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Potentiometric Determination of the Second-Stage Dissociation Constant of *N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic Acid in Various Water + Organic Solvent Mixtures

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The second-stage dissociation constant values of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) were determined at 25 ± 0.1 °C by potentiometric pH titration in different solvent mixtures. The organic solvents used are methanol, ethanol, dimethylformamide (DMF), dimethyl sulfoxide (DMSO), acetone, and dioxane. The ionization of BES depends on both the proportion and the nature of the organic cosolvent used. It is observed that the $\mathrm{p}K_{a_2}$ value is slightly influenced as the solvent is enriched in methanol and ethanol and remains practically constant in the presence of different amounts of DMF and DMSO. A pronounced change in the $\mathrm{p}K_{a_2}$ value is observed as the solvent is enriched in acetone or dioxane. These results are discussed in terms of various solvent characteristics. It is concluded that the electrostatic effect has only a relatively small influence on the dissociation equilibrium of the imino group. Other solvent effects such as solvent basicity and different stabilization of the conjugate acid free base by hydrogen-bonding interactions in aquoorganic solvent media relative to pure aqueous media as well as proton–solvent interaction play an important role in the acid dissociation equilibrium.

Introduction

The choice of alternative buffers has increased with the commercial availability of zwitterionic amino acids, mainly N-substituted taurines or N-substituted glycines prepared by Good and co-workers (*1*), so that organic buffers suitable for use in biochemistry now include *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES). Zwitterionic buffers—comparable to amino acids—show significant advantages over conventional buffers: insignificant penetration through biological membranes, maximum buffer capacity in a physiological pH range of 6.0–8.50, and no enzyme substrate or enzyme inhibitor properties. Though studies on the dissociation constants of acids and bases in mixed and nonaqueous solvents have been extensively investigated, relatively little work has been done to determine the dissociation constants of the biologically important zwitterionic buffers (*2–7*).

In the present paper we have determined the second-stage dissociation constant of BES in various water + organic solvent mixtures. The organic solvents used are methanol, ethanol, DMF, DMSO, acetone, and dioxane. The $\mathrm{p}K_{a_2}$ values have been discussed in terms of solvent properties.

Experimental Section

Chemicals and Solutions. *N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) was analytical grade (BDH) with a purity of 98% and was further purified according to Perrin (*8*). The organic solvents methanol, ethanol, DMF, DMSO, acetone, and dioxane were of high purity (analytical reagent or spectro grade products). Carbonate-free NaOH was standardized by titration with potassium hydrogen phthalate. The molarity of HNO_3 was checked by titration with standard NaOH solution. Generally dilute solutions were prepared by appropriate dilutions of the stock.

Procedure. Potentiometric pH measurements were made on solutions in a double-walled glass vessel at 25 ± 0.1 °C with a commercial Fisher combined electrode. The pH was measured with a Fisher Accumet pH/ion meter, model 2301, with a precision of ±0.002 pH unit. The temperature was controlled by circulation of water through the jacket, from a VEB Model E3E ultrathermostat bath and maintained within ±0.1 °C. Purified nitrogen was bubbled through the solution in order to maintain an inert atmosphere. Efficient stirring of the solution was achieved with a magnetic stirrer. All solutions were prepared in a constant ionic medium, 0.1 M KNO_3, by mixing the appropriate amounts of ligand, nitric acid, and potassium nitrate solutions. All the aqueous solutions of the different organic solvents were made by adjusting the total volume to 50 cm^3 by adding double-distilled water in each case. The concentration of hydrogen ion was decreased by the addition of sodium hydroxide, prepared in the ionic medium used for the solution. At each solvent percentage, at least 4 titrations (50 data points) were performed.

The pH meter readings have been corrected in accordance with the method described by Douheret (*9, 10*). This was carried out to account for the difference in acidity, basicity, dielectric constant, and ion activities in partially aqueous solutions relative to the pure solvent.

The concentration of free hydrogen ion, h, at each point of the titration is related to the emf, E, of the cell RE/TS/GE (RE and GE denote the reference and glass electrode, respectively) by the Nernst equation:

$$E = E^\circ + Q \log h \qquad (1)$$

where E° is a constant which includes the standard potential of the glass electrode and Q is the slope of the glass electrode response. The value of E° for the electrode was determined from a separate titration of nitric acid with sodium hydroxide, both of known concentration, under the same temperature and medium conditions as for the test solution titration. The data so obtained were analyzed by the program MAGEC (*11*). During the MAGEC calculation the autoprotolysis constant of water, K_w, was refined until the best value for Q was obtained. The value of Q at 25 °C was found to be 59.0 mV. The result obtained indicates the reversible Nernstian response of the glass electrode used.

Initial estimates of $\mathrm{p}K_{a_2}$ values were refined with the ESAB2M computer program (*12*) by minimizing the error

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Table I. Refined $pK_{a_2}*$ Values of *N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic Acid at Different Mass Fractions *w* for *w* organic solvents + (1 − *w*) Water Mixtures

organic solvent	*w*	pK_{a_2}	$pK_{a_2}*$ [a]
methanol	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	7.073 ± 0.05	7.068 ± 0.05
	0.20	7.136 ± 0.04	7.121 ± 0.04
	0.30	7.134 ± 0.05	7.094 ± 0.05
	0.40	7.029 ± 0.05	6.939 ± 0.05
	0.50	7.079 ± 0.05	6.954 ± 0.05
ethanol	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	6.997 ± 0.05	6.987 ± 0.05
	0.20	6.932 ± 0.04	6.899 ± 0.04
	0.30	7.100 ± 0.05	7.020 ± 0.05
	0.40	7.132 ± 0.05	7.000 ± 0.05
	0.50	7.132 ± 0.04	6.927 ± 0.04
DMF	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	7.115 ± 0.03	7.053 ± 0.03
	0.20	7.185 ± 0.05	7.055 ± 0.05
	0.30	7.222 ± 0.04	6.997 ± 0.04
	0.40	7.385 ± 0.03	7.055 ± 0.03
	0.50	7.535 ± 0.03	7.083 ± 0.03
DMSO	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	7.118 ± 0.03	7.086 ± 0.03
	0.20	7.206 ± 0.05	7.131 ± 0.05
	0.30	7.293 ± 0.04	7.133 ± 0.04
	0.40	7.518 ± 0.03	7.238 ± 0.03
	0.50	7.581 ± 0.04	7.156 ± 0.04
	0.55	7.693 ± 0.05	7.183 ± 0.05
acetone	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	7.172 ± 0.05	7.192 ± 0.05
	0.20	7.335 ± 0.04	7.375 ± 0.04
	0.30	7.360 ± 0.05	7.410 ± 0.05
	0.40	7.410 ± 0.04	7.480 ± 0.04
	0.50	7.526 ± 0.05	7.641 ± 0.05
dioxane	0.00	7.120 ± 0.03	7.120 ± 0.03
	0.10	7.147 ± 0.03	7.167 ± 0.03
	0.20	7.222 ± 0.03	7.262 ± 0.03
	0.30	7.397 ± 0.04	7.477 ± 0.04
	0.40	7.497 ± 0.05	7.617 ± 0.05
	0.50	7.810 ± 0.04	7.980 ± 0.04
	0.55	7.910 ± 0.03	8.105 ± 0.03

[a] $pK_{a_2}*$ = corrected pK_{a_2} values according to Douheret (*9*, *10*) ± uncertainties (refer to statistically determined uncertainties at small 95% confidence intervals).

squares sum:

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

The weight is calculated by

$$1/W_i = S_i^2 = S_V^2 + (\delta V_i/\delta E_i)^2 S_E^2 \qquad (3)$$

The minimization function (eq 2) was chosen, since the titrant volume, V_{calcd}, can be calculated from an explicit equation (*13*, *14*). The program ESAB2M minimizes eq 2 by using the Gauss–Newton nonlinear least-squares method (*15*) with the very efficient Levenberg–Marquardt algorithm (*16*, *17*).

In our potentiometric pH titrations we put $E° = 0$ ($E°$ = formal potential of the electrodic couple) in the input instructions during the ESAB2M calculations, since the program reads in pH in this case. Our calculations have been performed with a Gaussian error in V: $S_V = 0.005$.

Results and Discussion

The refined $pK_{a_2}*$ values of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES) in the different aquoorganic solvent mixtures are given in Table I. The value obtained in the present work for $pK_{a_2} = 7.12 \pm 0.03$ in pure water agrees with the literature data (*1*) ($pK_{a_2} = 7.15$). Examination of the results reveal three important features: (a) With an increase in the amphiprotic methanol or ethanol solvent concentration in the aqueous medium, the change in $pK_{a_2}*$ is quite small; in most cases it is slightly decreased. (b) With an increase in the DMF or DMSO concentration, the $pK_{a_2}*$ remains practically constant. (c) By increasing the acetone or dioxane (nonpolar solvents) concentration in the aqueous medium, the $pK_{a_2}*$ value increases.

Effect of the Amphiprotic Solvent. Solvent effects on acid–base phenomena in amphiphrotic media of intermediate and high dielectric constant (methanol, ethanol) are often successfully interpreted in terms of changes in the dielectric constant (electrostatic effects) and in the basicity (nonelectrostatic effects).

Consider an acid (HA) at molarity M in a mixed amphiprotic solvent (SH). It is assumed that the dielectric constant of the mixed solvent is sufficiently large to permit a certain amount of ionization. The proton activity a_H, as a measure of acidity, can be expressed (*18*) in terms of K_{HA} and K_{SH}, which are, respectively, the acidity constants of HA and SH_2^+:

$$a_H = \left(MK_{HA}K_{SH}\frac{\nu_{SH_2^+}\nu_{HA}}{\nu_{SH}\nu_{A^-}} \right)^{1/2} \qquad (4)$$

where $\nu_{SH_2^+}$, ν_{HA}, ν_{SH}, and ν_{A^-} are the activity coefficients of the species SH_2^+, HA, SH, and A^-, respectively. a_H is the activity of H^+.

A decrease in the dielectric constant usually causes $\nu_{SH_2^+}/\nu_{SH}$ and ν_{A^-}/ν_{HA} to increase, and it is possible that both of these ratios will increase by about the same amount (*19*). Hence, according to eq 4, a lowering of the dielectric constant due to addition of methanol or ethanol may have little effect on the acidity of an unbuffered solution of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid. With respect to the solvent basicity effect, the autoprotolysis constant of water is slightly influenced by addition of methanol or ethanol (pK of pure water is changed from 14.0 to 14.90 mol dm^{-3} for 0.5 mass fraction ethanol + water mixtures).

Accordingly, one can deduce that changing the medium basicity by increasing additions of methanol or ethanol to the aqueous medium has little influence on the second-stage dissociation constant of *N,N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES). Comparing the values of the pK_{a_2} of BES in 0.1 M KNO_3 water solution (Table I) and at the same salt concentration but in mixed water + ethanol or methanol solution, it can be seen that in ethanolic or methanolic solution the values of pK_{a_2} are only slightly changed compared with those for an aqueous medium. Such behavior is common for amino acids (*20*) and characterizes dipolar ions (*21*).

The results presented in Table I, with respect to ethanolic solutions, can conveniently be discussed in terms of $\Delta G_{\mathrm{ionizn}}$ defined as $2.303RT(\log K^{(w)} - \log K^{(s)})$, i.e., the difference between the standard free energies of ionization in the mixed solvent and in water (*21*). The ionization process can be represented by the general equation

$$HA + SH \rightleftharpoons SH_2^+ + A^- \qquad (5)$$

where HS 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 (*21*), only the first will be dominant. Therefore, although the difference in dielectric constants between pure water and the water + ethanol mixture is appreciable (78.3 vs 49.0 at 25 °C), 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 solvents are similar. Consequently the two effects will result in relatively small values of $\Delta G_{\mathrm{ionizn}}$.

Generally, it was recognized that properties of solvents such as dielectric constant and acidic or basic strengths play a major but not an exclusive role. Other factors such as stabilization of the different species existing in equilibrium through hydrogen bonding together with ion–solvent interactions and dispersion forces play an important role in the ionization of weak acids. Accordingly, the observed slight changes in pK_{a_2} of BES as the solvent is enriched in methanol or ethanol can be mainly interpreted as resulting from the following two factors.

(a) The relatively high stabilization of the conjugate base $(HOCH_2CH_2)_2NCH_2CH_2SO_3^-$ by a donor hydrogen bond in a pure aqueous medium relative to that in the presence of methanol or ethanol. This is due to the greater tendency of water molecules to donate hydrogen as compared with other solvent molecules (*22*). Thus, an increase in the methanol or ethanol 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_{a_2} value.

(b) The greater stabilization of the proton in methanol or ethanol + water mixtures relative to that in pure water through ion–solvent interactions (*23*, *24*). This effect will generate a low activity coefficient of the proton in alcohol + water mixtures compared to that in a pure aqueous medium, therefore causing a slight decrease in pK_{a_2}.

Effect of Dipolar Aprotic Solvents (DMF and DMSO). The observed constancy in the pK_{a_2} value of BES in the presence of varying amounts of DMF and DMSO can mainly be explained as resulting from the following two opposing effects: (a) A DMF + water or DMSO + water mixture is considered to be more basic than water (*25*). This behavior is based on the building up of a strong acceptor hydrogen bond from the (–NH⁺) group of the *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid in the former medium as compared to that in the latter one, thus facilitating the ionization process of the imino group, i.e., a low pK_{a_2} value. (b) The expected low stabilization of the conjugate *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid free base 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_{a_2} value.

Effect of a Low Basic Aprotic Acetone Solvent. The presence of acetone as a coorganic solvent exerts a pronounced effect on the pK_{a_2} of BES as compared to the effect of the other coorganic solvents studied (methanol, ethanol, DMSO, and DMF). Though ethanol and acetone have nearly the same dielectric constant (24.30 and 20.70, respectively), the pK_{a_2} values of BES in ethanol and in acetone + water mixtures of the same composition are different. This behavior can be considered as convincing evidence for the above-reported conclusion that the electrostatic effect will have a relatively small influence on the second-stage dissociation constant of *N*,*N*-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid. Thus, the observed increase in pK_{a_2} when the amount of the coorganic solvent acetone in the medium is increased can be mainly attributed to the following effects.

(a) Solvent basicity effect: acetone is considered to be a very weakly basic solvent (*26–29*); therefore, one can expect that the basicity of acetone mixtures with water is lower than that of a pure aqueous medium (*25*). This leads to lower ionization of the NH^+ group in the former media as compared to that in the latter one, i.e. a high pK_{a_2} value.

(b) Solute–solvent interaction effect: acetone is considered to be both a poorer acceptor as well as donor of hydrogen bonds compared to water (*30*). Thus, one may expect a low stabilization of the free BES conjugate base by hydrogen-bonding interactions in the presence of this coorganic solvent. This leads to a higher pK_{a_2} value in such media than that obtained in a pure aqueous solution.

(c) Proton–solvent interaction effect: it has been recognized that the special type of solvation of the H^+ ion, namely, hydrogen ion–solvent interaction, plays a vital role in acid base equilibria (*29*). Since acetone is a weakly basic solvent, one must expect that the H^+ ion becomes less stabilized in the presence of this coorganic solvent. Thus, increasing the acetone concentration in the aqueous medium results in a high activity coefficient of the proton, i.e., a high pK_{a_2} value.

Effect of an Aprotic Nonionizing Dioxane Solvent. The observed increase in the pK_{a_2} value of BES as the solvent is enriched in dioxane may be attributed to lowering of the dielectric constant which increases the fraction of associated ions to form Bjerrum ion pairs (*31*) and higher aggregates such as triple ions and dipole aggregates (*32*). In this aprotic nonionizing medium, the concentration of free ions is very low and acidity phenomena are governed largely by ionic association reactions, as Kolthoff and Bruckenstein have shown so convincingly (*33*).

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