Potentiometric Determination of the Second Stage Dissociation Constant of N-[*Tris*-(hydroxymethyl)-methyl]-2-aminoethane-sulphonic Acid (*TES*) in Different Solvent Mixtures

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Summary. The second stage dissociation constants of N-[*Tris*-(hydroxymethyl)methyl]-2-aminoethane sulphonic acid (*TES*) were determined at $25 \pm 0.1\,°C$ by potentiometric pH-titration in different mixed solvent mixtures. The organic solvents used were methanol, ethanol, dimethylformamide (*DMF*), dimethylsulfoxide (*DMSO*), acetone, and dioxane. The ionization of *TES* depends on both the proportion and the nature of the organic co-solvent used. It was observed that the pKa_2 value is slightly influenced as the solvent is enriched in methanol, ethanol and remains practically constant in presence of different amounts of *DMF* and *DMSO*. A pronounced change in the pKa_2 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 cationic group $-\overset{+}{N}H_2$. Other solvent effects such as solvent basicity, different stabilization of the conjugate acid free base by hydrogen bonding interactions in aquo-organic solvents media relative to pure aqueous media as well as proton-solvent interaction play an important role in the acid dissociation equilibrium.

Keywords. Dissociation constant, TES, potentiometry, Solvent mixtures.

Potentiometrische Bestimmung der zweiten Dissoziationskonstante von N-[*Tris*-(hydroxymethyl)-methyl]-2-aminoethansulfonsäure (*TES*) in verschiedenen Lösungsmittelmischungen

Zusammenfassung. Die Dissoziationskonstanten der zweiten Dissoziationsstufe von N-[*Tris*-(hydroxy-methyl)-methyl]-2-aminoethansulfonsäure (*TES*) wurden bei $25 \pm 0.1\,°C$ mittels potentiometrischer pH-Titration in verschiedenen Mischungen von Lösungsmitteln bestimmt. Die organischen Solventien waren Methanol, Ethanol, Dimethylformamid (*DMF*), Dimethylsulfoxid (*DMSO*), Aceton und Dioxan. Die Ionisierung von *TES* hängt sowohl vom Anteil als auch der Natur von Co-Solventien ab. Der pKa_2-Wert wird durch Anreicherung mit Methanol und Ethanol leicht beeinflußt, Anteile an *DMF* und *DMSO* bleiben wirkungslos. Anwesenheit von Aceton oder Dioxan ergibt eine deutliche Änderung im pKa_2-Wert. Diese Ergebnisse werden auf der Basis von Lösungsmittelcharakteristika diskutiert. Es kann geschlossen werden, daß der elektrostatische Effekt lediglich einen geringen Einfluß auf das Dissoziationsgleichgewicht der kationischen Aminogruppe hat. Andere Lösungsmitteleffekte wie die Lösungsmittelbasizität, verschiedenartige Stabilisierung duch Wasserstoffbrückenbindung in der konjugierten säurefreien Base im wäßrig-organischen Medium im Vergleich zu rein wäßrigem Medium und Proton-Solvens-Wechselwirkungen spielen eine wichtige Rolle im Säuredissoziations-gleichgewicht.

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-[Tris-(hydroxymethyl)-methyl]-2-aminoethane sulphonic acid (TES). Zwitterionic buffers – comparable to amino acids – show significant advantages over conventional buffers: insignificant penetration through biological membrane, maximum buffer capacity at a physiological pH range of 6.0–8.50 and no enzyme substrate or enzyme inhibitor properties. Though dissociation constants of acids and bases in mixed and non-aqueous 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 the second stage dissociation constant of TES has been determined in aqueous solutions containing varying proportions of organic solvents (methanol, ethanol, DMF, DMSO, acetone, and dioxane). The pKa_2 values are discussed in terms of solvent properties.

Experimental Part

N-[Tris-(hydroxymethyl)-methyl]-2-aminoethane sulphonic acid (TES) was analytical grade (BDH) with purity 98% and was further purified according to Perrin [8]. The organic solvents, methanol, ethanol, DMF, DMSO, acetone, and dioxane were of high purity (A. R. or spectro grade products). Carbonate-free NaOH was standardized by titration with KH-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.

Potentiometric pH measurements were made on solutions in a double-walled glass vessel at $25 \pm 0.1 \,°C$ 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 units. The temperature was controlled by circulation of water through the jacket, from a VEB model E3E ultrathermostat bath and maintained within $\pm 0.1 \,°C$. 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 test 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 test solutions contained an appropriate proportion (w/w) of the different organic solvents studied. The total volume was adjusted to $50 \, cm^3$ by adding double-distilled water. The concentration of hydrogen ion was decreased by the addition of sodium hydroxide, prepared in the ionic medium used for the test 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

$$E = E° + Q \log h, \tag{1}$$

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

analysed 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 result obtained indicates the reversible Nernstian response of the glass electrode used.

Initial estimates of pKa_2 values were refined with the ESAB2M computer program [12] by minimizing the error squares sum

$$U_v = \sum_i W_i (V_i - V_{\text{calc},i})^2. \tag{2}$$

The weight was calculated using

$$\frac{1}{W_i} = S_i^2 = S_v^2 + (\delta V_i / \delta E_i)^2 S_E^2. \tag{3}$$

The minimization function (2) was chosen, since the titrtant volume, V_{calc}, 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 efficiently Levenberg–Marquardt algorithm [16, 17].

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

Results and Discussion

The refined pKa_2 values of N-[Tris-(hydroxymethyl)-methyl]-2-aminoethane sulphonic acid (TES) in the different aquo-organic solvent mixtures are given in Table 1. Careful examination of the results reveal three important features:

(i)   With an increase in the amphiprotic methanol or ethanol solvent concentration in the aqueous medium, the change in pKa_2 is quite small, in most cases it is slightly decreased.

(ii) With an increase in the DMF or DMSO content, the pKa_2 remains practically constant.

(iii) by increasing the acetone or dioxane (non-polar solvents) concentration in the aqueous medium, the pKa_2 value increases.

Effect of the Amphiprotic Solvent

Solvent effects on acid–base phenomena in amphiprotic 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 (non electrostatic effects).

Considering an acid HA at molarity M in 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 the acidity constants of HA and SH_2^+,

$$a_H = \sqrt{M K_{HA} K_{SH} \frac{v_{SH_2^+} v_{HA}}{v_{SH} v_A^-}}. \tag{4}$$

Table 1. The refined pKa_2^* values of N-[$Tris$-(hydroxymethyl)-methyl]-2-aminoethane sulphonic acid (TES) in different organic solvent–water mixtures at 25 °C and $\mu = 0.1$ M KNO_3 (pKa_2^*: corrected pKa_2 values according to Douheret [9, 10], $\pm$ refer to standard deviations)

Organic solvent	%w/w solvent	pKa_2	pKa_2^*
Methanol	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.333 ± 0.04	7.328 ± 0.04
	20.0	7.396 ± 0.03	7.381 ± 0.03
	30.0	7.394 ± 0.04	7.354 ± 0.04
	40.0	7.291 ± 0.03	7.201 ± 0.03
	50.0	7.341 ± 0.04	7.216 ± 0.04
Ethanol	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.357 ± 0.04	6.347 ± 0.04
	20.0	7.314 ± 0.03	7.281 ± 0.03
	30.0	7.309 ± 0.04	7.229 ± 0.04
	40.0	7.167 ± 0.03	7.035 ± 0.03
	50.0	7.276 ± 0.04	7.071 ± 0.04
DMF	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.375 ± 0.04	7.313 ± 0.04
	20.0	7.445 ± 0.03	7.315 ± 0.03
	30.0	7.482 ± 0.05	7.257 ± 0.05
	40.0	7.645 ± 0.04	7.315 ± 0.04
	50.0	7.795 ± 0.03	7.343 ± 0.03
DMSO	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.378 ± 0.04	7.346 ± 0.04
	20.0	7.506 ± 0.05	7.431 ± 0.05
	30.0	7.509 ± 0.04	7.349 ± 0.04
	40.0	7.678 ± 0.03	7.398 ± 0.03
	50.0	7.841 ± 0.04	7.416 ± 0.04
Acetone	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.442 ± 0.04	7.462 ± 0.04
	20.0	7.595 ± 0.05	7.635 ± 0.05
	30.0	7.628 ± 0.04	7.678 ± 0.04
	40.0	7.670 ± 0.05	7.740 ± 0.05
	50.0	7.786 ± 0.04	7.901 ± 0.04
Dioxane	00.0	7.380 ± 0.03	7.380 ± 0.03
	10.0	7.353 ± 0.04	7.373 ± 0.04
	20.0	7.482 ± 0.05	7.522 ± 0.05
	30.0	7.657 ± 0.05	7.737 ± 0.05
	40.0	7.757 ± 0.05	7.877 ± 0.06
	50.0	8.070 ± 0.05	8.240 ± 0.05
	55.0	8.170 ± 0.06	8.365 ± 0.06

A decrease in the dielectric constant usually causes $v_{SH_2^+}/v_{SH}$ and v_A^-/v_{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 a little effect on the acidity of an unbuffered solution of TES. 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 a 50% w/w 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 TES. Comparing the values of the pKa_2 of TES in 0.1 M KNO$_3$ water solution (Table 1) 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 pKa_2 are only slightly changed compared with those for an aqueous medium. Such behaviour is common for amino-acids [20] and characterizes dipolar ions [21].

The data presented in Table 1 for ethanolic solution can conveniently be discussed in terms of $\Delta G_{(ion.)}$ defined as 2.303 $RT(\log K^{(w)} - \log K^{(s)})$, 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

$$HA + SH \rightleftharpoons SH_2^+ + A^-, \tag{5}$$

where HS denotes a molecule of solvent. The solutes involved in the ionization are charged. As a consequence, 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 constant 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 $\Delta G_{(ion.)}$.

Generally, it was recognized that properties of solvents such as dielectric constant, acidic or basic strengths play a major but not exclusive role. Other factors such as stabilization of the different species existing in equilibrium through hydrogen bonding together with ion solvent interaction and dispersion forces play an important role in the ionization of weak acids. Accordingly, the observed slight changes in pKa_2 of TES as the solvent is enriched in methanol or ethanol can be mainly interpreted as resulting from the following two factors:

(i) The relatively high stabilization of the conjugate base (HOCH$_2$)$_3$CNHCH$_2$-CH$_2$SO$_3^-$ by a donor hydrogen bond in a pure aqueous medium relative to that in 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 pKa_2 value.

(ii) The greater stabilization of the proton in methanol or ethanol–water mixtures relative to that in pure water through ion-solvent interaction [23, 24]. This

effect will generate a low activity coefficient of the proton in alcohol–water mixtures compared to that in pure aqueous medium, therefore, causing a slight decrease in pKa_2.

Effect of Dipolar Aprotic Solvents (DMF and DMSO)

The observed constancy in the pKa_2 value of TES 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 [25]. This behaviour is based on the building up of a strong acceptor hydrogen bond from the $(-\overset{+}{N}H_2)$ group of TES in the former medium as compared to that in the latter one, thus facilitating the ionization process of the cationic $-\overset{+}{N}H_2$ group i.e. a low pKa_2 value.

(ii) The expected low stabilization of the conjugate TES free base by a hydrogen bond donates from solvent molecules in DMF or DMSO–water mixture compared to that obtained in pure aqueous medium. This in turn results in a high pKa_2 value.

Effect of a Low Basic Aprotic Acetone Solvent

The presence of acetone as co-organic solvent exerts a pronounced effect on the pKa_2 of TES as compared to the effect of the other co-organic solvents studied (methanol, ethanol, DMSO and DMF). Though, ethanol and acetone have nearly the same dielectric constant (24.30 and 20.70 respectively), the pKa_2 values of TES in ethanol and in acetone–water mixtures of the same composition are different. This behaviour can be considered as a convincing evidence for the above reported conclusion that the electrostatic effect will have a relatively small influence on the second stage dissociation constant of TES. Thus, the observed increase in pKa_2 when the amount of the co-organic solvent acetone in the medium is increased can be mainly attributed to the following effects:

(i) Solvent basicity effect: acetone is considered to be 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 ionisation of the cationic $\overset{+}{N}H_2$ group in the former media as compared to that in the latter one, i.e. a high pKa_2 value.

(ii) Solute–solvent interactions effect: acetone is considered to be both poorer acceptor as well as donor of hydrogen bonds compared to water [30]. Thus, one may expect a low stabilization of the free TES conjugate base by hydrogen bonding interactions in presence of this co-organic solvent. This leads to higher pKa_2 value in such media than that obtained in pure aqueous solution.

(iii) 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, therefore, one must expect that the H^+ ion becomes less stabilized in presence of this co-organic solvent. Thus, increasing the acetone proportion in the aqueous medium results in a high activity coefficient of the proton, i.e., a high pKa_2 value.

Effect of an Aprotic Nonionizing Dioxane Solvent

The observed increase in the pKa_2 value of *TES* upon enrichment of the solvent with dioxane may attributed to a lowering of the dielectric constant which increases in turn 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].

TES is an organic acid of the class of Good buffers [1]. It is a potentially useful zwitterionic buffer for use in biochemistry at physiological *pH* range (6.0–8.50) due to its low toxicity. The experimental *pH* and pKa_2 data reported in this paper for *TES* in various binary aqueous-organic solvent mixtures may be of great use in a number of areas of chemistry, biochemistry and biology.

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