

Thermodynamic Study of the Second-Stage Dissociation of 2-Aminoethanesulfonic Acid (Taurine) in Water at Different Ionic Strength and in Dioxane-Water Media

Mohamed Magdy Khalil¹, Rehab Khaled Mahmoud^{*2}, Saif Elden Babiker³

¹Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

^{*2}Department of Chemistry, Faculty of Science, Beni-Suef University, Beni-Suef, Egypt

³Medical Research Center Jazan University

*radwaraft@yahoo.com

Abstract- The second-stage dissociation constant pK_2 of 2-Aminoethanesulfonic acid (taurine) was determined in aqueous solution at different ionic strengths and different temperatures, using pH -metric technique. The thermodynamic quantities (ΔG° , ΔH° , and ΔS°) were studied and discussed. The protonation equilibria for the Taurine in nonaqueous solutions were studied by pH -potentiometry. The dissociation constants, pK_a , of taurine and the thermodynamic parameters for the successive and overall protonation processes of taurine were derived at different temperatures in three different mixtures of water and dioxane (mole fractions of dioxane were 0.083, 0.174 and 0.33). Titrations were also carried out in (water + dioxane) with ionic strengths of 0.15, 0.20 and 0.25 mol $\cdot$ dm⁻³ NaNO₃, and the resulting dissociation constants are reported in this paper. A detailed thermodynamic analysis of the effects of organic solvent, dioxane, temperature and ionic strength on the protonation processes of taurine is presented and discussed to determine the factors that control these processes.

Keywords- Protonation Constants; Taurine; Potentiometry; Solution Studies

I. INTRODUCTION

Taurine (2-amino-ethanesulfonic acid) is one of the most abundant amino acids in the human body. A number of researchers classify taurine as vitamin-like substance due to its diverse biological activities [1]. The biosynthesis of taurine is believed to be incomplete in astrocytes and neurons, but the metabolic cooperation between these two cell types is essential for the completion of the metabolic pathway for taurine [2]. Taurine is ubiquitously distributed, but is enriched in electrically excitable tissues such as the brain, retina, heart, and skeletal muscles [3]. The regulatory role of taurine has been implicated in a plethora of functions such as an anti-inflammatory molecule [4, 5], osmolyte, anti-oxidant [3, 6, 7], trophic factor [8, 9], and as a neuromodulator [10-12]. Clinically, taurine has been used with varying degrees of success for the treatment of a variety of conditions, including, but not limited to, cardiovascular diseases, hypercholesterolemia, epilepsy, macular degeneration, Alzheimer's disease, hepatic disorders, alcoholism, cystic fibrosis, and, most recently in in vitro fertilization [13-14]. Acid dissociation constants are important parameters to indicate the extent of ionization of molecules in solutions at different pH values. The acidity constants of organic reagents play a fundamental role in many analytical procedures such as acid-base titration, solvent extraction, complex formation, and ion transport. It has been shown that the acid-base properties affect the toxicity [15], chromatographic retention behavior, and pharmaceutical properties [16] of organic acids and bases. This empirical approach, although sometimes inescapable, is generally wasteful and it is more fruitful to determine the physico-chemical properties of the reagents and their resulting metal complexes, and in particular their protonation constants. Determining those values could lead to a better understanding of the cause of specificity and selectivity of relevant analytical reactions. With this in view, the thermodynamic protonation constants, pK_a , of taurine in different mole fractions of dioxane were determined and the thermodynamic functions associated with the protonation process evaluated in this study. Much of the theoretical foundation of modern organic chemistry is based on the observation of the effects on acid-base equilibrium of changing molecular structure [17]. The determination of the dissociation constant of an acid in mixtures of organic oxygen compounds with water provides useful data for the theoretical understanding of the ionization process in these media. There is still possibility to work on the determination of dissociation constants of biologically important substances for which the data at various temperatures and in different solvents mixture is not available frequently.

Potentiometry is regarded as the primary technique for the study of solution equilibria because of its precision, accuracy, reliability, experienced data analysis and relatively cheap instrumentation. Recently, many techniques, such as voltammetry [18], spectrophotometry [19] and NMR [20], have been used with good results for equilibrium studies, but potentiometry (e.g. [21, 22]) still maintains its role as the principal analytical technique in this field.

In this study, taurine refers to a class of compounds used for various analytical and biological applications. A good knowledge of its ionization in dioxane-water media is highly desirable. For this reason, knowledge of the constants for the

taurine is a prerequisite for gaining an understanding of their mechanisms of action in both chemical and biological processes. So, the ionization constants of taurine were determined potentiometrically in dioxane-water mixtures of different proportions. Also, the effect of structure on the ionization constants was discussed.

II. EXPERIMENT

A. Materials and Solutions

The 2-Aminoethanesulfonic acid (taurine) used in this study is a commercially available chemical (Sigma) products, and was used without further purification. The B.D.H. "AnalaR" *p*-dioxane was purified by the procedure of Weissberger and Proskauer [23]. It was refluxed over pellets of KOH for 8 to 10 h and, distilled, and the middle fraction of the distillate was refluxed over metallic sodium for 5 to 6 h and re-distilled. The middle fraction was used. Its purity was established by determining the freezing point which varied from 184.75 to 184.95 K (uncorrected) against the reported range of 184.80 to 185.15 K [24-25]. Carbonate-free sodium hydroxide solution was prepared by dissolving the AnalaR pellets in bidistilled water, and the solution was standardized potentiometrically with potassium hydrogen phthalate (Merck. AG). The nitric acid, sodium hydroxide, and sodium nitrate were from Merck p.a.

B. Apparatus and Procedure

The *pH* titrations were performed using a Metrohm 702 titroprocessor equipped with a 665 dosimat (Switzerland). The titroprocessor and the electrode were calibrated with a computer program (GLEE, glass-electrode evaluation) [26] that was used for the calibration of glass electrode by means of a strong acid-strong base titration. This program provides an estimate of the carbonate contamination of the base, the pseudo-Nernstian standard potential, slope of the electrode, and optionally, the concentration of the base and pK_w . It uses a non-linear, least-squares refinement to fit a modified Nernst equation (Eq. (1)),

$$E = E^0 + s \log[H^+], \quad (1)$$

where E is the measured electrode potential, E^0 and s are parameters of the refinement and represent the standard electrode potential and slope, and $[H^+]$ represents the hydrogen ion concentration.

In acidic solutions the hydrogen ion concentration is obtained from the mineral acid concentration, T_H , as calculated from Eq. (2), that is, $\log[H^+] = \log(T_H)$,

$$T_H = \frac{(a_H v_0 + \gamma b_H v)}{(v_0 + v_1 + v)}, \quad (2)$$

where a_H is the concentration, ($\text{mol} \cdot \text{dm}^{-3}$), of the acid, of which $v_0 \text{ cm}^3$ were added to the titration vessel; b_H is the concentration, ($\text{mol} \cdot \text{dm}^{-3}$), of the base in the burette (by convention given a negative sign), v_1 is the volume (cm^3) of the background electrolyte solution added to the titration vessel; and v , (cm^3), is the volume of the base added from the burette; and γ is a correction factor for the base concentration, where γ is refined and the calculated base concentration is γb_H .

In alkaline solutions the effective concentration of the base is usually reduced by the presence of a small amount (preferably <1%) of carbonate contamination. The extent of this contamination can be estimated by means of a gran plot [26]. Initially E^0 was estimated from the acidic region and s was taken as the ideal Nernstian slope (59.16 mV), then Eqs. (3) and (4) were fitted by linear least-squares regression.

Acidic region:

$$(v_1 + v_1 + v) 10^{\frac{E-E^0}{s}} = m^a v + c^a, \quad (3)$$

Alkaline region:

$$(v_1 + v_1 + v) 10^{\frac{E-E^0}{s} - pK_w} = m^b v + c^b, \quad (4)$$

A typical Gran plot was constructed. From the slopes and intercepts of the fitted lines two estimates were obtained for the volume of the base consumed at the equivalence point $v_e^a = -c^a / m^a$ for the acidic region and $v_e^b = -c^b / m^b$ for the alkaline region. Assuming that the difference is due to carbonate, the effective base concentration is reduced by the factor v_e^a / v_e^b in the alkaline region. The mineral acid concentration in the alkaline region is then given by Eq. (5).

$$T_H = \frac{(a_H \nu_0 + \gamma \left(\frac{\nu_e^a}{\nu_e^b}\right) b_H \nu)}{(\nu_0 + \nu_1 + \nu)}, \quad (5)$$

where T_H is negative and $\log[H^+] = -pK_w - \log(T_H)$.

Given with these estimates of $\log[H^+]$, the standard potential and slope can be obtained by least-squares fitting of Eq. (1) with standard buffer solutions. Based on the scale of the U.S. National Bureau of Standard [27], the *pH*-metric titrations were carried out at the desired temperature in a purified nitrogen atmosphere. The temperature was controlled by circulation of water through the jacket, from ultrathermostat bath and the variation was maintained within ± 0.1 °C. Efficient stirring of the solution was achieved with a magnetic stirrer. All test solutions were prepared by mixing the appropriate amounts of ligand, nitric acid and sodium nitrate solutions. All the test solutions contained an appropriate proportion (w/w) of the different organic solvent (dioxane) studied. The total volume was adjusted to 50 cm³ by adding double-distilled water. All the potentiometric titrations were carried out under magnetic stirring and bubbling purified pre-saturated N₂(g) through the solution, to exclude O₂(g) and CO₂(g) inside. For each temperature, various ionic strength values were investigated and for each ionic strength at least two measurements were performed. The estimated precision was ± 0.15 mV and ± 0.001 mL for the e.m.f. and titrant volume readings, respectively.

All calculations were performed using computer program based on unweighted linear least-squares fitting. The *pH* values in the dioxane +water solvent mixtures were corrected using the equation $pH^* = pH(R) - \delta$, where pH^* is the corrected reading and $pH(R)$ is the *pH*-meter reading obtained in a partially aqueous organic solvent, by Douheret [28]. This was carried out to account for the difference in acidity, basicity, dielectric constant, and ion activities in the partially aqueous solutions relative to the pure solvent.

C. Calculation

To account for the differences in acidity, basicity, dielectric constant and ion activities for the partially aqueous solutions relative to pure aqueous ones the *pKa* values were calculated adopting the Irving and Rossotti technique as described [29-30].

From the titration, ($\bar{n}_H$) the average number of protons associated with the Taurine, and the free ligand exponents at several *pH* values, were calculated using the formula of Irving and Rossotti [29-30]. Firstly, using the *pH*-metric titration curves, the average number of moles of protons per mole of ligand ($\bar{n}_H$) at several *pH* values for the above-mentioned ligand was calculated using the equation:

$$\bar{n}_H = \left\{ yC_L + \frac{(V_a - V_b \text{ or } V_a - V_d)C_b}{V_o} \right\} (C_L)^{-1}, \quad (6)$$

where y is the number of dissociable protons ($y=1$), V_a , V_b and V_d are the volumes of NaOH consumed to reach the same *pH* values in curves a, b and d, respectively C_b and C_L are the concentrations of NaOH and ligand, respectively, and V_o is the original volume (25 cm³). The adequacy of a proposed regression chemical model to fit the experimental data and the reliability of parameter *pKa* can be estimated and examined by the goodness-of-fit test [31]. Thermodynamic quantities (ΔG° , ΔH° , and ΔS°) associated with the protonation equilibria of phenolic acids were calculated by the following equation

$$\Delta G^\circ = -RT \ln K, \quad (7)$$

Both the standard enthalpies (ΔH°) and entropy (ΔS°) changes are correlated with the Gibb's free energy change by the relation:

$$\Delta G^\circ = \Delta H^\circ - T \Delta S^\circ \quad (8)$$

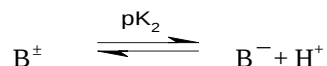
III. RESULTS AND DISCUSSION

In aqueous solution, turine is present in the zwitterionic form. Although the overall change is neutral, it has a large dipole moment and is represented as B[±], a dipolar molecule. The acid-base equilibrium of the zwitter ion form of turine can be represented as follows in Scheme 1



Scheme 1

or, more simply,



The pK_1 values of the taurine investigated were too low (≤ 2.30) and existed only in strongly acidic solutions. In this situation, the criterion for the constancy of ionic strength is violated; hence, all data obtained were characterized by a large scatter [32]. Thus, the value of the dissociation constant of the strongly acidic sulfo group ($pK_1 = 1.68$ [33]) cannot be considered to be accurate. Therefore, these values were not used in the calculations, since the pH-metric data were measured in the range of $2 \leq \text{pH} \leq 11$. The pK_2 of taurine was determined potentiometrically using a computer program based on *Irving and Rossotti* pH titration technique [34-35]. The details regarding the potentiometric method were reported in the experiment section. The value obtained (9.05) at 25 °C and $I = 0.10 \text{ mol dm}^{-3} \text{ NaNO}_3$ was compared with those of other works [33, 36-40] and it was shown that the results agreed within a very reasonable range (Table 1). The maximum deviation in pK_2 values never exceeded than 0.2 units. Such deviations between the results obtained by different physical methods are not unusual; examples are plentiful in the literature [33, 36-40].

TABLE 1 SECOND DISSOCIATION CONSTANT OF TAURINE IN WATER

Taurine	pKa		
	literature	References	This work
	8.93	[29]	9.05 ± 0.02
	8.95	[30]	
	8.93	[31]	
	9.01	[32]	
	8.90	[33]	
	9.08	[34]	
	9.00	[36]	

The amino group within taurine is characterized by a lower acid dissociation constant as compared with β -alanine ($pK_2 = 10.36$ [32]); this can be apparently attributed to the fact that the sulfo group has a stronger negative inductive effect as compared to that of the carboxylic group, which results in a decrease in electron density on the nitrogen atom of the taurine amino group and hence, decrease in its basicity.

The values of pK_2 at different temperature and different ionic strengths are included in Table 2.

TABLE 2 SECOND DISSOCIATION CONSTANTS (pK_2) OF TAURINE AT DIFFERENT IONIC STRENGTHS AND DIFFERENT TEMPERATURES

Ionic Strength	Temperature / °C			
	25	35	45	55
0	9.161 ± 0.02	9.022 ± 0.01	8.909 ± 0.02	8.849 ± 0.02
0.02	9.153 ± 0.01	9.015 ± 0.03	8.903 ± 0.03	8.844 ± 0.01
0.06	9.120 ± 0.02	8.981 ± 0.02	8.871 ± 0.04	8.812 ± 0.02
0.1	9.070 ± 0.04	8.930 ± 0.02	8.810 ± 0.02	8.768 ± 0.02
0.15	9.052 ± 0.01	8.902 ± 0.01	8.807 ± 0.01	8.744 ± 0.03
0.2	9.011 ± 0.02	8.860 ± 0.01	8.758 ± 0.04	8.710 ± 0.01
0.25	8.975 ± 0.03	8.848 ± 0.04	8.724 ± 0.02	8.670 ± 0.02

The plot of pK_2 vs. $\sqrt{I}$ is linear as shown in Fig. 1. This is in full agreement with the Debye-Hückel equation [41]. The thermodynamic equilibrium constants (at $I = 0.00$) were determined by applying linear regression analysis.

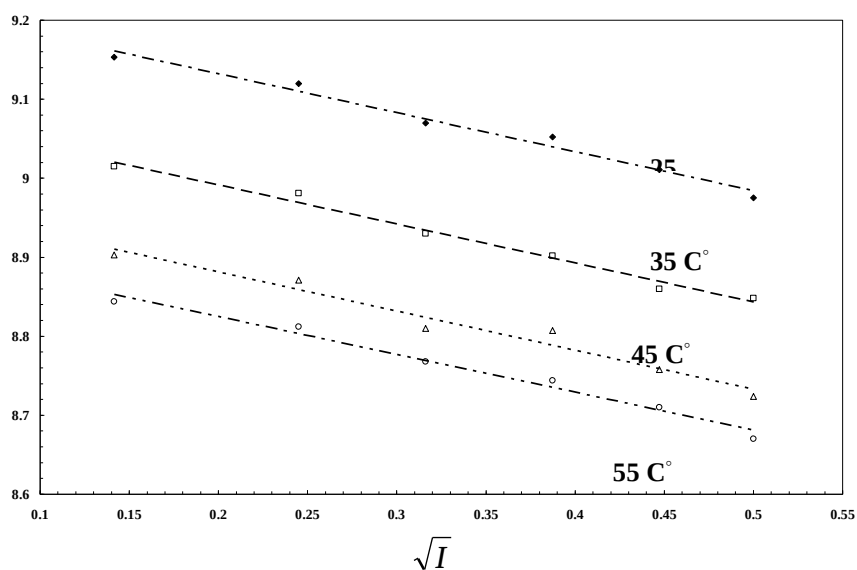


Fig. 1 Plot of pK_2 values of the taurine versus $\sqrt{I}$ at different temperatures

The thermodynamic quantities (ΔG° , ΔH° , and ΔS°) associated with the dissociation of taurine were also studied at each ionic strength, and the values obtained are given in Table 3. The enthalpy changes for the dissociation process were positive (endothermic). The positive values of ΔG° for the dissociation processes of the taurine denote that the processes were not spontaneous. In addition, the negative values of entropy changes pointing to increased ordering due to association. A plot of pK_2 vs. $1/T$ gives a straight line as in Fig. 2.

TABLE 3 THERMODYNAMIC QUANTITIES FOR THE DISSOCIATION PROCESSES OF TAURINE AT DIFFERENT IONIC STRENGTHS

I	$\Delta G \text{ kJ mol}^{-1}$	$\Delta H \text{ KJ mol}^{-1}$	$-\Delta S \text{ KJ K}^{-1} \text{ mol}^{-1}$
0	52.29	22.83	98.76
0.02	52.24	22.62	99.33
0.06	52.05	22.52	99.03
0.1	51.77	22.26	98.91
0.15	51.67	22.20	98.71
0.2	51.43	21.98	98.62
0.25	51.23	21.95	98.18

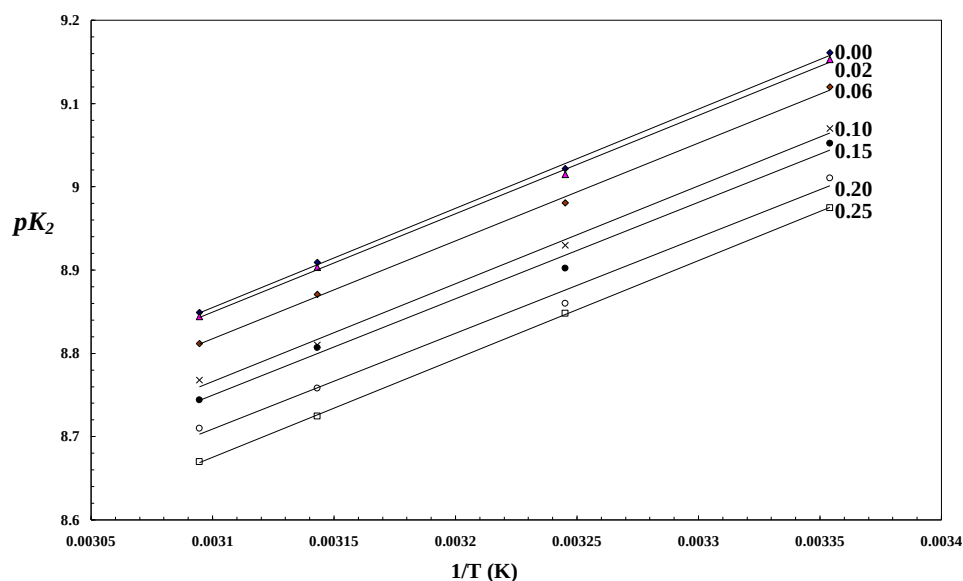


Fig. 2 Plot of pK_2 values of the taurine versus $1/T$ (K) at different ionic strengths

It is marked in Fig. 3 that as the composition of organic solvent increases, the pK_a values also increase. The dielectric constant of a solvent is the measure of a solvent's efficiency to separate oppositely charged ions. According to Coulomb's Law the attraction of forces (F in Newton) between two ions charged with q^- and q^+ (in coulombs) separated by distance r (in meter) is:

$$F = 8.988 \times 10^9 q / \epsilon r^2$$

where ϵ is the dielectric constant of the solvent. The attractive force is inversely proportional to the dielectric constant [42-43]. The larger the value of the dielectric constant, the smaller the attraction forces between the two ions (H^+ and B^- for dissociation reaction of base)



and thus the larger the acidity constant pK_a values. The dielectric constants of water and dioxane are 78.5 and 2.21 respectively. With an increase of dioxane concentration, the dielectric constant value of solvent will decrease resulting in a decrease of acidity constant or increase in pK_a values [42-43].

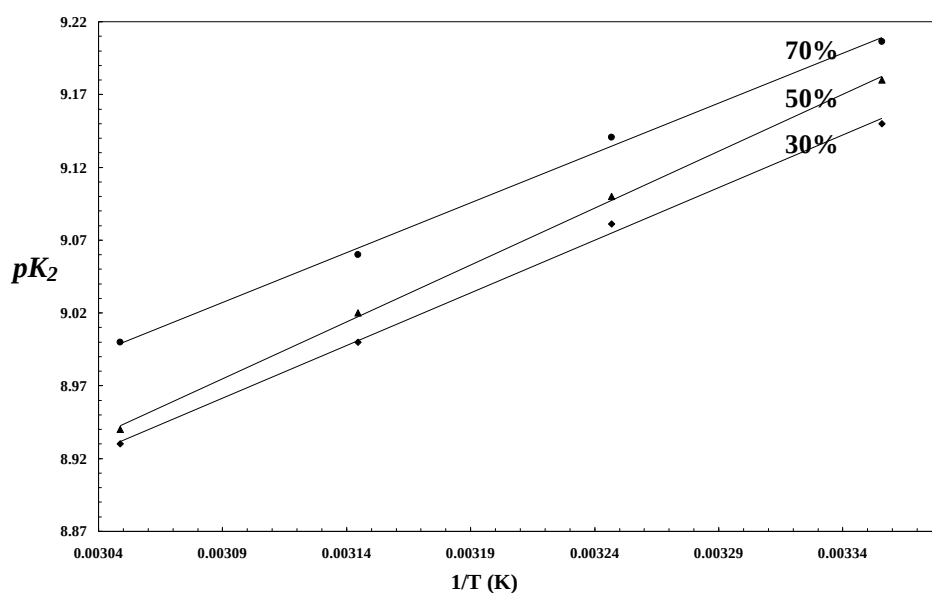


Fig 3. Plot of dissociation constants, pK_2 , of taurine versus $1/T$ in $0.1 \text{ mol} \cdot \text{dm}^{-3} \text{ NaNO}_3$ solutions at different (water + dioxane) mixtures

When the ionization of an acid gives a net increase of ions, a decrease in the dielectric constant of the solvent should be accompanied by a decrease in the protonation constant (increase of pK_a) of a weak acid dissolved in it. A solvent of low dielectric constant increases the electrostatic forces between the ions and facilitates the formation of molecular species, and should increase pK_a , as borne out for taurine as in Table 4. The pK_2 values increase with the increase of dioxane content (mole fraction of dioxane) because of the decrease in the dielectric constant of bulk solvent. The plot of pK_a versus mole fraction of dioxane (Fig. 3) shows a linear relationship of the form $pK_a = mX_z + c$, where m , X_z and c represent the slope, mole fraction of dioxane and intercept, respectively. A similar behavior was found for several other acids such as acetic, propionic and formic acids [44], and for a number of β -diketones in aqueous dioxane [45]. Similarly linear plots were also obtained for some other mixed water solvents, e.g., acetic, propenoic, butyric and benzoic acids in (methanol + water) mixtures [46]. Regarding the variation of pK_a values with the solvent composition, one can postulate that the zwitterionic to neutral form ratio decreases as the dioxan content increases. This can be inferred from considerations on specific solute-solvent interactions and structural changes in taurine from water to (dioxin + water) media. The present study supports the idea that the solvent effect on the protonation constant for the taurine can be utilized to predict whether the zwitterion or the neutral form is the predominant species and how the zwitterionic to neutral form ratio changes with the concentration of organic component in (water + organic) solvent mixtures. The basicity of a compound is the result of various factors such as: (i) the solvent effect: solvation power, the tendency of forming hydrogen bonds, selective solvation, dielectric constant and the composition of the solution in the first solvation layer in the case of mixed solvents and (ii) structural effect, electronic effect, steric effect and the formation of hydrogen bonding. Since equilibrium constants in general are temperature dependent, a variation in temperature during measurements would naturally have a deleterious effect on the quality of the recorded data. Of much higher significance, however, is the fact that the measuring electrode itself is highly temperature sensitive, including its response to changes ("slope") in $[H^+]$. The sum of these effects is usually of the order of magnitude $1 \text{ mV} \cdot \text{K}^{-1}$, and it is therefore vital that experiments are performed with a temperature control of at least 0.05 K [47].

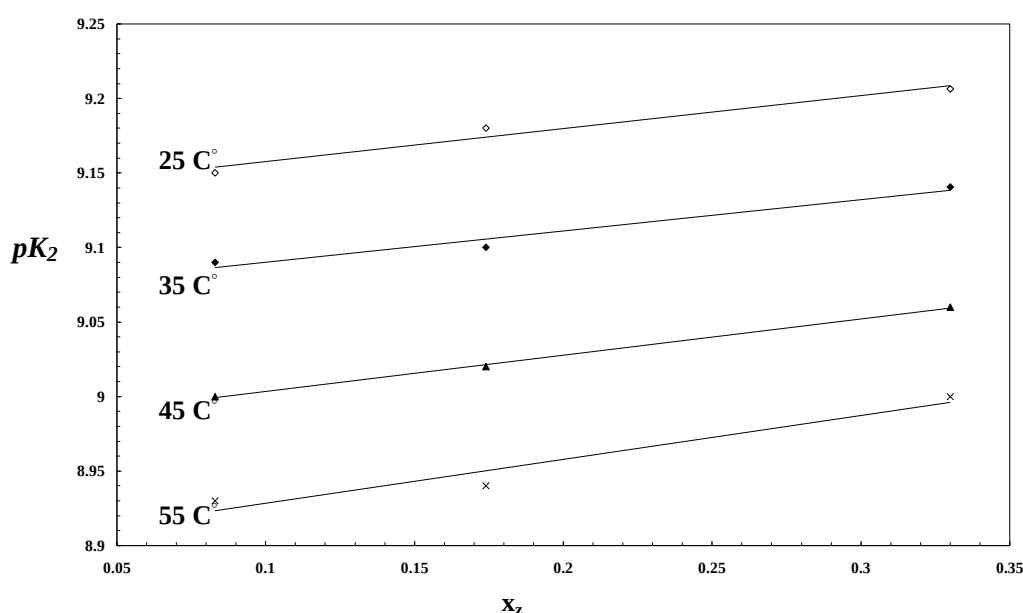
TABLE 4 SECOND DISSOCIATION CONSTANTS FOR TAURINE IN WATER + DIOXANE MIXTURES AT DIFFERENT TEMPERATURE AND $I = 0.1 \text{ MOL} \cdot \text{DM}^{-3} \text{NaNO}_3$

Temperature/ °C	% w/w Dioxane (mole fraction of dioxane, X_z)		
	30%(0.083)	50%(0.174)	70%(0.33)
25	9.15	9.18	9.21
35	9.08	9.10	9.14
45	9.00	9.02	9.06
55	8.93	8.94	9.00

The second dissociation constants of taurine were determined in water +30, +50, and +70 wt % dioxane at four different temperatures from 25 to 55 °C at intervals of 10 °C. The plot of pK_2 vs. $1/T$ gives straight line Fig. 4. The values of pK_2 , together with their standard deviations are listed in Table 5. As can be seen from table 5, the slight increase in the value of ΔG° from +30 to +70 wt % dioxane could possibly be explained by the decrease in the dielectric constant of the mixed solvent, which could then cause a increase in the electrostatic free energies of the various ions in the solution produced by the dissociation process. The values of ΔH° decreased with increasing organic content of the solvent mixture, suggesting that the second dissociation process was becoming increasingly exothermic. The similarity between ΔH° values of the dissociation processes of turine in water and water + dioxane mixtures indicates similar pattern of solvation in these media. The values of ΔS° were found to increase with increase in dioxane content of the solvent medium for all the taurine, thereby proving that it is dependent of solvent composition. The net entropy changes associated with the protonation process can be attributed to a combination of the following: (i) charge neutralization (ii) subsequent liberation of ordered water molecules (i) and (iii) loss of rotational and vibrational degrees of freedom of the ligand. Only (iii) gives a small negative contribution to entropy, the contributions of (i) and (ii) together are more than the offset caused by (iii) and hence the protonation process has large positive ΔS° values. The protonation of these acids then appears to be entropy driven as expected from the degree of charge neutralisation and polarizability of the resulting anions. Furthermore, the change in ΔH° with change in medium was relatively small and within experimental error. For this reason, the plots of pK_a versus X_z give practically the same slopes at different temperatures (Fig. 4). It is therefore justifiable to assume that ΔH° is independent of solvent composition over the range studied. The positive values of ΔG° for the dissociation of taurine studied denote that the processes are not spontaneous.

TABLE 5 THERMODYNAMIC QUANTITIES FOR THE DISSOCIATION PROCESSES OF TAURINE IN WATER + DIOXANE MIXTURES AT DIFFERENT TEMPERATURE AND $I = 0.1 \text{ MOL} \cdot \text{DM}^{-3} \text{NaNO}_3$

% w/w Dioxane	$\Delta G \text{ KJ mol}^{-1}$	$\Delta H \text{ KJ mol}^{-1}$	$-\Delta S \text{ KJ K}^{-1} \text{ mol}^{-1}$
30	52.23	13.10	132.36
50	52.40	14.96	125.61
70	52.55	13.86	128.76

Fig. 4 Plot of pK_2 values of taurine versus x_z of dioxane solvent in $0.1 \text{ mol} \cdot \text{dm}^{-3} \text{NaNO}_3$ solution at different temperatures

The errors introduced in the determination of pKa are reflected in the values of all of the thermodynamic functions. Therefore, an estimate of the error is necessary to show the reliability of these results. The values of pKa were determined generally with a precision of 0.02 and did not exceed 0.03, and hence the error in ΔG° was estimated to be between 0.03 and 0.04 kJ $\cdot$ mol⁻¹. A high electrolyte concentration was used in order keep variations of the activity coefficients at a minimum. Precise thermodynamic data can only be obtained provided an inert electrolyte of fairly high concentration (≥ 0.10 mol $\cdot$ dm⁻³) is used. The main differences between different procedures for the study of ionic equilibria in aqueous and nonaqueous solutions are due to the activity coefficients. As in most equilibria in nonaqueous medium, a background electrolyte is added to maintain constant ionic strength about 0.1 mol $\cdot$ dm⁻³ NaNO₃. This is allowed in some water-dioxane mixtures, but not in solvents of low dielectric constants where the solubility of the electrolyte is very low.

REFERENCES

- [1] C. Buddhala, H. Prentice and J-Yen Wu, "Modes of Action of Taurine and Granulocyte Colony-stimulating Factor in Neuroprotection," *J. Exp. Clin. Med.*, vol. 4(1), pp. 1-7, 2012.
- [2] V. Vitvitsky, S. K. Garg and R. Banerjee, "Taurine biosynthesis by neurons and astrocytes," *J Biol Chem.*, pp. 286, 2011.
- [3] S. S. Oja and P. Saransaari, "Pharmacology of taurine," *Proc West Pharmacol.*, vol. 50, 2007.
- [4] M. Sun, Y. Zhao, Y. Gu and C. Xu, "Anti-inflammatory mechanism of taurine against ischemic stroke is related to down-regulation of PARP and NF-kappaB," *Amino Acids*, 2011.
- [5] J. Miao, J. Zhang, L. Zheng, X. Yu, W. Zhu and S. Zou, "Taurine attenuates Streptococcus uberis-induced mastitis in rats by increasing T regulatory cells," *Amino Acids*, 2011.
- [6] S. Schaffer, K. Takahashi and J. Azuma, "Role of osmoregulation in the actions of taurine," *Amino Acids*, vol. 19, 2000.
- [7] S. W. Schaffer, J. Azuma and M. Mozaffari, "Role of antioxidant activity of taurine in diabetes," *Can J Physiol Pharmacol*, vol. 87, 2009.
- [8] R. Hernandez-Benitez, H. Pasantes-Morales, I. T. Saldana and Ramos-Mandujano, "Taurine stimulates proliferation of mice embryonic cultured neural progenitor cells," *J Neurosci Res*, vol. 88, 2010.
- [9] L. Lima and S. Cubillos, "Taurine might be acting as a trophic factor in the retina by modulating phosphorylation of cellular proteins," *J Neurosci Res*, vol. 53, 1998.
- [10] W. Q. Chen, H. Jin, M. Nguyen, J. Carr, Y. J. Lee, C. C. Hsu and M. D. Faiman, et al, "Role of taurine in regulation of intracellular calcium level and neuroprotective function in cultured neurons," *J Neurosci Res*, vol. 66, 2001.
- [11] A. Idrissi and E. Trenkner, "Growth factors and taurine protect against excitotoxicity by stabilizing calcium homeostasis and energy metabolism," *J Neuro. Sci.*, vol. 19, 1999.
- [12] T. M. Foos and J. Y. Wu, "The role of taurine in the central nervous system and themodulation of intracellular calcium homeostasis," *Neurochem Res.*, vol. 27, 2002.
- [13] M. Bidri, P. Choay, "Taurine: a particular aminoacid with multiple functions," *Ann Pharm Fr.* vol. 61, 2003.
- [14] T. C. Birdsall, "Therapeutic applications of taurine," *Altern Med Rev.* vol. 3, 1998.
- [15] Y. H. Zhao, L. H. Yuan and L. S. Wang, "Quantative Structure- Activity Relationships of Organic Acids and Bases," *Bull. Environ. Contam. Toxicol.*, vol. 57, pp. 242-249, 1996.
- [16] H. Rochester, "Acidity Functions," Academic Press: New York, 1971.
- [17] L. P. Hammet, "Physical Organic Chemistry," McGraw-Hill: New York, 1940.
- [18] R. M. Cigala, F. Crea, C. De Stefano, G. Lando, D. Milea and S. Sammartano, "The inorganic speciation of tin (II) in aqueous solution," *Geochim. Cosmochim. Ac.*, vol. 87, pp. 1, 2012.
- [19] F. Crea, D. Cucinotta, C. De Stefano, D. Milea, S. Sammartano and G. Vianelli, "Modeling solubility, acid-base properties and activity coefficients of amoxicillin, ampicillin and (+)6-aminopenicillanic acid, in NaCl (aq) at different ionic strengths and temperatures," *Eur. J. Pharm. Sci.*, vol. 47, pp. 661-677, 2012.
- [20] P. Cardiano, D. Cucinotta, C. Foti, O. Giuffrè and S. Sammartano, "Potentiometric, Calorimetric, and ¹H NMR Investigation on Hg²⁺-Mercaptocarboxylate Interaction in Aqueous Solution," *J. Chem. Eng. Data*, vol. 56, pp. 1995-2004, 2011.
- [21] S. Berto, P. G. Daniele, G. Lando, E. Prenesti and S. Sammartano, "Methodological Aspects in the Study of Alkali Metal Ion Weak Complexes Using Different ISEs Electrodes," *Int. J. Electrochem. Sci.*, vol. 7, pp. 10976-10986, 2012.
- [22] S. Cataldo, A. Gianguzza, A. Pettignano, D. Piazzese and S. Sammartano, Complex Formation of Copper(II) and Cadmium(II) with Pectin and Polygalacturonic Acid in A queous Solution. An ISE-H⁺ and ISE- Me²⁺ Electrochemical Study *Int. J. Electrochem. Sci.*, vol. 7, pp. 6722-6737, 2012.
- [23] A. Weissberger and E. S. Proskauer, "Organic Solvents," Interscience, New York, 1955.
- [24] A. T. Vogel, A Text Book of Practical Organic Chemistry. Longmans-Green, London, 1956.
- [25] B. E. Conway, "Electrochemical Data," Elsevier, New York, 1952.
- [26] P. Gans, O. Sullivan and B. Glee, "a new computer program for glass electrode calibration," *Talanta*, vol. 51, pp. 33-37, 2000.
- [27] R. G. Bates, "Determination of pH-Theory and Practice," 2nd ed, Wiley Interscience, New York, 1975.
- [28] G. Douheret, "Liquid junction potentials and effects of the medium glass-calomel electrode in such mixtures," *Bull. Soc. Chem. Fr.*, pp. 3122-3133, 1968.

- [29] A. E. Fazary, A. F. Mohamed and N. Lebedeva, "Protonation equilibria studies of the standard α -amino acids in NaNO_3 solutions in water and in mixtures of water and dioxane," J. Chem. Thermodyn., vol. 38, pp. 1467-1473, 2006.
- [30] A. E. Fazary and M. Taha, "Thermodynamics of the second-stage dissociation of 2-[N-(2-hydroxyethyl)-Nmethylaminomethyl]-propenoic acid (HEMPA) in water at different ionic strengths and different solvent mixtures," J. Chem. Thermodyn, vol. 37, pp. 43-48, 2005.
- [31] M. Ionization Constants of Acids and Bases (Wiley, London, 1962). Meloun, J. Havel and E. H. Gfeldt, "Computation of Solution Equilibria," Ellis Horwood, Chichester, 1988.
- [32] A. Albert, "Quantitative studies on the avidity of naturally occurring substances for trace metals. Amino-acids having only two ionizing groups," Biochem. J, vol. 47, pp. 531, 1950.
- [33] Yu. S. Petrova and L. K. Neudachina, "Potentiometric Study of Complexation between Taurine and Metal Ions," Russian J. of Inorg. Chem., vol. 58(5), pp. 617, 2013.
- [34] H. M. Irving and H. S. Rossotti, "Methods for computing successive stability constants from experimental formation curves," J. Chem. Soc., pp. 3397-3405, 1953.
- [35] H. M. Irving and H. S. Rossotti, "The calculation of formation curves of metal complexes from pH titration curves in mixed solvents," J. Chem. Soc., pp. 2904-2910, 1954.
- [36] S. P. Datta and A. K. Grzybowski, "The stability constants of the silver complexes of some aliphatic amines and amino-acids," J. Chem. Soc. pp. 1091-1095, 1959.
- [37] J. Maslowska and L. Chruscinski, "Potentiometric studies on mixed complexes of Ni (II) with taurine, dl-methionine and dl-ethionine in aqueous solution," Polyhedron, vol. 3, pp. 1329-1331, 1984.
- [38] E. Bottari and M. R. Festa, "Taurine as a ligand. An investigation of Ag(I)- and Cd(II)-taurine complex equilibria by direct and competitive techniques, and use of the Ag(I)-taurine complex electrode to assess taurine in solution," Talanta, vol. 46, pp. 91-98, 1998.
- [39] E. C. O' Brien, E. Farkas, and A. Rockenbauer, "Metal complexes of taurine. The first reported solution equilibrium studies for complex formation by taurine at physiological pH; the copper (II)-glycylglycinate-aurine and the copper (II)-glycylaspartate-aurine systems," J. Inorg. Biochem., vol. 77(3), pp. 135-139, 1999.
- [40] S. H. Hansen, M. L. Andersen, C. Cornett, R. Gradinaru and N Grunnet, "A role for taurine in mitochondrial function," journal of Biomedical Science, vol. 17(1), pp. S23, 2010.
- [41] S. S. Mian, K. H. Donald and R. M. Vincent, "Activity corrections for ionic equilibria in aqueous solutions," Can. J. Chem., vol. 58, pp. 1253-1257, 1980.
- [42] L. I. Song, M. Paleologon, and W. C. Purdy, "Determination of the acidity constants of chlorinated phenolic compounds by liquid chromatography," J. Chromotogra. Sci., vol. 29, pp. 66-69, 1991.
- [43] Saeeduddin and A. W. K. Khanzada, "Thermodynamic dissociation constant studies of caffeine at different temperatures and in organic water solvent mixture," Pakistan J. Pharmaceutical Sciences vol. 17, no. 1, pp. 83-87, 2004.
- [44] P. J. Glover, "Equilibria in solution. I. Ion solvation and mixed solvent interaction," J. Am. Chem. Soc., vol. 87, pp. 5275-5279, 1965.
- [45] Harned B.B. Owen, "The Physical Chemistry of Electrolytic Solutions," 3rd edn. Reinhold, New York, 1958.
- [46] L. G. Van Uitert, G. Hass, W. C. Fernelius and B. E. Douglas, "Studies on Coordination Compounds. II. the Dissociation Constants of β -Diketones in Water-Dioxane Solutions," J. Am. Chem. Soc., vol. 75, pp. 455-457, 1953.
- [47] A. K. Covington, Ion Selective Electrodes. In: Durst R.A. (ed.) National Bureau of Standards Special Publication 314, Chapt. 4. Washington, DC, 1969.