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Apparent Second-Stage Dissociation Constants of Some Zwitterionic Buffers for Biochemical and Physiological Research in Various Hydroorganic Media

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The apparent second-stage dissociation constants of 3-(*N*-morpholino)propanesulfonic acid, 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid, and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulfonic acid) were determined at (25.0 ± 0.1) °C and $I = 0.1$ mol dm^{-3} (KNO_3) by potentiometric pH titration in pure water and different hydroorganic solvent media. The organic solvents used were methanol, ethanol as amphiprotic hydrogen bond acceptor−donor (HBA-D) solvents, *N*,*N*-dimethylformamide, dimethyl sulfoxide, acetone, dioxane as hydrogen bond acceptor solvents, and acetonitrile as (HBA-HBD) solvent. The ESAB2M computer program was used to refine the initial estimates of the apparent second-stage dissociation constants of the three zwitterionic buffers studied. pK_{a2}* values change with increasing organic content of the solvent mixture. The results obtained are discussed in terms of average macroscopic properties of the mixed solvents. The implications of the results with regard to specific solute−solvent interactions, particularly stabilization of zwitterionic species, are discussed. The effects of coorganic solvents on the acid dissociation equilibria have been interpreted using solvatochromic quantitative values of Kamlet−Taft hydrogen bond acidity and basicity (α, β) and dipolarity−polarizability π^* of the solvent.

Introduction

Attention has been drawn by Good and co-workers (Good et al., 1966; Ferguson et al., 1980) to the use of zwitterionic amino acids and zwitterionic N-substituted amino sulfonic acids as buffers of biochemical interest. These compounds are all ampholytes (with zwitterionic structures) and are useful buffers compatible with most media of physiological interest. Potentially useful zwitterionic buffers for use in biochemistry now include 3-(*N*-morpholino)propanesulfonic acid (MOPS), 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO), and *N*-(2-hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulfonic acid) (HEPPSO) because of their low toxicity. Zwitterionic N-substituted amino propanesulfonic acids exhibit improved properties in terms of the following criteria: high solubility in water, stability in solution, minimum salt effects, and insignificant penetration through biological membranes. Though studies on the dissociation constants of acids and bases in various hydroorganic media have been extensive, relatively little work has been done to determine the second-stage dissociation constants of zwitterionic buffers (Roy et al., 1984; Azab et al., 1994). Through our extended studies of the apparent second-stage dissociation of zwitterionic compounds in various hydroorganic media, we have now determined the pK_{a2} of MOPS, AMPSO, and HEPPSO by potentiometric pH titration in pure water and various water + ethanol, water + methanol, water + dimethylformamide, water + dimethyl sulfoxide, water + acetonitrile, water + acetone, and water + dioxane mixtures containing different mass fractions of the organic solvent ranging between 0 and 0.55. Methanol and ethanol were chosen as representatives of amphiprotic hydrogen bond acceptor−donor (HBA-D) solvents. We have chosen DMF, DMSO, acetone, and dioxane as hydrogen bond acceptor (HBA) solvents and acetonitrile as (HBA-HBD) solvent. All the solvents chosen are of frequent use in biochemical and biological studies. The dependence of the apparent dissociation constant values on the composition of the solvent mixtures has been investigated in order to examine solvent−solute interactions. By virtue of the presence of hydroxy groups, AMPSO and HEPPSO have high hydrogen-bonding capability. Thus, the solvent effects on the ionization of AMPSO and HEPPSO in various water + organic solvent mixtures are of particular interest, especially with reference of specific solute−solvent interactions. Quantitative interpretation of the solvent effects on the acid dissociation equilibria of the different zwitterionic buffer ligands have been made using the solvatochromic parameters values of Kamlet−Taft α, β, and π^*(Kamlet et al., 1983).

Experimental Section

Chemicals. Reagent-grade 3-(*N*-morpholino)propanesulfonic acid (MOPS), 3-[(1,1-dimethyl-2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (AMPSO), and (*N*-(2-hydroxyethyl)piperazine-*N*′-(2-hydroxypropanesulfonic acid) (HEPPSO) were from Sigma Chemical Co., St. Louis, MO. We determined by potentiometric pH titration the molecular weight of MOPS, AMPSO, and HEPPSO to verify/determine the purity, especially for acidic/basic contaminants. The purity averaged 99.5% for the three compounds, with a standard deviation of 0.05%. Methanol, ethanol, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetonitrile, acetone, and dioxane were from Merck AG, Darmstadt, Germany. A CO_2-free solution of potassium hydroxide (Merck AG) was prepared and standardized against multiple samples of primary-standard potassium hydrogen phthalate (Merck AG) under CO_2-free conditions. KNO_3 was from Merck AG, Darmstadt, Germany. The exact concentrations of the stock solutions of the ligand (buffers) was determined by titration. Hydro-

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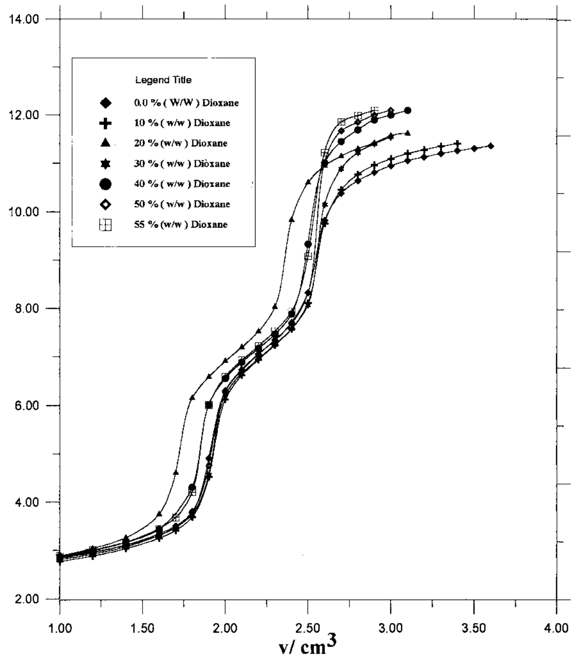


Figure 1. pH* against volume of 0.0806 mol dm^{-3} KOH for MOPS in dioxane + water mixtures at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3.

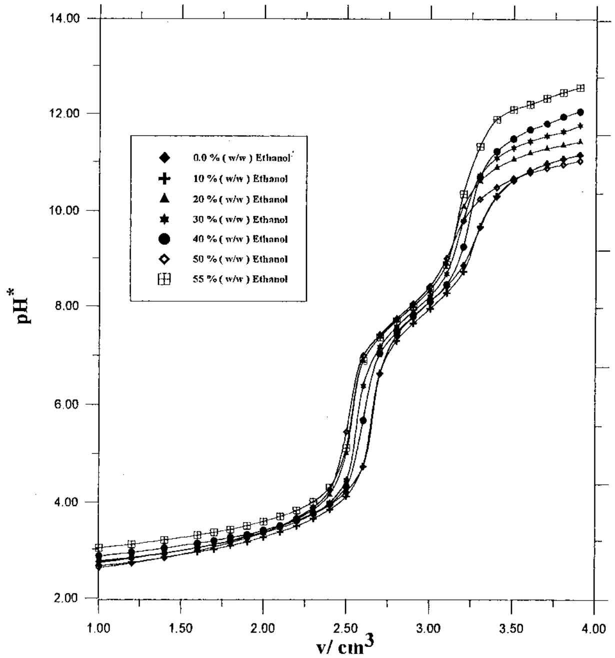


Figure 3. pH* against volume of 0.0806 mol dm^{-3} KOH for HEPPSO in ethanol + water mixtures at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3.

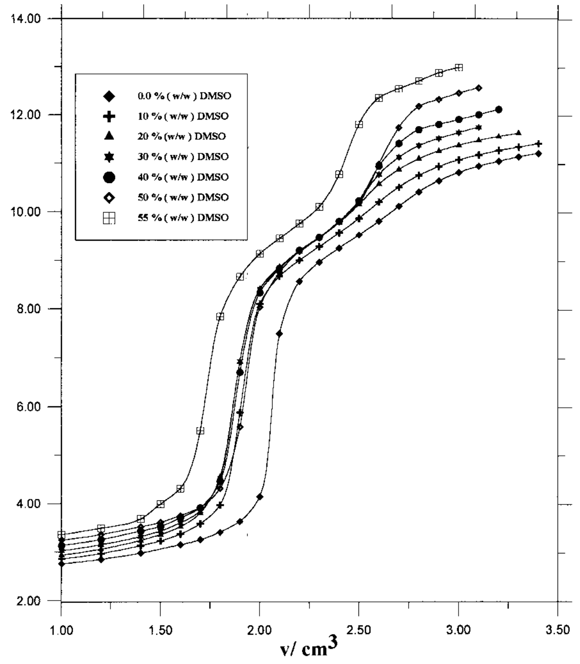


Figure 2. pH* against volume of 0.0806 mol dm^{-3} KOH for AMPSO in DMSO + water mixtures at 25 °C and $I = 0.1$ mol dm^{-3} KNO_3.

organic solvent mixtures containing different mass fractions of the organic solvents were prepared by mass.

Procedure. Potentiometric pH measurements were made on solutions in a double-walled glass vessel at (25.0 ± 0.1) °C with a commercial Fisher combination electrode (catalog no. 13-639-104) contaning calomel referance electrode. A Fisher Accumet pH/ion meter model 825 MP was used. The instrument was calibrated against standard aqueous buffers of pH 4.00 (phthalate buffer) and 9.20 (borate buffer). This calibration was required for the calculation of the dissociation constants in pure water only. The instrument was rechecked after each experiment. The electrode system was calibrated in aqueous medium in terms of hydrogen ion concentration instead of activities. Provided the ionic strength of the test solution remains constant, the free hydrogen ion activity can be expressed in terms of concentration (May et al., 1985). Thus, all the constants determined in pure water in this work are concentration constants. In the measurements in hydroorganic solvents calibration of the electrode system was done in the working medium by the MAGEC program (May et al., 1985) using the data for titration of nitric acid with potassium hydroxide, both of known concentration, under the same temperature and medium condition, $I = 0.1$ mol dm^{-3} (KNO_3). During the MAGEC calculation the calibration parameters (standard potential of the cell and value of ionic product of the medium) were used to test the Nernstian response of the potentiometric cell. The temperature was controlled by a Fisher Scientific Isotemp Refrigerated Circular model 9000 water thermostat, and it was maintained within ±0.1 °C. Purified nitrogen was bubbled through the solution to maintain an inert atmosphere. Efficient stirring of the solution was achieved with a magnetic stirrer. All test solutions (1×10^{-3} mol dm^{-3} zwitterionic buffer ligand + 4×10^{-3} mol dm^{-3} HNO_3) were prepared in a constant ionic medium, 0.1 mol dm^{-3} KNO_3, by mixing the appropriate masses of ligand, nitric acid, potassium nitrate, and the different organic solvents studied. At least four titrations were performed in each mixture. The concentration of free hydrogen ion, C_{H^+}, at each point of the titration in hydroorganic media was calculated from the measured emf, E, of the cell RE/TS/GE (RE and GE denote the reference and glass electrodes, respectively, and TS is the test solution) using the Nernst equation

$$E = E° + Q \log C_{H^+} \qquad (1)$$

where $E°$ is a constant that includes the standard potential of the glass electrode.

It is to be assumed that the activity coefficient is constant, an assumption usually justified by performing the experiments with a medium of high ionic strength (0.1

mol dm^{-3} KNO_3). Values for K_W for water in water + organic solvent systems were taken from the literature (Bates, 1964; Woolley et al., 1970, 1972; Gutbezahl et al., 1953; Harned et al. 1939). The protonation constants were then determined by use of the Bjerrum function (Bjerrum, 1921)

$$\bar{n} = (H_T - h + K_W/h)/A_T = (\beta_1 h + 2\beta_2 h^2)/(1 + \beta_1 h + \beta_2 h^2) \quad (2)$$

where $\bar{n}$ is calculated from the experimental quantities, h, the total concentration of titratable hydrogen ion H_T, and the total reagent concentration A_T. The pK_{a2} values were determined from the overall protonation constants calculated by the linearization method of Irving and Rossotti (Irving et al., 1953). Initial estimates of pK_{a2} values were refined with the ESAB2M computer program (Stefano et al., 1987) by minimizing the error squares sum

$$U_V = \Sigma_i W_i (V_i - V_{calcd,i})^2 \quad (3)$$

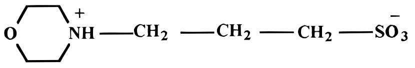
where V_i and $V_{calcd,i}$ are experimental and calculated values of the titrant volume for every point i of the titration curve. The weight is calculated by

$$1/W_i = S_i^2 = S_V^2 + (\delta V_i/\delta E_i)^2 \cdot S_E^2 \quad (4)$$

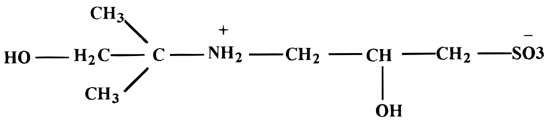
Here S_i is the estimated overall variance and S_V and S_E are estimates of standard deviation in titrant volume and potential, respectively. Titrant volume, $V_{calcd,i}$, can be calculated from an explicit equation (Arena et al., 1979; Rigano et al., 1984). Our calculation was performed with a Gaussian error in V of $S_V = 0.005$.

Results and Discussion

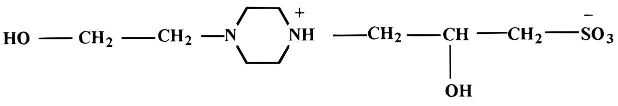
MOPS, AMPSO, and HEPPSO, possess the following zwitterionic structures



(MOPS)



(AMPSO)



(HEPPSO)

The second dissociation constant step involves the deprotonation of the substituted methylammonium of AMPSO and the deprotonation of the cationic group $-NH^+$ of MOPS and HEPPSO. If the above zwitterions are designated $Z^{\pm}$, the second dissociation step can be represented by

$$Z^{\pm} \rightleftharpoons H^+ + Z^-$$

Representative titration curves from which the initial estimates of the apparent dissociation constants have been calculated are shown in Figures1−3. The refined pK_{a2}* values in different solvent mixtures are given in Tables 1−3. The values obtained in the present work for the apparent dissociation constant values pK_{a2} of the zwitterionic buffers studied in pure water agree with literature data (Perrin, 1979; Ferguson et al., 1980).

Table 1. Refined pK_{a2}*[a] Values of MOPS at Different Mass Fractions w of Organic Solvent + (1 − w) Water at (25.0 ± 0.1) °C and I = 0.1 mol dm^{-3} KNO_3

organic solvent	w	pK_{a2}*
methanol	0.00	7.18 ± 0.04
	0.10	7.13 ± 0.02
	0.20	7.08 ± 0.02
	0.30	7.03 ± 0.03
	0.40	7.97 ± 0.04
	0.50	6.77 ± 0.04
	0.55	6.75 ± 0.03
ethanol	0.00	7.18 ± 0.04
	0.10	7.13 ± 0.03
	0.20	7.01 ± 0.02
	0.30	6.96 ± 0.03
	0.40	6.63 ± 0.03
	0.50	6.51 ± 0.03
	0.55	6.39 ± 0.02
DMF	0.00	7.18 ± 0.04
	0.10	7.01 ± 0.02
	0.20	7.00 ± 0.03
	0.30	6.80 ± 0.02
	0.40	6.91 ± 0.02
	0.50	6.86 ± 0.02
	0.55	7.09 ± 0.02
DMSO	0.00	7.18 ± 0.04
	0.10	7.09 ± 0.01
	0.20	7.03 ± 0.02
	0.30	6.92 ± 0.04
	0.40	6.81 ± 0.03
	0.50	6.64 ± 0.02
	0.55	6.55 ± 0.02
acetonitrile	0.00	7.18 ± 0.04
	0.10	7.11 ± 0.02
	0.20	7.22 ± 0.02
	0.30	7.59 ± 0.03
	0.40	7.91 ± 0.02
	0.50	8.01 ± 0.04
	0.55	7.98 ± 0.02
acetone	0.00	7.18 ± 0.04
	0.10	7.21 ± 0.03
	0.20	7.17 ± 0.01
	0.30	7.13 ± 0.02
	0.40	7.15 ± 0.04
	0.50	7.16 ± 0.04
	0.55	7.19 ± 0.02
dioxane	0.00	7.18 ± 0.04
	0.10	7.16 ± 0.01
	0.20	7.18 ± 0.02
	0.30	7.22 ± 0.01
	0.40	7.29 ± 0.02
	0.50	7.37 ± 0.02
	0.55	7.45 ± 0.03

[a] pK_{a2}* = Corrected pK_{a2} values ± uncertainties at the 95% confidence level.

According to Bates (Bates, 1964), solvent effects on the dissociation equilibria of the zwitterionic buffers under investigation can be expressed by eq 5

$$pK_a^* - pK_a = (121.6n/\bar{r})\,(1/\epsilon_2 - 0.0128) \quad (5)$$

where $\bar{r}$ is the common radius of all the ions, ϵ_2 is the relative permittivity of the mixed organic solvent, $n = 2$ for HA, A pairs of the charge type A^0B^-, $n = 4$ for the charge type A^-B^{2-}, $n = 0$ for the charge type A^+B^0, and 0.0128 is the reciprocal of the permittivity of water at 25 °C. The apparent dissociation constants for some dicarboxylic acids in different hydroorganic media calculated by

Table 2. Refined pK_{a2}^{*} [a] Values of AMPSO at Different Mass Fractions w of Organic Solvent + (1 − w) Water at (25.0 ± 0.1) °C and $I = 0.1$ mol dm^{-3} KNO_3

organic solvent	w	pK_{a2}^{*}
methanol	0.00	9.11 ± 0.03
	0.10	9.10 ± 0.02
	0.20	9.22 ± 0.03
	0.30	9.17 ± 0.03
	0.40	9.10 ± 0.04
	0.50	9.04 ± 0.03
	0.55	8.95 ± 0.03
ethanol	0.00	9.11 ± 0.03
	0.10	9.07 ± 0.01
	0.20	9.02 ± 0.02
	0.30	8.93 ± 0.03
	0.40	8.76 ± 0.02
	0.50	8.66 ± 0.04
	0.55	8.63 ± 0.04
DMF	0.00	9.11 ± 0.03
	0.10	9.07 ± 0.03
	0.20	8.89 ± 0.03
	0.30	8.82 ± 0.03
	0.40	8.61 ± 0.04
	0.50	8.51 ± 0.02
	0.55	8.32 ± 0.02
DMSO	0.00	9.11 ± 0.03
	0.10	9.00 ± 0.02
	0.20	9.07 ± 0.03
	0.30	8.88 ± 0.03
	0.40	8.84 ± 0.05
	0.50	8.73 ± 0.03
	0.55	8.72 ± 0.02
acetonitrile	0.00	9.11 ± 0.03
	0.10	9.06 ± 0.04
	0.20	9.31 ± 0.02
	0.30	9.42 ± 0.02
	0.40	9.56 ± 0.03
	0.50	9.82 ± 0.03
	0.55	10.22 ± 0.01
acetone	0.00	9.11 ± 0.03
	0.10	9.12 ± 0.01
	0.20	9.14 ± 0.03
	0.30	9.13 ± 0.03
	0.40	9.09 ± 0.04
	0.50	9.22 ± 0.03
	0.55	9.13 ± 0.03
dioxane	0.00	9.11 ± 0.03
	0.10	9.22 ± 0.01
	0.20	9.11 ± 0.02
	0.30	9.19 ± 0.03
	0.40	9.27 ± 0.03
	0.50	9.33 ± 0.03
	0.55	9.40 ± 0.03

[a] pK_{a2}^{*} = Corrected pK_{a2} values ± uncertainties at the 95% confidence level.

Table 3. Refined pK_{a2}^{*} [a] Values of HEPPSO at Different Mass Fractions w of Organic Solvent + (1 − w) Water at (25.0 ± 0.1) °C and $I = 0.1$ mol dm^{-3} KNO_3

organic solvent	w	pK_{a2}^{*}
methanol	0.00	7.79 ± 0.04
	0.10	7.90 ± 0.04
	0.20	7.78 ± 0.04
	0.30	7.73 ± 0.02
	0.40	7.71 ± 0.03
	0.50	7.61 ± 0.04
	0.55	7.57 ± 0.03
ethanol	0.00	7.79 ± 0.04
	0.10	7.78 ± 0.03
	0.20	7.72 ± 0.03
	0.30	7.57 ± 0.03
	0.40	7.49 ± 0.04
	0.50	7.54 ± 0.04
	0.55	7.39 ± 0.03
DMF	0.00	7.79 ± 0.04
	0.10	7.77 ± 0.03
	0.20	7.70 ± 0.04
	0.30	7.58 ± 0.03
	0.40	7.42 ± 0.02
	0.50	7.51 ± 0.03
	0.55	7.53 ± 0.03
DMSO	0.00	7.79 ± 0.04
	0.10	7.70 ± 0.03
	0.20	7.64 ± 0.02
	0.30	7.54 ± 0.02
	0.40	7.38 ± 0.02
	0.50	7.37 ± 0.02
	0.55	7.36 ± 0.03
acetonitrile	0.00	7.79 ± 0.04
	0.10	7.87 ± 0.03
	0.20	8.06 ± 0.03
	0.30	8.11 ± 0.03
	0.40	8.21 ± 0.02
	0.50	8.41 ± 0.03
	0.55	8.64 ± 0.03
acetone	0.00	7.79 ± 0.04
	0.10	7.96 ± 0.03
	0.20	7.94 ± 0.03
	0.30	7.88 ± 0.03
	0.40	7.84 ± 0.04
	0.50	7.89 ± 0.03
	0.55	7.94 ± 0.03
dioxane	0.00	7.79 ± 0.04
	0.10	7.81 ± 0.04
	0.20	7.94 ± 0.04
	0.30	7.89 ± 0.04
	0.40	8.04 ± 0.04
	0.50	8.08 ± 0.04
	0.55	8.10 ± 0.04

[a] pK_{a2}^{*} = Corrected pK_{a2} values ± uncertainties at the 95% confidence level.

Azab (Azab et al., 1997) increase markedly as the permittivity decreases. The pK_{a2} for zwitterionic buffers usually increases or decreases somewhat with increasing the organic solvent concentration. The effects of coorganic solvents on the acid dissociation equilibria can be interpreted using the solvatochromic quantitative values of Kamlet−Taft hydrogen bond acidity and basicity (α, β) and dipolarity−polarizability π^{*} of the solvent (Kamlet et al., 1983).

The results presented in Tables 1−3, with respect to the amphiprotic hydrogen bond acceptor−donor (HBA-D) ethanol solvent ($\pi^{*} = 0.54$, $\beta = 0.77$, and $\alpha = 0.83$), can conveniently be discussed in terms of $\Delta G_{(\mathrm{protonation})}$ defined as $2.303RT$ ($\log K^{(\mathrm{w})} - \log K^{(\mathrm{s})}$) i.e., the difference between the standard free energies of protonation in the mixed solvent and in water (Gordon, 1975). Protonation of the solvent (SH) by $Z^{\pm}$ can be represented by the general equation:

$$Z^{\pm} + SH \rightleftharpoons SH_2^{\ +} + Z^{-}$$

Since the solutes involved in the ionization are charged, only charge transfer will be dominant (Gordon, 1975). Therefore, although the difference in the parameter π^{*} between pure water and water + ethanol mixture is appreciable, this will have little effect on the protonation constants of the solutes. Also, solvation in mixed ethanol + water should not differ much from that in water, since the structures of the two solvents are similar. This behavior can be quantitatively attributed to the small difference in the solvatochromic parameter α between pure water and ethanol solvents. The α scale of solvent HBD acidities describes the ability of the solvent to donate a proton in a solvent-to-solute hydrogen bond. Consequently, with an increase in ethanol concentration in the aqueous medium, only a small change in $\Delta G_{(\mathrm{protonation})}$ will occur. The observed slight changes in $\mathrm{p}K_{a2}$* with methanol content can be attributed to the two following factors.

(a) The relatively high stabilization of the conjugate bases by donor hydrogen bonds in a pure aqueous medium relative to that in the presence of methanol.

(b) The greater stabilization of the proton in methanol + water mixtures relative to that in pure water through ion−solvent interaction (Bennetto et al., 1966; Tomkins, 1966).

In the presence of increasing concentrations of hydrogen bond acceptor solvents (HBA), dimethylformamide, and dimethyl sulfoxide with high values of the solvatochromic parameter β ($\beta = 0.69$ for DMF and 0.76 for DMSO), deprotonation is facilitated because the solvent mixture solvates the proton more than the zwitterion. The β scale of HBA basicities provides a measure of the solvent's ability to accept a proton (donate an electron pair) in a solute-to-solvent hydrogen bond. Thus the $\mathrm{p}K_{a2}$* values decrease with increasing content of the dipolar aprotic solvents DMF and DMSO.

The observed small increase in the $\mathrm{p}K_{a2}$* as the medium is enriched in dioxane may be due to the fact that the release of the proton is rendered more difficult in the presence of this cosolvent. This behavior is probably attributed to the lower β value of dioxane ($\beta = 0.37$). The observed increase of the $\mathrm{p}K_{a2}$* in the presence of varying amounts of low basic aprotic acetonitrile and acetone solvents may be attributed to a low stabilization of the free conjugate bases of the zwitterionic buffers by hydrogen-bonding interaction.

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