

Potentiometric Determination of the Apparent Dissociation Constants of Some N-Substituted 3-Amino-2-hydroxypropanesulfonic Acids in Various Hydroorganic Media

Hassan A. Azab,* Fikria S. Deghaidy, Adel S. Orabi, and Nader Y. Farid

Chemistry Department, Faculty of Science, Suez Canal University, Ismailia, Egypt

The apparent dissociation constants of 3-[*N*-(tris(hydroxy methyl)methyl)amino]-2-hydroxypropane sulfonic acid (TAPSO), 3-[*N,N*-bis(2-hydroxyethyl)amino]-2-hydroxypropane sulfonic acid (DIPSO), and 3-[*N*-morpholino]-2-hydroxypropane sulfonic acid (MOPSO) were determined at $(25.00 \pm 0.10)^\circ\text{C}$ and $I = 0.1 \text{ mol dm}^{-3}$ (KNO_3) by potentiometric pH titration in pure water and different hydroorganic solvent media. The organic solvents used were methanol, ethanol, *N,N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetonitrile, acetone, and dioxane. $\text{p}K_{\text{az}}^*$ changes with an increase in the concentration of organic solvent. These results are discussed in terms of solvent characteristics. Solvent basicity and stabilization of the conjugate acid free base by hydrogen-bonding interactions in hydroorganic solvent media relative to pure aqueous media as well as proton-solvent interaction play an important role in the dissociation equilibrium of the *N*-substituted 3-amino-2-hydroxypropanesulfonic acid buffers studied.

Introduction

Good and co-workers (Good et al., 1966) recommended a series of zwitterionic buffer compounds compatible with most media of physiological and biochemical importance. These ampholytes were either amines or *N*-substituted amino acids. The choice of alternative buffers had greatly increased with the commercial availability of seven zwitterionic biochemical buffer substances prepared by Ferguson (Ferguson et al., 1980). These compounds are aminoalkane sulfonates 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 extensively investigated, relatively little work has been done to determine the dissociation constants of the new zwitterionic biochemical 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 3-[*N*-(tris(hydroxymethyl)methyl)amino]-2-hydroxypropanesulfonic acid (TAPSO), 3-[*N,N*-bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO), and 3-[*N*-morpholino]-2-hydroxypropanesulfonic acid (MOPSO). TAPSO, DIPSO, and MOPSO are potentially useful zwitterionic buffers for use in biochemistry because of their low toxicity. In the present paper we determine the apparent second-stage dissociation constants of the biologically important zwitterionic buffers TAPSO, DIPSO, and MOPSO by potentiometric pH titrations 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 and 0.55. 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. We have chosen DMF and DMSO as aprotic polar solvents because their dielectric constants are relatively high, but lower than that of water. Methanol and ethanol were chosen as representatives of amphiprotic solvents. Acetone and acetonitrile have been chosen as low basic aprotic solvents and dioxane as an aprotic nonionizing solvent. All the solvents chosen are of frequent use in biochemical and biological studies.

Experimental Section

Chemicals. Reagent grade 3-[*N*-(tris(hydroxymethyl)methyl)amino]-2-hydroxypropanesulfonic acid (TAPSO), 3-[*N,N*-bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO), and 3-[*N*-morpholino]-2-hydroxypropanesulfonic acid (MOPSO) were from Sigma Chemical Co., St. Louis, MO. We determined by potentiometric pH titrations the molecular weight of TAPSO, DIPSO, and MOPSO 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%. The solvents used methanol, ethanol, *N,N*-dimethylformamide (DMF), dimethylsulfoxide (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 concentration of the stock solutions of the ligand (buffers) was determined by titration. Hydroorganic solvent mixtures containing different mass fractions of the organic solvents were prepared by mixing weighed quantities of water and cosolvent.

Procedure. pH potentiometric measurements were made on solutions in a double-walled glass vessel at $(25.0 \pm 0.1)^\circ\text{C}$ with a commercial Fisher combined electrode. A Fisher Accumet 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). The instrument was rechecked after each experiment. The electrode system was calibrated in aqueous medium in terms of hydrogen ion concentration instead of activities. Thus, all the constants determined in this work are concentration constants. Calibration of the electrode system was done in the working medium by the MAGEC program (May and Williams, 1985) using the data of titration of nitric acid with potassium hydroxide, both of known concentration, under the same temperature and medium condition, $I = 0.1 \text{ mol dm}^{-3}$ (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. The temperature was controlled by a Fisher Scientific Isotemp Refrigerated Circular Model 9000 water thermostat, and it was maintained within ± 0.1 °C. Efficient stirring of the solution was achieved with a magnetic stirrer. All test solutions ($1 \times 10^{-3} \text{ mol dm}^{-3}$ zwitterionic buffer ligand + $4 \times 10^{-3} \text{ mol dm}^{-3} \text{ HNO}_3$) were prepared in a constant ionic medium, $0.1 \text{ mol dm}^{-3} \text{ KNO}_3$, by mixing the appropriate amounts of ligand, nitric acid, potassium nitrate, and the proportion of the different organic solvents studied. At each mixture, at least four titrations were performed. The concentration of free hydrogen ion, C_{H^+} , at each point of the titration 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

$$E = E^\circ + Q \log C_{\text{H}^+} \quad (1)$$

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 ($0.1 \text{ mol dm}^{-3} \text{ KNO}_3$). Values for K_{W} for water in water + organic solvent systems have been taken from the literatures (Bates, 1964; Woolley and Hepler, 1972; Woolley et al., 1970; Gutbezahl and Grunwald, 1953; Harned and Fallon, 1939). The protonation constants were then determined by use of the Bjerrum function (Bjerrum, 1921). The $\text{p}K_{\text{a}2}$ values were determined from the overall protonation constants calculated by the linearization method of Irving and Rossotti (Irving and Rossotti, 1953). Initial estimates of $\text{p}K_{\text{a}2}$ values were refined with the ESAB2M computer program (Stefano et al., 1987) by minimizing the error squares sum

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

where V_i and $V_{\text{calcd},i}$ are experimental and calculated values of the titrant for every point i of the titration curve. Our calculation has been performed with a Gaussian error in V of $S_V = 0.005$.

Results and Discussion

3-[*N*-(Tris(hydroxymethyl)methyl)amino]-2-hydroxypropanesulfonic acid (TAPSO), 3-[*N,N*-bis(2-hydroxyethyl)amino]-2-hydroxypropanesulfonic acid (DIPSO), and 3-[*N*-morpholino]-2-hydroxypropanesulfonic acid (MOPSO)

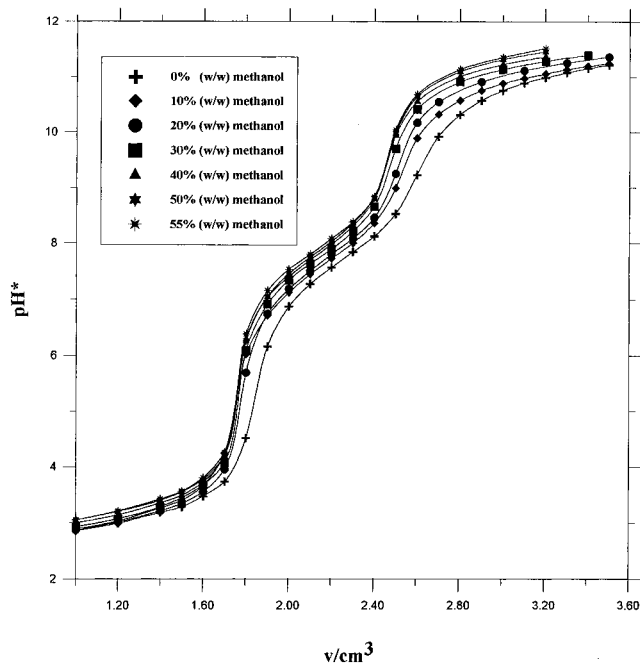
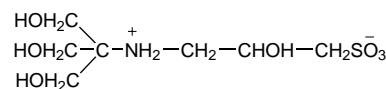
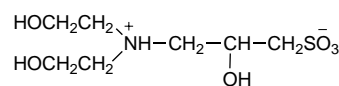


Figure 1. pH^* vs volume of $0.0806 \text{ mol dm}^{-3} \text{ KOH}$ for TAPSO in methanol + water mixtures at 25 °C and $I = 0.1 \text{ mol dm}^{-3} \text{ KNO}_3$.

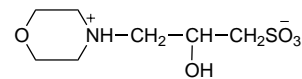
possess the following zwitterionic structures, respectively.



(TAPSO)



(DIPSO)

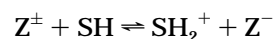


(MOPSO)

The second dissociation step involves the deprotonation of the substituted methylammonium of TAPSO and the deprotonation of the cationic group N^+H of DIPSO and MOPSO. If the above zwitterions are designated Z^\pm , the second dissociation step can be represented by



A representative titration curve from which the initial estimates of the apparent dissociation constants of TAPSO in methanol + water mixtures have been calculated is shown in Figure 1. The refined $\text{p}K_{\text{a}2}^*$ values of TAPSO, DIPSO, and MOPSO in different solvent mixtures are given in Tables 1–3. The values obtained in the present work for the apparent dissociation constant values $\text{p}K_{\text{a}2}$ of the zwitterionic buffers studied in pure water agree with the literature data (Ferguson et al., 1980). The results presented in Tables 1–3, with respect to ethanolic solution, can conveniently be discussed in terms of $\Delta G_{(\text{protonation})}$ (Gordon, 1975). Protonation of the solvent by Z^\pm can be represented by the general equation



where Z^\pm represents the zwitterionic form of TAPSO,

Table 1. pK_{a2}^* ^a (Apparent Dissociation Constant) Values of TAPSO at Different Mass Fractions w for w Organic Solvent + (1 - w) Water Mixtures at (25.0 ± 0.1) °C and $I = 0.1 \text{ mol dm}^{-3} \text{ KNO}_3$

organic solvent	w	pK_{a2}^*
methanol	0.00	7.60 ± 0.03
	0.10	7.61 ± 0.02
	0.20	7.63 ± 0.02
	0.30	7.63 ± 0.02
	0.40	7.64 ± 0.02
	0.50	7.67 ± 0.02
ethanol	0.00	7.60 ± 0.03
	0.10	7.61 ± 0.02
	0.20	7.56 ± 0.02
	0.30	7.48 ± 0.02
	0.40	7.45 ± 0.02
	0.50	7.45 ± 0.02
DMF	0.00	7.60 ± 0.03
	0.10	7.46 ± 0.02
	0.20	7.45 ± 0.02
	0.30	7.41 ± 0.02
	0.40	7.46 ± 0.02
	0.50	7.44 ± 0.02
DMSO	0.00	7.60 ± 0.03
	0.10	7.62 ± 0.02
	0.20	7.53 ± 0.02
	0.30	7.49 ± 0.01
	0.40	7.38 ± 0.02
	0.50	7.30 ± 0.02
acetone	0.00	7.60 ± 0.03
	0.10	7.52 ± 0.02
	0.20	7.58 ± 0.01
	0.30	7.61 ± 0.01
	0.40	7.65 ± 0.02
	0.50	7.80 ± 0.01
dioxane	0.00	7.60 ± 0.03
	0.10	7.55 ± 0.02
	0.20	7.58 ± 0.01
	0.30	7.66 ± 0.01
	0.40	7.75 ± 0.01
	0.50	7.86 ± 0.01
acetonitrile	0.00	7.60 ± 0.03
	0.10	7.75 ± 0.02
	0.20	7.83 ± 0.01
	0.30	7.93 ± 0.01
	0.40	8.05 ± 0.01
	0.50	8.30 ± 0.01
	0.55	8.37 ± 0.01

^a pK_{a2}^* = corrected pK_{a2} values ± uncertainties referring to statistically determined uncertainties at small 95% confidence intervals.

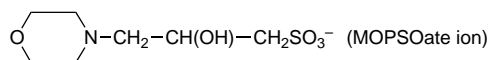
DIPSO, and MOPSO, respectively, and SH denotes a molecule of solvent. Since the solutes involved in the ionization are charged, then of the two basic steps in solute-solvent interactions, i.e., charge transfer and charge separation (Gordon, 1975), only the first will be dominant. Also, the solvation in mixed ethanol + water as solvents should not differ much from that in water, since the structures of the two solvents are similar. Consequently, with an increase in the amphiprotic ethanol solvent concentration, small change in $\Delta G_{(\text{protonation})}$ may be observed, which may lead to small change in pK_{a2}^* of TAPSO, DIPSO, and MOPSO; in most cases, it is decreased. The observed slight changes in pK_{a2}^* of TAPSO, DIPSO, and MOPSO as the solvent is enriched in methanol can be mainly interpreted as resulting from the two following factors:

Table 2. pK_{a2}^* ^a (Apparent Dissociation Constant) Values of DIPSO at Different Mass Fractions w for w Organic Solvent + (1 - w) Water Mixtures at (25.0 ± 0.1) °C and $I = 0.1 \text{ mol dm}^{-3} \text{ KNO}_3$

organic solvent	w	pK_{a2}^*
methanol	0.00	7.54 ± 0.01
	0.10	7.54 ± 0.03
	0.20	7.52 ± 0.04
	0.30	7.44 ± 0.02
	0.40	7.27 ± 0.02
	0.50	7.19 ± 0.03
ethanol	0.00	7.54 ± 0.01
	0.10	7.47 ± 0.03
	0.20	7.35 ± 0.03
	0.30	7.17 ± 0.03
	0.40	6.95 ± 0.05
	0.50	6.79 ± 0.04
DMF	0.00	7.54 ± 0.01
	0.10	7.45 ± 0.02
	0.20	7.34 ± 0.02
	0.30	7.26 ± 0.02
	0.40	7.17 ± 0.03
	0.50	6.96 ± 0.03
DMSO	0.00	7.54 ± 0.01
	0.10	7.46 ± 0.01
	0.20	7.40 ± 0.02
	0.30	7.26 ± 0.02
	0.40	7.06 ± 0.02
	0.50	6.94 ± 0.01
acetone	0.00	7.54 ± 0.01
	0.10	7.51 ± 0.02
	0.20	7.49 ± 0.02
	0.30	7.53 ± 0.03
	0.40	7.53 ± 0.02
	0.50	7.57 ± 0.02
dioxane	0.00	7.60 ± 0.03
	0.10	7.54 ± 0.01
	0.20	7.59 ± 0.02
	0.30	7.53 ± 0.02
	0.40	7.55 ± 0.02
	0.50	7.62 ± 0.02
acetonitrile	0.00	7.73 ± 0.01
	0.10	7.77 ± 0.01
	0.20	7.54 ± 0.01
	0.30	7.42 ± 0.02
	0.40	7.61 ± 0.02
	0.50	7.72 ± 0.01
	0.40	7.81 ± 0.01
	0.50	7.93 ± 0.01
	0.55	8.02 ± 0.02

^a pK_{a2}^* = corrected pK_{a2} values ± uncertainties referring to statistically determined uncertainties at small 95% confidence intervals.

(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. Thus, when the medium is enriched with methanol the activity coefficient of the conjugate base increases, thereby causing a slight increase in the pK_{a2}^* values.

(b) The greater stabilization of the proton in methanol + water mixtures relative to that in pure water through

Table 3. pK_{a2}^* (Apparent Dissociation Constant) Values of MOPSO at Different Mass Fractions w for w Organic Solvent + (1 - w) Water Mixtures at (25.0 ± 0.1) °C and $I = 0.1 \text{ mol dm}^{-3} \text{ KNO}_3$

organic solvent	w	pK_{a2}^*
methanol	0.00	6.81 ± 0.02
	0.10	6.92 ± 0.04
	0.20	6.90 ± 0.03
	0.30	6.73 ± 0.02
	0.40	6.69 ± 0.02
	0.50	6.60 ± 0.02
ethanol	0.55	6.71 ± 0.02
	0.00	6.81 ± 0.02
	0.10	6.83 ± 0.01
	0.20	6.72 ± 0.01
	0.30	6.52 ± 0.02
	0.40	6.45 ± 0.02
DMF	0.50	6.30 ± 0.02
	0.55	6.24 ± 0.02
	0.00	6.81 ± 0.02
	0.10	6.83 ± 0.02
	0.20	6.69 ± 0.02
	0.30	6.68 ± 0.02
DMSO	0.40	6.59 ± 0.02
	0.50	6.59 ± 0.02
	0.55	6.54 ± 0.02
	0.00	6.81 ± 0.02
	0.10	6.88 ± 0.01
	0.20	6.73 ± 0.03
acetone	0.30	6.75 ± 0.02
	0.40	6.61 ± 0.02
	0.50	6.41 ± 0.02
	0.55	6.35 ± 0.01
	0.00	6.81 ± 0.02
	0.10	6.93 ± 0.02
dioxane	0.20	6.99 ± 0.03
	0.30	6.89 ± 0.02
	0.40	6.89 ± 0.03
	0.50	6.97 ± 0.03
	0.55	6.95 ± 0.04
	0.00	6.81 ± 0.02
acetonitrile	0.10	6.90 ± 0.02
	0.20	6.87 ± 0.01
	0.30	6.94 ± 0.01
	0.40	7.05 ± 0.02
	0.50	7.07 ± 0.01
	0.55	7.08 ± 0.02
	0.00	6.81 ± 0.02
	0.10	6.93 ± 0.01
	0.20	7.07 ± 0.01
	0.30	7.23 ± 0.02
	0.40	7.33 ± 0.02
	0.50	7.46 ± 0.02
	0.55	7.50 ± 0.02

^a pK_{a2}^* = corrected pK_{a2} values ± uncertainties referring to statistically determined uncertainties at small 95% confidence intervals.

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}^* .

In the presence of increasing concentrations of the dipolar aprotic solvents with high donating ability (DMF and DMSO), deprotonation is facilitated because the solvent mixture solvates the proton more than the zwitterion. This leads to lower pK_{a2}^* values in such media than that obtained in pure aqueous solution. The observed small increase in the pK_{a2}^* of TAPSO, DIPSO, and MOPSO as the medium is enriched in the aprotic nonionizing dioxane solvent may be due to the fact that the release of the proton from the amino nitrogen ($-N^+H_2$) of the zwitterionic form

of TAPSO and from the imino group ($-NH^+$) of the zwitterionic form of DIPSO and MOPSO is rendered more difficult in the presence of this cosolvent. The observed small increase of the pK_{a2}^* values of TAPSO, DIPSO, and MOPSO 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 N-substituted 3-amino-2-hydroxypropanesulfonic acid buffers studied by hydrogen-bonding interaction in the presence of these coorganic solvents.

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