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Medium Effect on the Apparent Second Stage Dissociation Constants of Some Zwitterionic Buffers for Physiological Research in Various Water + Organic Solvent Mixtures

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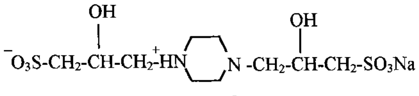
The apparent second stage dissociation constants of piperazine-*N*,*N*-bis[2-hydroxypropanesulfonicacid] (POPSO), *N*-[2-hydroxyethy1]piperazine-*N′*-3-propane sulfonic acid (HEPPS), 2-[*N*-cyclohexylamino]-ethanesulfonic acid (CHES) and mono[tris(hydroxymethyl)aminomethane] phosphate (Trizma) were determined 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 and ethanol as amphiprotic hydrogen bond acceptor−donor (HBA-D) solvents, *N*,*N*-dimethylformamide (DMF), dimethy1 sulfoxide, acetone, and dioxane as hydrogen bond acceptor solvents, and acetonitrile as an HBA−HBD solvent. The ESAB2M computer program was used to refine the initial estimates of the apparent second-stage dissociation constants of the four 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 interacions, 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

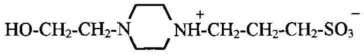
Good et al. (1966) recommended a series of zwitterionic buffer compounds compatible with most media of physiological and biochemical interest. These ampholytes were either amines or N-substituted amino acids. The choice of alternative buffers had greatly increased with the commerical availability of seven zwitterionic biochemical buffer substances prepared by Ferguson et al. (1980). These compounds are aminoalkanesulfonates and show significant advantages over conventional buffers: high solubility in water, stability in solution, minimum salt effects, insignificant penetration through biological membranes, no complex formation with biological samples, and no enzyme substrate or enzyme inhibitor properties.

Though studies on the dissociation constants of acids and bases in various hydroorganic media have been investigated, relatively little work has been done to determine the second stage dissociation constants of the new zwitterionic buffer substances (Roy et al., 1984; Azab, 1993; Azab et al., 1993, 1994).

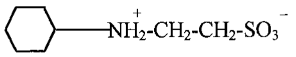
Organic buffers compatible with most media of physiological and biochemical importance now include Piperazine-*N*,*N′*-bis[2-hydroxypropanesulfonic acid] (POPSO), [*N*-[2-hydroxyethy1]piperazine-*N′*-3-propanesulfonic acid] (HEPPS), 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES), and mono[tris(hydroxymethy1)aminomethane]phosphate (Trizma), which possess the following zwitterionic structures, respectively. POPSO, HEPPS, CHES, and Trizma are potentially useful zwitterionic buffers for use in biochemistry because of their low toxicity.



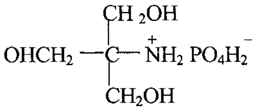
POPSO



HEPPS



CHES



$$OHCH_2-\overset{CH_2OH}{\underset{CH_2OH}{C}}-\overset{+}{N}H_2\ PO_4H_2^-$$

TRIZMA PHOSPHATE

Through our extended studies of the apparent second-stage dissociation of zwitterionic compounds in various hydroorganic media, we have determined the apparent second stage dissociation constants of the biologically important zwitterionic buffers POPSO, HEPPS, CHES, and Trizma by potentiometric pH-titration in pure water and various water + ethanol, water + methanol, water + dimethylformamide, water + dimethyl sulfoxide, water + acetone, water + acetonitrile, and water + dioxane mixtures containing different mass fractions of the organic solvent ranging between 0.0 and 0.55. We have chosen DMF, DMSO, acetone, and dioxane as hydrogen bond acceptor (HBA) solvents and acetonitrile as a HBA−HBD solvent. Methanol and ethanol were chosen as representatives of amphiprotic hydrogen bond acceptor−donor (HBA−D) solvents. All the solvents chosen are of frequent use in biochemical and biological studies. The solvent effects on the ionization of POPSO, HEPPS, and Trizma in various water + organic solvent mixtures are of particular interest, especially with reference of specific solute−solvent interactions because of the presence of hydroxy groups. The solvatochromic parameter values of Kamlet−Taft α, β, and

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π^* (Kamlet et al., 1983) have been used for quantitative interpretation of the solvent effects on the acid dissociation equilibria of the different zwitterionic buffer ligands. 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.

Experimental Section

Chemicals. Reagent grade piperazine-*N*,*N*′-bis[2-hydroxypropanesulfonic acid] (POPSO), *N*-[2-hydroxyethy1]-piperazine-*N*′-3-propanesulfonic acid] (HEPPS), 2-[*N*-cyclohexylamino]ethanesulfonic acid (CHES), and mono[tris-(hydroxymethyl)aminomethane]phosphate (Trizma) were from Sigma Chemical Co., St. Louis, MO. We determined the molecular weight of POPSO, HEPPS, CHES, and Trizma by potentiometric pH-titration to verify/determine the purity, especially for acidic/basic contaminants. The purity averages 99.5% for the four compounds, with a standard deviation of 0.05%.

Methanol, ethanol, *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetone, acetonitrile, and dioxane were from Fisher Scientific Co. A CO_2-free solution of potassium hydroxide (Fisher Scientific Co.) was prepared and standardized aganist multiple samples of primary-standard potassium hydrogen phthalate (Fisher Scientific Co.) under CO_2-free conditions. KNO_3 was from Fisher Scientific Co. The exact concentration of the stock solutions of the ligand (buffer) was determined by titration. Hydroorganic solvent mixtures containing different mass fractions of the organic solvents were prepared by mixing weighed quantities of water and solvent.

Procedure. Potentiometric pH measurements were made on solutions in a double-walled glass vessel at 25.0 ± 0.1 °C with a Fisher combined electrode (catalog no. 13-639-104) containing a calomel reference electrode. A Fisher accument pH/ion meter model 825 MP was used. The instrument was calibrated against standard buffers of pH 4.00 (phthalate buffer) and 9.20 (borate buffer). This calibration was required for the calculation of the p*K*s values 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 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 conditions, $I = 0.1$ 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) have been used to test the Nernstian response of the potentiometric cell.

A Fisher Scientific Isotemp refrigerated circular model 9000 water thermostat controlled the temperature, 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 proportion of the different organic solvent studied. In each mixture, at least three titrations were performed. The concentration of the 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 electrode, respectively and TS is the test solution using the Nernst equation

$$E = E° + Q \log C_{H^+} \tag{1}$$

where $E°$ is a constant which 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 of K_w for water in water + organic solvent systems have been taken from the literature (Bates, 1964; Wooley and Hepler, 1972; Wooley et al. 1970; Gutbezahl and Grunwald, 1953; Harned and Fallon, 1939). The protonation constants were then determind by use of the Bjerrum function (Bjerrum, 1921)

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

which is calculated from the following experimental quantities: *h*, the total concentration of titrable hydrogen ion H_T, and the total reagent concentration A_T.

The pK_{a2}^* values were determind from the overall protonation constants calculated by the linearization method of Irving and Rosotti (1953). Intitial estimates of pK_{a2}^* values were refined with the ESAB2M computer program (Stefano et al., 1987) by minimizing the error square sum

$$U_v = \sum_i W_i \, (V_i - V_{calcd,i})^2 \tag{3}$$

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

$$1/W_i = S_i^{\,2} = S_v^{\,2} + (\delta V_i/\delta E_i)^2 S_E^{\,2} \tag{4}$$

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 has been performed with a Gaussian error in *V* of $S_v = 0.005$.

Results and Discussion

The second dissociation involves the deprotonation of the substituted methylammonium of CHES and the deprotonation of the cationic group $-N^+H$ of POPSO and HEPPS. The dissociation constant of Trizma involves the deprotonation of the cationic $-NH_2^+$ group. If the zwitterions are designated $Z^{\pm}$, the second dissociation step can be represented by

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

Representative titration curves from which the intitial estimates of the apparent dissociation constants have been calculated are shown in Figures 1−4. The refined pK_{a2}^* values of POPSO, CHES, HEPPS, and Trizma in different solvent mixtures are given in Tables 1−4. The values obtained in the present work for the apparent dissociation constant values pK_{a2} of the zwitterionic buffers studied in

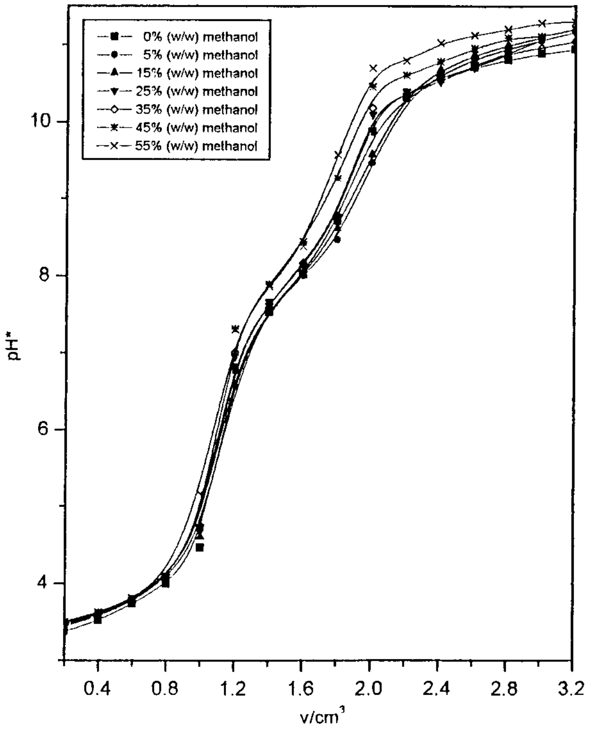

Figure 1. pH* vs volume of 0.0690 mol dm^{-3} KOH for POPSO in methanol + water mixtures at 25 °C and I = 0.1 mol dm^{-3} KNO$_3$.

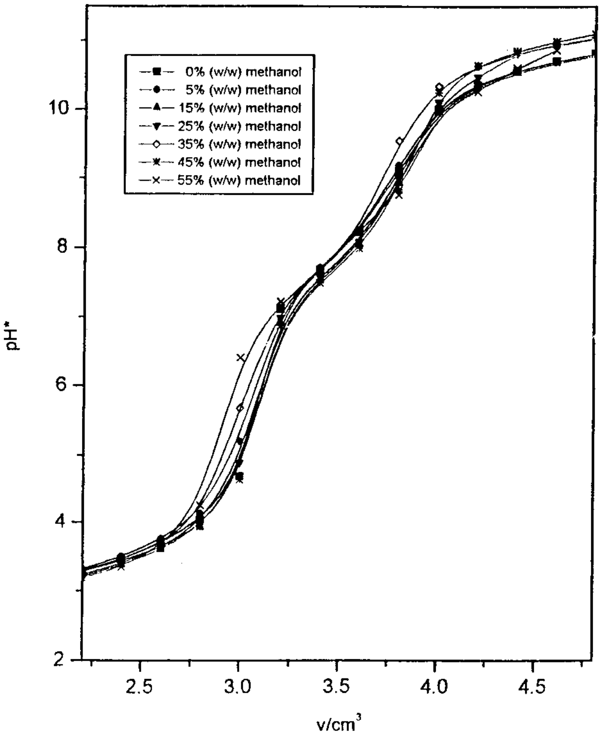

Figure 2. pH* vs volume of 0.0690 mol dm^{-3} KOH for HEPPS in methanol + water mixtures at 25 °C and I = 0.1 mol dm^{-3} KNO$_3$.

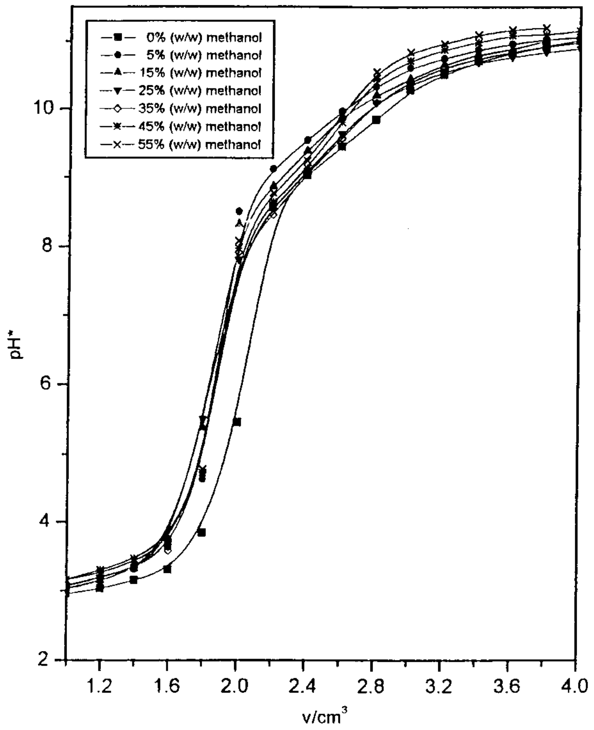

Figure 3. pH* vs volume of 0.0690 mol dm^{-3} KOH for CHES in methanol + water mixtures at 25 °C and I = 0.1 mol dm^{-3} KNO$_3$.

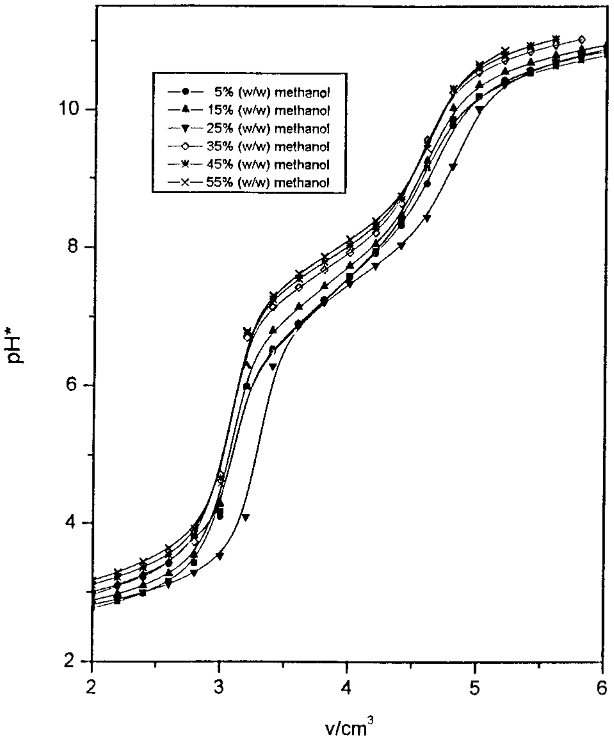

Figure 4. pH* vs volume of 0.0690 mol dm^{-3} KOH for Trizma in methanol + water mixtures at 25 °C and I = 0.1 mol dm^{-3} KNO$_3$.

pure water agree with the literature data (Ferguson et al., 1980; Roy et al., 1984).

According to previous studies (Bates, 1964) a purely electrostatic effect on the dissociation equilibria of the zwitterionic buffers under investigation can be expressed by eq 5, where $\bar{r}$ is the common radius of all the ions, (ϵ_2)

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

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-}, and n = 0 for the charge type A^+B^0, while 0.0128 is the reciprocal of the permittivity of water at 25 °C. The apparent dissociation constants for some dicarboxylic acids in different hydrooganic media calculated before (Azab et al., 1997) increase markedly as the permittivity decreases. The pK_{a2}* for zwitterionic buffers, however, is not, as might be expressed from eq 5, unaffected by a change in the permittivity. It usually increases or

Table 1. Refined pK_{a2}* [a] (Apparent Dissociation Constant) Values of POPSO 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	7.60 ± 0.02
	0.05	7.45 ± 0.04
	0.15	7.52 ± 0.03
	0.25	7.51 ± 0.04
	0.35	7.41 ± 0.04
	0.45	7.46 ± 0.02
	0.55	7.55 ± 0.03
ethanol	0.00	7.60 ± 0.02
	0.05	7.37 ± 0.03
	0.15	7.28 ± 0.04
	0.25	7.40 ± 0.04
	0.35	7.38 ± 0.03
	0.45	7.30 ± 0.03
	0.55	7.22 ± 0.03
DMF	0.00	7.60 ± 0.02
	0.05	7.43 ± 0.03
	0.15	7.44 ± 0.03
	0.25	7.38 ± 0.02
	0.35	7.43 ± 0.02
	0.45	7.46 ± 0.03
	0.55	7.42 ± 0.03
DMSO	0.00	7.60 ± 0.02
	0.05	7.47 ± 0.03
	0.15	7.51 ± 0.02
	0.25	7.52 ± 0.04
	0.35	7.62 ± 0.02
	0.45	7.54 ± 0.03
	0.55	7.50 ± 0.04
acetone	0.00	7.60 ± 0.02
	0.05	7.57 ± 0.04
	0.15	7.75 ± 0.02
	0.25	7.79 ± 0.03
	0.35	7.86 ± 0.02
	0.45	7.94 ± 0.03
	0.55	8.02 ± 0.02
acetonitrile	0.00	7.60 ± 0.02
	0.05	7.59 ± 0.03
	0.15	7.71 ± 0.01
	0.25	7.81 ± 0.03
	0.35	7.89 ± 0.01
	0.45	8.05 ± 0.02
	0.55	8.24 ± 0.03
dioxane	0.00	7.60 ± 0.02
	0.05	7.64 ± 0.01
	0.15	7.85 ± 0.02
	0.25	7.99 ± 0.03
	0.35	8.25 ± 0.02
	0.45	8.36 ± 0.01
	0.55	8.48 ± 0.04

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

Table 2. Refined pK_{a2}* [a] (Apparent Dissociation Constant) Values of HEPPS 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	8.04 ± 0.02
	0.05	7.79 ± 0.03
	0.15	7.84 ± 0.03
	0.25	7.82 ± 0.04
	0.35	7.76 ± 0.04
	0.45	7.75 ± 0.03
	0.55	7.70 ± 0.03
ethanol	0.00	8.04 ± 0.02
	0.05	7.81 ± 0.03
	0.15	7.78 ± 0.03
	0.25	7.88 ± 0.02
	0.35	7.86 ± 0.03
	0.45	7.79 ± 0.04
	0.55	7.70 ± 0.04
DMF	0.00	8.04 ± 0.02
	0.05	8.11 ± 0.03
	0.15	8.12 ± 0.03
	0.25	8.06 ± 0.02
	0.35	8.11 ± 0.04
	0.45	8.14 ± 0.04
	0.55	8.08 ± 0.04
DMSO	0.00	8.04 ± 0.02
	0.05	8.01 ± 0.03
	0.15	8.05 ± 0.03
	0.25	8.08 ± 0.04
	0.35	8.16 ± 0.03
	0.45	8.08 ± 0.04
	0.55	8.10 ± 0.02
acetone	0.00	8.04 ± 0.02
	0.05	8.17 ± 0.03
	0.15	8.40 ± 0.03
	0.25	8.45 ± 0.04
	0.35	8.52 ± 0.03
	0.45	8.72 ± 0.02
	0.55	8.86 ± 0.03
acetonitrile	0.00	8.04 ± 0.02
	0.05	8.19 ± 0.02
	0.15	8.46 ± 0.01
	0.25	8.53 ± 0.03
	0.35	8.56 ± 0.01
	0.45	8.78 ± 0.02
	0.55	8.89 ± 0.03
dioxane	0.00	8.04 ± 0.02
	0.05	8.22 ± 0.02
	0.15	8.25 ± 0.01
	0.25	8.42 ± 0.04
	0.35	8.51 ± 0.03
	0.45	8.87 ± 0.02
	0.55	8.92 ± 0.03

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

decreases somewhat with an increase in 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., 1977, 1983). These solvatochromic parameters may be used to quantify and rationalize multiple interacting solvent effects on the dissociation equilibria of the different zwitterionic buffer ligands studied.

The results presented in Tables 1−4, 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 ionization in the mixed solvent and in water (Gordon, 1975). Protonation of the solvent (SH) by $Z^{\pm}$ can be represented by the general equation

$$Z^{\pm} + SH \rightleftharpoons SH_2^{\ +} + Z^{-} \qquad (6)$$

Since the solutes involved in the ionization are charged, only charge transfer will be dominant (Gordon 1975). Therefore, although the difference in the dipolarity polarizability solvatochromic parameter π^* between pure water and the water + ethanol mixture is appreciable, it will have little effect on the protonation constants of the solutes. Also the solvation in mixed ethanol + water as solvent should not differ much from that in water, since the structures of the two are similar.

Table 3. Refined pK_{a2}*[a] (Apparent Dissociation Constant) Values of CHES 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	9.32 ± 0.02
	0.05	9.39 ± 0.03
	0.15	9.47 ± 0.03
	0.25	9.71 ± 0.04
	0.35	9.91 ± 0.04
	0.45	9.22 ± 0.04
	0.55	9.02 ± 0.03
ethanol	0.00	9.32 ± 0.02
	0.05	9.28 ± 0.04
	0.15	9.04 ± 0.04
	0.25	8.76 ± 0.04
	0.35	8.53 ± 0.04
	0.45	8.42 ± 0.04
	0.55	8.27 ± 0.04
DMF	0.00	9.32 ± 0.02
	0.05	9.16 ± 0.03
	0.15	8.96 ± 0.04
	0.25	8.71 ± 0.04
	0.35	8.35 ± 0.04
	0.45	8.19 ± 0.04
	0.55	7.90 ± 0.04
DMSO	0.00	9.32 ± 0.02
	0.05	9.19 ± 0.04
	0.15	8.96 ± 0.04
	0.25	8.87 ± 0.04
	0.35	8.64 ± 0.04
	0.45	8.49 ± 0.04
	0.55	8.19 ± 0.04
acetone	0.00	9.32 ± 0.02
	0.05	8.85 ± 0.04
	0.15	8.91 ± 0.04
	0.25	9.30 ± 0.01
	0.35	9.27 ± 0.02
	0.45	8.67 ± 0.04
	0.55	8.65 ± 0.04
acetonitrile	0.00	9.32 ± 0.02
	0.05	9.28 ± 0.02
	0.15	9.40 ± 0.02
	0.25	9.41 ± 0.02
	0.35	9.45 ± 0.03
	0.45	9.53 ± 0.02
	0.55	9.82 ± 0.02
dioxane	0.00	9.32 ± 0.02
	0.05	9.48 ± 0.03
	0.15	9.57 ± 0.04
	0.25	9.65 ± 0.03
	0.35	9.69 ± 0.03
	0.45	9.39 ± 0.02
	0.55	9.34 ± 0.02

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

Table 4. Refined pK_{a2}*[a] (Apparent Dissociation Constant) Values of TRIZMA 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	8.05 ± 0.04
	0.05	7.80 ± 0.03
	0.15	7.90 ± 0.03
	0.25	7.91 ± 0.03
	0.35	7.82 ± 0.02
	0.45	7.68 ± 0.03
	0.55	7.63 ± 0.03
ethanol	0.00	8.05 ± 0.04
	0.05	7.86 ± 0.03
	0.15	7.67 ± 0.01
	0.25	7.41 ± 0.02
	0.35	7.29 ± 0.04
	0.45	7.16 ± 0.03
	0.55	7.05 ± 0.03
DMF	0.00	8.05 ± 0.04
	0.05	7.81 ± 0.04
	0.15	7.54 ± 0.03
	0.25	7.38 ± 0.03
	0.35	7.24 ± 0.03
	0.45	7.03 ± 0.03
	0.55	7.15 ± 0.03
DMSO	0.00	8.05 ± 0.04
	0.05	7.77 ± 0.03
	0.15	7.53 ± 0.02
	0.25	7.42 ± 0.03
	0.35	7.22 ± 0.03
	0.45	7.41 ± 0.03
	0.55	7.45 ± 0.03
acetone	0.00	8.05 ± 0.04
	0.05	7.51 ± 0.03
	0.15	7.53 ± 0.03
	0.25	7.57 ± 0.03
	0.35	7.66 ± 0.02
	0.45	7.76 ± 0.03
	0.55	7.82 ± 0.03
acetonitrile	0.00	8.05 ± 0.04
	0.05	7.80 ± 0.03
	0.15	7.81 ± 0.01
	0.25	7.83 ± 0.03
	0.35	7.91 ± 0.01
	0.45	8.05 ± 0.02
	0.55	8.24 ± 0.03
dioxane	0.00	8.05 ± 0.04
	0.05	7.84 ± 0.01
	0.15	7.85 ± 0.02
	0.25	7.86 ± 0.03
	0.35	7.97 ± 0.02
	0.45	7.99 ± 0.01
	0.55	8.05 ± 0.04

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

This behavior can be quantitatively attributed to the small difference in the solvatochromic parameter α between pure water and methanol solvents.

The observed slight changes in pK_{a2}* of POPSO, HEPPS, CHES and Trizma as the solvent is enriched in methanol can be mainly interpreted as resulting from the following two factors.

(a) The first is 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. This is due to the greater tendency of water molecules to donate proton in a solvent-to-solute hydrogen bond ($\alpha = 1.17$). Considering only this effect, an increase in the methanol proportion in the aqueous medium will result in an increase in the activity coefficient of the conjugate base, thereby causing a slight increase in the pK_{a2}* values.

(b) The second is 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). This effect will generate a low activity coefficient of the proton, therefore causing a slight decrease in pK_{a2}* values.

Factor a may predominate in the case of CHES due to the absence of the hydroxyl group which result in a small increase in pK_{a2}* with increasing organic content of the solvent mixture. In the presence of hydrogen bond acceptor solvents (HBA), dimethlyformamide and dimethyl sulfoxide with high values of the solvatochromic parameter β (β = 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 (hydrogen bond acceptor) basicities provides a measure of the solvent's ability to accept at proton (donate an electron pair) in a solute-to-solvent hydrogen bond. Thus the pK_{a2}* values decrease with increasing the content of the dipolar aprotic solvents with high donicity DMF and DMSO, in case of the zwitterionic buffers CHES and Trizma in hydroorganic media containing different mass fractions of DMF or DMSO.

The observed constancy in the pK_{a2}*values of POPSO and HEPPS in the presence of varying amounts of DMF and DMSO can mainly be explained as resulting from the following two opposing effects.

(i) A DMF + water or DMSO+ water mixture is considered to be more basic than water. This behavior is based on the building up of a strong acceptor hydrogen bond from the $-NH^+$ group of the zwitterionic buffers studied in the former media as compared to that in the latter one, thus facilitating the ionization process of the $-NH^+$ cationic group, i.e., a low pK_{a2}*value.

(ii) The second effect is the expected low stabilization of the conjugate free bases of each of piperazine-*N*,*N*′-bis[2-hydroxypropanesufonic acid]) and *N*-[2-hydroxyethy1]-piperazine-*N*′-3-propanesulfonic acid by a hydrogen bond donated from solvent molecules in a DMF or DMSO + water mixture compared to that obtained in a pure aqueous medium. This in turn results in a high pK_{a2}* value.

The observed increase in the pK_{a2}* values of POPSO, HEPPS, CHES, and Trizma when the amount of the coorganic solvents acetone and acetonitrile (low basic aprotic solvents) in the medium is increased can be mainly attributed to a low stabilization of the free conjugate bases of these zwitterionic buffers by hydrogen bonding interaction.

The observed small increase in the pK_{a2}* values of POPSO, HEPPS, CHES, and Trizma as the medium is enriched in the aprotic nonionizing dioxane solvent may be attributed to the fact that the release of the proton from the amino nitrogen ($-NH_2^+$) of the zwitterionic form of CHES and Trizma and from the imino group ($-NH^+$) of the zwitterionic form of POPSO and HEPPS is rendered more diffcult in the presence of this cosolvent. This behavior is probably attributed to the lower β values of dioxane ($\beta = 0.37$).

The effects of coorganic solvents on the acid dissociation equilibria of the four zwitterionic buffers under investigation may be interpreted using the free energy of transfer of the proton from water to water −S (mixed solvent) in the relation

$$pK_{a2} - pK_{a2}^* = \Delta G^\circ_{tr}(H^+)_{w\rightarrow w(W)} + \Delta G^\circ_{tr}(Z)_{w\rightarrow w(S)} - \Delta G^\circ_{tr}(Z^\pm)_{w\rightarrow w(S)} \quad (7)$$

The $\Delta G^\circ_{tr}(H^+)_{w\rightarrow w(S)}$ values change with increasing content of the solvent mixture (Bates, 1964) leading to changes in pK_{a2}* values of the different zwitterionic buffer ligands (POPSO, HEPPS, CHES, and Trizma) according to eq 7. Useful information regarding the possible variation in microheterogeneity of the solvation shells around the solute may be obtained.

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