, the strength of dissolved acids and bases.

It should also be emphasized that solvent mixtures can be more convenient than individual solvents owing to enhanced solubility efficiency, increased sharpness of color change of indicators during titration and more manageable shape of acid-base titration curves (Niazi *et al.*, 2006). 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. Previously, the solvent effect on the protonation equilibrium was believed to be guided chiefly by electrostatic interactions (Born model). However, recent studies have revealed that the change in macroscopic properties such as the dielectric constant of the solvent cannot be the sole factor (Shamel *et al.*, 2010).



**Scheme 1. Chemical Structures of Inosine 5'-monophosphate (IMP)**

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. In continuation of previous work (Gharib *et al.*, 2008.; Gharib *et al.*, 2010.; Farajtabar and Gharib, 2010.; Gharib and Sadeghi, 2007.; Jabbari *et al.*, 2008; Gharib *et al.*, 2006) in this study the protonation constants of IMP have been determined in different aqueous ethanol mixtures to examine the dependence of acid-base equilibrium on solvent composition.

## MATERIALS AND METHODS

### Experimental section

#### Chemicals

Sodium salt of Inosin monophosphate was obtained from Fluka, as analytical reagent grade material and used without further purification. Ethanol was from Merck (reagent grade) and was used as received. Sodium perchlorate was from Merck and was dried under vacuum at room temperature for at least 48 h before use. NaOH solution was prepared from a titrisol solution (Merck) and its concentration was determined by several titrations with standard HCl solution. The aqueous stock solutions of inosin monophosphate were freshly prepared daily, and their concentrations were determined each time by titration with NaOH solution. Perchloric acid (Merck) was used as supplied. All dilute solutions were prepared from double-distilled water with conductance equal to  $(1.3 \pm 0.1) \mu\text{S}$ .

#### Apparatus

The electromotive force was measured using a Metrohm model 781 pH ion-meter. A combined glass-pH electrode (model 6.0258.000) was modified by replacing its aqueous KCl solution with  $0.01 \text{ mol dm}^{-3} \text{ NaCl} + 0.09 \text{ mol dm}^{-3} \text{ NaClO}_4$  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 ( $-\log [\text{H}^+]$ ) 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  $(25 \pm 0.1^\circ\text{C})$  and a constant ionic strength of  $0.1 \text{ mol dm}^{-3}$  sodium perchlorate. The protonation constants were evaluated from the measurements of absorbance versus emf by titration of 25 mL Sodium salt of Inosin monophosphate  $[(1.0 \text{ to } 3.0) \times 10^{-3} \text{ mol dm}^{-3}]$  with  $0.1 \text{ mol dm}^{-3}$  sodium hydroxide solution with both the same ionic strength and mole fraction of organic solvent [(0 to 60) % methanol v/v].

In the first step, the electrode system calibration was performed by Gran's method (Pehrsson *et al.*, 1976). 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 ( $0.1 \text{ mol dm}^{-3} \text{ NaOH}$ ). The potential was allowed to stabilize after each addition of the titrant, and the recorded emf values were then used to obtain  $E^\circ$ . The procedure was continued to  $\text{pH} = 2$  (lower than the  $\text{pK}$  of base). In the second step, 25 mL of an acidic solution ( $0.01 \text{ mol dm}^{-3} \text{ HClO}_4$ ) of Sodium salt of Inosin monophosphate  $[(1.0 \text{ to } 3.0) \times 10^{-3} \text{ mol dm}^{-3}]$  at the same conditions of temperature, ionic strength and solvent composition was titrated with a sodium hydroxide solution ( $0.1 \text{ mol dm}^{-3}$ ). 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 (Gameiro *et al.*, 2000). 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 following equation:

$$E_{cell} \text{ (mV)} = E_{cell}^{\circ} + k \log [H^+] + k \log \gamma_{H^+} + E_{LJ} \quad (1)$$

In this equation  $E_{cell}^{\circ}$  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  $\gamma_{H^+}$  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:

$$E_{cell} = E'_a + k \log [H^+] \quad (2)$$

In this equation  $E'_a$  is  $E_{cell}^{\circ} + k \log \gamma_{H^+} + E_{LJ}$ . 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  $\log [H^+]$  (Jaime Ferrer *et al.*, 2007.; Garib *et al.*, 2010). In the acidic region, the hydrogen ion concentration can be expressed as following equation:

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

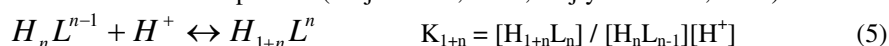
Where  $M_{HClO_4}$  and  $M_{NaOH}$  are the molarities of perchloric acid and sodium hydroxide, and  $V_0$  and  $V_1$  are the initial volume of perchloric acid and the added volume of sodium hydroxide solution, respectively. Finally:

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

## RESULTS AND DISCUSSION

The protonation constants of 5'-IMP have been determined spectrophotometrically based on the relation  $A = f$  (pH) (Beck and Nagypal, 1990). The measured absorbance,  $A$ , [(250 to 310) nm in the interval of 0.5 nm] and  $p_cH$  from the spectrophotometric titration were conducted using the computer program which employs a least-squares method (Leggett, 1985.; Meloun *et al.*, 1986.; Monajjemi *et al.*, 2003). The data in the computer program were fitted to eq 5 by minimizing the error square sum of the difference 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 inosine 5'-monophosphate in different aqueous solutions of ethanol, eq 5, are listed in Table 1 together with the values reported in the literature for comparison (Major *et al.*, 2002; Vojtylova *et al.*, 2009).



Where  $HL$  represents inosine 5'-monophosphate and  $n$  may be 0, 1 or 2 for the different protonation equilibria of the base. In Figure 1, the species mole fractions of the 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. The inosine 5'-monophosphate ( $IMP^{2-}$ ) shown in Scheme 1 may bind with two protons at the phosphate group and one at the purine moiety, (N-7). It was proposed that  $H_3(IMP)^+$  releases its first proton from  $P(O)(OH)_2$ , the second one from  $H^+$  (N-7) and the third one again from the phosphate group. A fourth proton is released in the alkaline pH range from the neutral H (N-1) site (Da Costa *et al.*, 2000.; Gharib *et al.*, 2008). The assignments agree well with the previous conclusion.

### Solvent Effect

The protonation constants of inosine 5'-monophosphate in water-ethanol mixed solvents have same behaviors.  $\log K_1$  and  $\log K_2$  base increases with increasing proportion of organic solvents in the mixtures (Table- 1).

**Table 1. Average values of the protonation constants of IMP at 25 °C and constant ionic strength (0.1 mol dm<sup>-3</sup> NaClO<sub>4</sub>) and different aqueous ethanol mixtures**

Ethanol % (v/v)	$\log K_1$	Log $K_2$	Ref	Ethanol % (v/v)	$\log K_1$	Log $K_2$	Ref
0	8.99 ± 0.05	5.91 ± 0.05	This work	50	9.85 ± 0.05	7.35 ± 0.05	This work
10	9.13 ± 0.05	6.11 ± 0.05	This work	60	10.04 ± 0.05	7.73 ± 0.05	This work
20	9.23 ± 0.05	6.42 ± 0.05	This work	0	9.02	6.22	20
30	9.39 ± 0.05	6.85 ± 0.05	This work	0	8.88	6.04	20
40	9.57 ± 0.05	7.01 ± 0.05	This work	0	8.99 ± 0.05	5.90 ± 0.06	20

<sup>a</sup> The values reported in the literature for pure water are also listed for comparison.

**Table 2. Solvatochromic parameters and the dielectric constants of different aqueous ethanol mixtures at 25 °C**

Ethanol % (v/v)	$\alpha$	$\beta$	$\pi^*$	$E_T^N$	$\epsilon$	Ethanol % (v/v)	$\alpha$	$\beta$	$\pi^*$	$E_T^N$	$\epsilon$
0	1.23	0.49	1.14	1.00	79.5	30	0.95	0.61	1.09	0.84	55.0
10	1.13	0.52	1.14	0.95	73.7	35	0.91	0.63	1.06	0.82	47.5
15	1.08	0.55	1.13	0.92	70.2	40	0.89	0.66	1.04	0.80	41.1
20	1.03	0.57	1.12	0.90	66.0	45	0.87	0.68	1.00	0.78	35.4
25	0.98	0.59	1.11	0.87	61.6						

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 (Barbosa *et al.*, 1998). 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,  $\epsilon$ , should be linear according to this equation:

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

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  $L^- + H^+ \leftrightarrow HL$  and  $n = 0$  for the charge type  $HL + H^+ \leftrightarrow H_2L^+$ . Therefore, a change in polarity of the medium has a major role in  $\log K_1$  of inosine 5'-monophosphate, and in this case, the correlation between  $\log K_2$  with the reciprocal of the dielectric constant of the aqueous alcohol mixtures is linear (Figure 2).

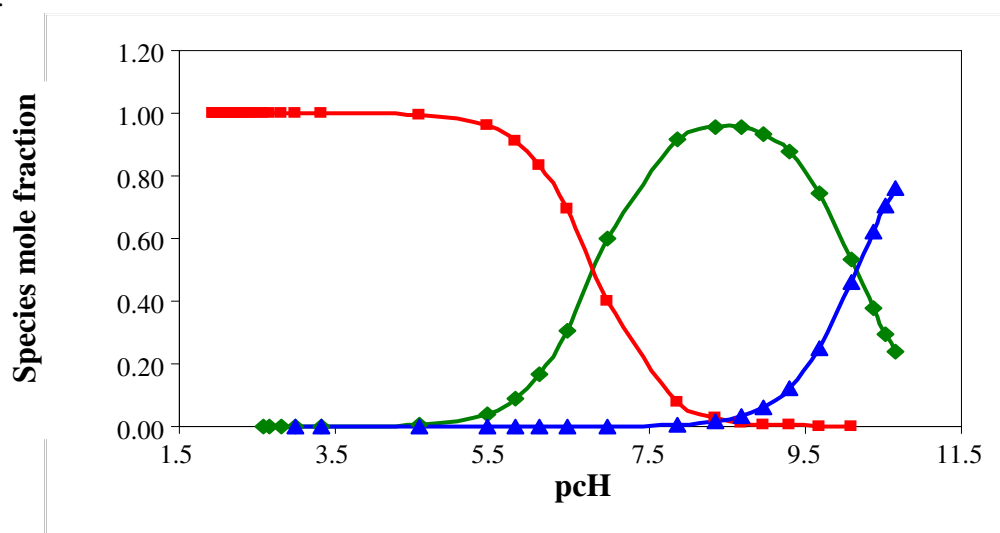


Figure 1. Distribution diagrams of the different species of IMP ( $\blacksquare$ :  $H_2L^+$ ;  $\blacklozenge$ :  $HL$ ;  $\blacktriangle$ :  $L^-$ ), in water at 25 °C and ionic strength of  $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$ .

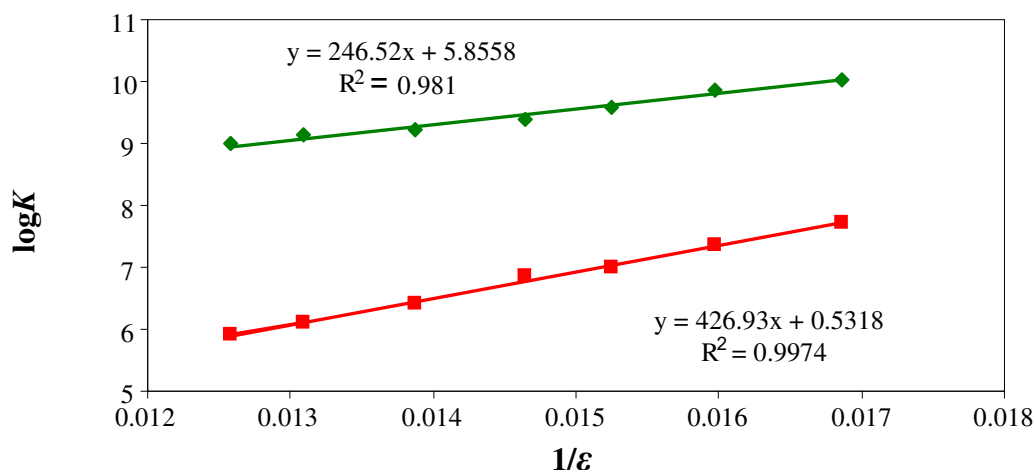


Figure 2. Plots of the experimental values of: ( $\blacklozenge$ :  $\log K_1$ ;  $\blacksquare$ :  $\log K_2$ ) of 5'-IMP versus the reciprocal of dielectric constant of the different mixed solvents at 25 °C and an ionic strength of  $0.1 \text{ mol dm}^{-3} \text{ NaClO}_4$ .

However, the linearity of the correlation between  $\log K_1$  of inosine 5'-monophosphate with the reciprocal of the dielectric constant of ethanol-water is poor (with correlation coefficients between 1 and 0.98, respectively) (Figure 2). 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. Therefore, it is necessary to elucidate the nature of solute-solvent interactions for a better understanding of solvent effects. However, it is now understood that the anion and cation species are selectively solvated by acidic or basic solvents, respectively (Barbosa *et al.*, 1997). 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 (Izutsu, 2002). 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 5'-IMP increase, when the organic solvent percentage increases in the mixtures.

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. Among these scales (more than 40), the most comprehensive are the solvato-chromic 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 Reichardt,  $E_T$ , based on the solvato-chromic behavior of pyridinium N-phenoxide betaine dye. This dye is the most solvato-chromic compound reported to date (Reichardt, 2004). 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 tetra methyl silane, 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 (Maleki *et al.*, 1999). A very good linear correlation of all  $\log K$  versus  $E_T^N$  was obtained in different aqueous solutions of ethanol and ethanol

$$\log K_1 (\text{IMP, ethanol}) = 6.70(\pm 0.13) + 3.16(\pm 0.14)E_T^N \quad (7a)$$

$N = 9$ ,  $\text{rss} = 2.64 \times 10^{-3}$ ,  $\text{ose} = 0.02$ ,  $r^2 = 0.99$ ,  $f = 167.79$

$$\log K_2 (\text{IMP, ethanol}) = 11.24(\pm 0.17) - 7.16(\pm 0.18)E_T^N \quad (7b)$$

$N = 9$ ,  $\text{rss} = 4.32 \times 10^{-3}$ ,  $\text{ose} = 0.02$ ,  $r^2 = 1.00$ ,  $f = 1516.99$

In these equations  $N$ ,  $\text{rss}$ ,  $\text{ose}$ ,  $r^2$ , 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 (Krygowskiet *et al.*, 1985), and the dielectric constant values were obtained from the literature (Puranik *et al.*, 1994) 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  $\log K$  versus the KAT parameters (Taft *et al.*, 1984.; Kamlet *et al.*, 1983) (Kamlet, Abboud, Taft) was obtained from following equation:

$$\log K_1 (\text{IMP, ethanol}) = 9.20(\pm 0.82) - 2.11(\pm 0.49) + 1.45(\pm 0.51)\pi^* \quad (8a)$$

$N = 9$ ,  $\text{rss} = 6.02 \times 10^{-4}$ ,  $\text{ose} = 1.00 \times 10^{-2}$ ,  $r^2 = 1.00$ ,  $f = 902.04$

$$\log K_2 (\text{IMP, ethanol}) = 9.53(\pm 0.63) - 5.59(\pm 0.74)\alpha - 1.29(\pm 0.31)\pi^* \quad (8b)$$

$N = 9$ ,  $\text{rss} = 4.33 \times 10^{-3}$ ,  $\text{ose} = 2.69 \times 10^{-2}$ ,  $r^2 = 1.00$ ,  $f = 707.95$

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 (Blokzijl and Engberts, 1993). Using the solvato-chromic solvent parameters,  $\beta$ , and  $\pi^*$ , which have been introduced in previous reports (Gharib *et al.*, 2008; Gharib *et al.*, 2010; Farajtabar and Gharib, 2010.; Gharib and Sadeghi, 2007; Jabbari *et al.*, 2008), the multiparametric equation, eq 9, has been proposed for use in the so-called linear solvation energy relationship.

$$\text{Log}K = A_0 + a\alpha + b\beta + p\pi^* \quad (9)$$



Where  $A_0$  represents the regression value, and  $\pi^*$  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  $\beta$  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 coefficient is a measure of 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 multi parameters 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,  $\text{ose}$ , of  $\log K$ . The KAT parameters have been taken from our previous report (Gharib *et al.*, 2010).

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  $\pi^*$  did not give good results in all cases,  $r^2 = 0.95$  to  $0.97$ . However, the correlation analysis of  $\log K$  values with dual-parameter equations indicates significant improvement with regard to the single- or multi parameter models which is also consistent with the results obtained with normalized polarity parameter,  $E_T^N$ , regression analysis.

The coefficient of  $\beta$  in the correlation analysis of the dual parameter of the KAT equation in the case of  $\log K_1$  of IMP is negative and has a major role (more than 60 % in aqueous solutions of ethanol) for both systems. So,  $\log K_1$  values decrease with an increase in the hydrogen bond basicity parameter. Also, the positive sign of  $\pi^*$  in this case indicates that a decrease in the polarity of the mixed solvents decreases the  $\log K_1$  values. However, the coefficients of  $\beta$  and  $\pi^*$  in correlation analysis of  $\log K_2$  of both bases are negative. The negative sign of  $\pi^*$  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  $\beta$  with a major role in dual-parameter correlation analysis of about (81 and 87) % in water-ethanol as well as (68 and 64) % in water-methanol mixtures for IMP, 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.

## CONCLUSION

The protonation constants of Inosine 5'-monophosphate ( $K_1$  to  $K_2$ ) were determined in binary mixtures of water with ethanol containing (0, 10, 20, 30, 40, 50 and 60) % (v/v) using a combination of potentiometric and spectrophotometric methods at  $(25 \pm 0.1^\circ\text{C})$  and constant ionic strength ( $0.1 \text{ mol dm}^{-3}$  sodium perchlorate). Normalized polarity parameter ( $E_T^N$ ) and Kamlet, Abboud, and Taft (KAT) parameters were used for analyze of protonation constants and a very good linear correlation of  $\log K$  versus the normalized polarity parameter was obtained.

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