Thermodynamic First Dissociation Constant of Amino Acids in Dioxan-Water Mixture by Potentiometric Method

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***Abstract*—This paper mainly focusing on the thermodynamic first dissociation constants of the amino acids have been determined in Dioxane-Water mixture systems at a temperatures (298.15K). It is observed that ionization constant of amino acids in dioxane-water mixture systems increases with increase in temperature. The standard electrode potential E^0 of the cell;**

Pt/H_2(g)(p=1atm) Amino acids ; HCl; x% dioxane; Hg_2Cl_2 | Hg

m_1 m_2 (100-x)% water

have been determined in 0%, 10%; 20% ;30% ;40%;50% ;60% and 70% of dioxane-water mixture at a temperatures 298.15K and molality ranging from 0.005 to 0.1 mol kg^{-1}. E^o of the cell can be computed by modified Davies equation. The experimental results and standard thermodynamic properties are reported.

Keywords- Thermodynamic, Dioxan, Hydrochloric acid, amino acid and Davies equation.

I. INTRODUCTION

Proteins play a key role in nearly all biological processes. The basic structural units of proteins are amino acids. The side chains of these building blocks are differ in hydrogen-bonding capacity, shape, size, charge, hydrophobicity and chemical reactivity[1-5]. These side chains contribute to the structure and function of proteins. Theoretical and computer simulation studies on the thermodynamic properties of amino acids and the role of electrostatics in particular, in this context, become very important in developing a molecular view of how different residues interact with each other and with solvent . Such studies can investigate on protein structure, function and conformational stability, nature of active sites of enzymes, steric and electrostatic complementarities in protein-ligand, protein-DNA interactions etc[6-7].

The physicochemical properties of amino acids in aqueous solutions provide valuable information on solute-solute and solute-solvent interactions [9-14]. This is important in understanding the stability of proteins, in several biochemical and physiological processes in a living cell [15-18]. Potentiometry is regarded as the primary technique for the study of solution equilibria due to reliability, accuracy and cheap apparatus. Recently, several techniques, are avialable such as voltammetre [19], spectrophotometry [20] and NMR [21], have been used with good results for equilibrium studies, but potentiometer (e.g. [22-23]) still maintains its role as the principal analytical technique in this field.

In this study, amino acids are used for various analytical and biological applications. A good knowledge of its ionization in dioxane-water media is highly desirable. Thus the known values of the constants for the Amino acids, act as antibiotics, hormones, food additives, poisons or pain-killers and it is primarily because of their medicinal properties that the study of peptides has become one of the most active areas of current research [24].

II. MATERIALS AND METHODS

A Chemical and reagents

Amino acids are from (Sigma-Aldrich), Dioxane, NaOH and HCl (Merck) were used. Sodium hydroxide and hydrochloric acid were provided as M/10 standard solutions, diluted as per need. All the chemicals were purchased from E. Merck of A.R. grade used without further purification. The concentration of β-alanine was in weight percent and that of electrolyte in mol kg^{-1}correct to a micro mol kg^{-1}.The solutions were prepared in double distilled water after removal of dissolve gases.

B Procedure

For the EMF measurements a pH voltmeter (Metrohm 780) with a resolution of 0.1 mV and 0.1 K was used, together with a combined pH glass electrode (Metrohm 6.0258.010) with a 3 M KCl (Metrohm 6.2308.020) inner reference electrolyte and an integrated Pt1000 temperature sensor. When not in use, the electrode was stored in a storage solution (Metrohm 6.2323.000). Before each readings, electrode wascarefully rinsed with distilled water and dried. The cells were completely filled with the electrolyte solutions, placed in a water bath.The experiments were carried out under nitrogen atmosphere inside a glove box to prevent carbon dioxidefrom air absorbing into the electrolyte solutions. Now taking each reading the EMF (E / mV) and the temperature in the cell (T / K) were recorded

Amino acids dissociate in aqueous solutions chemical reactions occurs as below

$$^{-}O_2C-R-NH_3^{+} + H_2O \rightleftharpoons {}^{-}O_2C-R-NH_2 + H_3O^{+}$$

where R is a group of hydrogen atoms or any other (in)organic groups forming an amino acid and the presence of any protonated carboxyl group is neglected. The mole balances of the components in cell (II) were:

$$\bar{n}_{-O_2CRNH+} = n_{-O_2CRNH+} + n_{-O_2CRN}$$

$$\bar{n}_{NaOH} = n_{Na+}$$

$$\bar{n}_{H_2O} = n_{H_2O} + n_{H_3O+} + n_{OH-}$$

Electro neutrality resulted are

$$n_{Na+} + n_{H_3O+} = n_{-O_2CRN} + n_{OH-}$$

At equilibrium conditions, reactions are presented as

$$K_w(T) = \frac{a_{H_3O+}a_{OH-}}{a^2_{H_2O}}$$

$$K_2(T) = \frac{a_{-O_2CRN}a_{H_3O+}}{a_{-O_2CRNH+}a_{H_2O}}$$

C Determination of the Thermodynamic Parameters

To the experimentally determined dissociation constants, the well-known thermodynamic relations are applied

$$\Delta_r G_m = -RT \ln K \qquad \textbf{(1)}$$

$$\Delta_r H_m = -R\frac{d \ln K}{d(1/T)} \qquad \textbf{(2)}$$

the change of standard state properties (T=T°=298.15 K) for the dissociation of an amino acid in water are calculated from eq(2) &(3)

$$\ln K_i = \frac{A}{T} + B + C \ln(T) \qquad \textbf{(3)}$$

where i=1 for alanine, methionine,glycine,phenylalanine and proline. The further thermodynamic relations can be calculated as

$$\Delta_r S_m = \frac{(\Delta_r H_m - \Delta_r G_m)}{T} \qquad \textbf{(4)}$$

$$\Delta_r C_{p,m} = \frac{d\Delta_r H_m}{dT} \qquad \textbf{(5)}$$

III. RESULT AND DISCUSSION

The e.m.f. of the cells(C-1),(C-2) to (C-3) can be represented as :

$$E = E^0 - k \log m_H.m_{Cl} - k \log \gamma_H.\gamma_{Cl} \qquad \textbf{(6)}$$

Which becomes to equation (2) since it has been established that modified Davies equation is valid in this case The values of first dissociation constants of amino acids are in the best agreement with the results with an average relative deviation in ln(K_1) of 0.31 %. The results by May, Gillespie, Dey, and Majumdar deviate by 0.87 %, 1.24 %, 1.49 %, and 1.10 %, respectively. The differences between the correlated values of this work and the litterature values for amino acids are larger than those of protonated. The activity coefficients of the compounds into consideration when determining the first dissociation constants from experimental data; hence, the activity coefficients were set to unity for all species. In Figure-2, ln(K_1) is plotted against the molality of amino acids. The extrapolations to zero amino acid molality were done by linear regression.

$$E + k \log m_H.m_{Cl} - \frac{2kA'\sqrt{\mu}}{1+\sqrt{\mu}} = E^0 - k\beta\mu \qquad \textbf{(7)}$$

Where k= 2.303 (RT/F), A′=Appropriate Debye-Hückel constant. Since all the three electrolytes HCl, NaCl and KCl were dissociated, these values of ionic strengths, μ in cells (C-1),(C-2) to (C-3) would be given by m_1, m_1+m_2 and m_1+m_3 respectively. All other terms in equation (7) except E^0 and β are known, the value of k and A being known from table

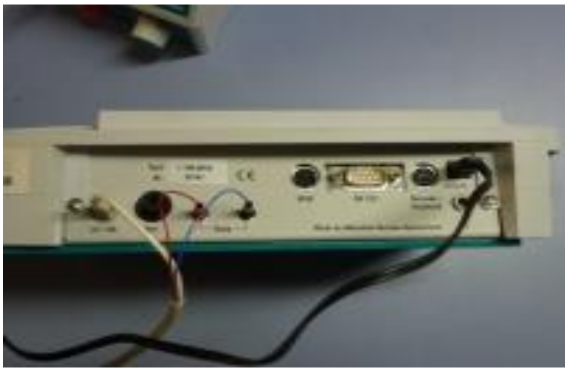

Figure-1 of Metrohm 780 pH Meter

TABLE I. FIRST DISSOCIATION CONSTANTS OF AMINO ACIDS IN DIFFERENT DIOXAN-WATER MIXTURE AT 298.5K

Dioxane %	I/ε ×10^2	Glysine	α-alanine	β-alanine	L-Valanine	L-Asparagine	L-Methionine	L-Threonine	L-proline
0.0	1.25	2.30	2.30	3.67	2.30	2.05	2.25	2.14	2.01
10	1.41	2.42	2.43	3.71	2.41	2.14	2.31	2.21	2.10
20	1.60	2.54	2.58	3.80	2.58	2.39	2.43	2.40	2.23
30	1.91	2.71	2.76	4.01	2.73	2.50	2.61	2.56	2.56
40	2.32	2.98	2.99	4.26	3.00	2.71	2.84	2.84	2.72
50	2.89	3.13	3.27	4.52	3.21	2.99	2.98	3.01	2.96
60	3.82	3.45	3.69	4.82	3.59	3.31	3.16	3.27	3.39
70	5.56	3.71	3.83	4.91	3.41	3.53	3.41	3.75	3.76

TABLE II. VALUES OF LN (K_1)AND THERMODYNAMICS QUANTITIES OF AMINO ACIDS

T/K	Glysine	α-alanine	β-alanine	L-Valanine	L-Asparagine	L-Methionine	L-Threonine	L-proline
298.5	-18.21	-22.16	-24.44	-26.12	-16.31	-12.05	-15.52	-25.61
$\Delta_r G^\circ_m$/kJ·mol $^{-1}$	78.23	77.12	78.95	73.91	71.23	77.62	79.54	73.12
$\Delta_r H^\circ_m$/kJ·mol $^{-1}$	45.14	48.73	46.80	43.11	44.22	43.23	42.32	48.71
- ΔS KJ K^{-1} mol^{-1}	97.16	98.10	98.34	98.56	98.79	98.91	98.19	98.16

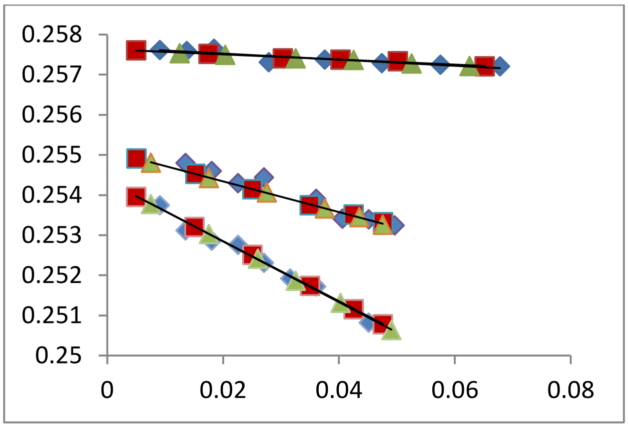

Figure 2. Plot of Y against μ; X = 10%,20%,30% Dioxane-water

On the setting up of all cells, thermostat, emf, assembly etc. The cells were completely filled with the electrolyte solutions and placed in an air thermostate (±0.05). The experiments were performed under nitrogen atmosphere inside a glove box to prevent the carbon dioxide from air absorbing into the electrolytic solutions. Now the cell were connected to potentiometer (±0.1mV). A stop-cock was opened and kept as such for ≈ 3 hours until the equilibrium of the cell was setup and the constant e.m.f was noted at different temperatures and concentrations. This e.m.f was used to calculate the various thermodynamic parameters and ion-solvent interaction E^0 cell from the experiment and mH and mCl from molalities of the experimental solution. The left hand side of the Equation. (7) (=Y,say) is plotted against the corresponding values of μ at 298.5 K of dioxane-water mixture for 10%, 20%,30%,40%,50%,60%, and 70%, shown in figure. II, and III. Figures show that the points of HCl alone, mixture of HCl; NaCl and HCl; KCl lie on the line within 0.1 mV. In figure II to III, the intercepts of the plots at μ =0 give the values of Em^0 . The standard electrode potential for the Hg |Hg_2Cl_2, Cl^- electrode in 10%, 20%,30%,40%,50%,60% and 70%of dioxane-water mixture is presented by means of a general expression of the method of least square to equation (8).

$$Em^0 = a + b (T - 298.15) + c (T - 298.15)^2 \qquad \textbf{(8)}$$

where T is temperature in K. The values of the constants a, b and c for hydrochloric acid in dioxane-water are shown

in Table. The electrode potentials on molal scale (Em^0) were converted to those on molar (Ec^0) and mole fractional (E_N^0) scale according to these equation,

$$E_C{}^0 = Em^0 + 2k \log(d_0/g\ cm^{-3}) \qquad \textbf{(9)}$$

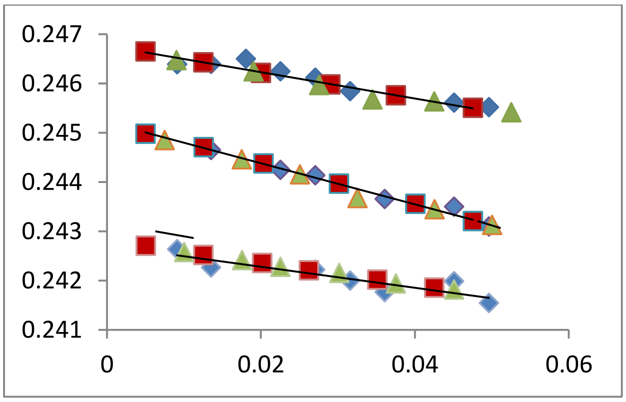

Figure 3. Plot of Y against μ; X = 40%,50%,70% Dioxane-water

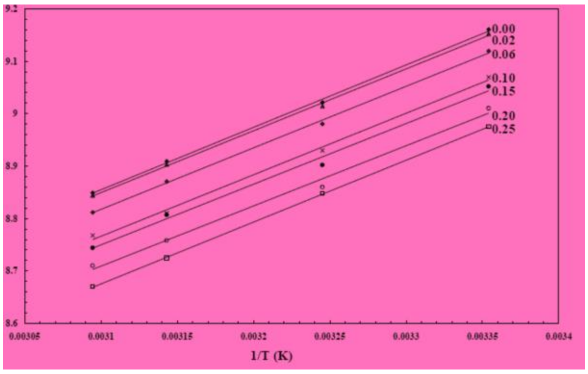

Figure 4. Plot of pK_1 values of amino acids versus 1/T (K) at different ionic strengths

The thermodynamic quantities ($\Delta G°$, $\Delta H°$, *and* $\Delta S°$) associated with the dissociation of amino acids were also studied at each ionic strength and the values. The enthalpy changes for the dissociation process were positive (endothermic). The positive values of $\Delta G°$ for the dissociation processes of the amino acids 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_1 vs. $1/T$ gives a straight line as in Fig. 4.

IV. CONCLUSION

In this study, The first dissociation constants of protonated amino acids have been determined from potentiometer. Parts of the results extend the temperature range of available literature data of the investigated compounds. From pK_a values Gibb's free energy can be calculated. The experimental results and derived values of the standard state thermodynamic properties are reported. The values of the dissociation constants and the thermodynamic relations presented in this work give information about the use of amino acids.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.

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