CHAPTER - II : PART-II
THERMODYNAMICS OF TRANSFER OF L-ASPARTIC ACID, L-PHENYL ALANINE AND L-HISTIDINE FROM WATER TO ETHANOL-WATER MIXTURES AT 25°C

Introduction

The medium effects on diprotonation or protolytic equilibria of weak Bronsted acids of different charge types have been studied extensively\textsuperscript{24-30}. Earlier it was believed that these effects are mainly dependent on the dielectric constant of solvent medium. Study of an equilibria in several isodielectric mixtures did not confirm this supposition\textsuperscript{31}. It was, therefore, thought that the dielectric constant is not the sole factor in these protolytic equilibria, the chemical nature of solute-solvent interactions also plays a major role.

The free energies of transfer of charged species from one solvent to another may formally be represented as 'electrostatic' and 'non-electrostatic'\textsuperscript{32}. The electrostatic term\textsuperscript{33} depends on a size parameter, particularly geometry and charge distribution within organic ions\textsuperscript{34}. The non-electrostatic contributions arise due to solvation effects embodied in the overall transfer of neutral and charged species. The limitations of the Born equation and the importance of specific solvation effects are emphasised by Bates et al.\textsuperscript{32}.
Medium effects on deprotonation of several Bronsted acids, have been analysed by Kundu et al.\textsuperscript{30} after thoroughly dissecting the effect, into various components. Earlier, Nowak et al.\textsuperscript{29} had investigated the basicity effect on the dissociation of some phenols. A perusal of earlier work on these aspects revealed that generally the medium effects had been observed, only for the dissociation of neutral acids or charged ions. We, therefore, thought it appropriate to investigate this aspect for the dissociation of zwitter ion of amino acids.

This is a continuation of our earlier work\textsuperscript{35} on the effect of dioxane-water mixtures on the dissociation of phenols, benzoic acids, anilines, pyridines and salicylic acids.

\textbf{Experimental}

Ethanol was purified by the standard method. The water used was distilled in all glass apparatus after prior deionization and distillation with alkaline permanganate. The mixed solvents were prepared by volume. The amino acids viz., l-aspartic acid, l-phenylalanine and l-histidine were from Aldrich Chemicals (A.R. grade).

The pH meter used for measurements was digital Elico model (accuracy $\pm$ 0.01 pH) with a glass and calomel
electrode assembly. The Calvin-Bjerrum titration technique was adopted to determine the pK values of the amino acids. The pK values of the -COOH group in phenylalanine and the first -COOH group in histidine could not be determined with accuracy as these are in the pH range of 2 to 2.5. The H⁺ ion concentrations in any solution was obtained by converting the observed B values (pH meter readings) after taking into account the solvent correction given by Bates. The details regarding calculation, maintenance of inert atmosphere during titration etc. form a part of earlier papers.

The solubilities of amino acids in several ethanol-water mixtures are available. The unit for solubility is mole litre⁻¹. These values were directly taken from the literature for the purpose of free energy calculations. The available solubility values for l-histidine and l-phenylalanine were expressed in g/100 g of solvent. These were converted into moles/lit. for uniformity.

Results and Discussion

a) Free energies of transfer

Values of thermodynamic dissociation constants, pK₀ at zero ionic strength were calculated by introducing a correction factor for the ionic strength of solution

\[ pK^0 = pK + 2A \sqrt{I} \left( 1 + B a_i \sqrt{I} \right) \]
where \( a \) is the ionic radius of a zwitter ion. The activity coefficient, \( \gamma \) necessary for the calculations of I, in ethanol-water mixtures were obtained from the works of Bates\(^{37} \).

The constants \( A \) and \( B \) were calculated from the dielectric constant, \( \varepsilon \) and densities, \( d \), of ethanol + water mixtures at 298 K by using the following relationships:

\[
A = 1.8246 \times 10^6 \ d^{1/2} \ (\varepsilon T)^{3/2}
\]

\[
B = 50.29 \ d^{1/3} \ (\varepsilon T)^{1/3}
\]

Having determined the dissociation constants \( pK^0 \) of amino acids in water and in ethanol-water mixtures, we have calculated \( \Delta G^0_{tr}(\text{diss}) \), the free energy of transfer from the solvent mixture (s) to water (W) from the equation

\[
\Delta G^0_{tr(diss)} = -RT\ln 10 \ (\Delta pK^0)
\]

The free energy calculated in this way is the sum of electrostatic free energy, \( \Delta G^0_{tr(el)} \), and the non-electrostatic (chemical) free energy, \( \Delta G^0_{tr(\text{chem})} \). Hence,

\[
\Delta G^0_{tr(\text{chem})} = \Delta G^0_{tr(diss)} - \Delta G^0_{tr(el)}.
\]

\( \Delta G^0_{tr(el)} \) was calculated from the Born equation,

\[
\Delta G^0_{tr(el)} = 122 \left( \frac{1}{\varepsilon_S} - 0.0128 \right) \left( \frac{1}{r(H^+)} + \frac{1}{r(A^-)} \right)
\]
The ionic radius of the solvated $H^+$ ion was taken as 2.8 Å$^3$. The radius of an amino acid anion was taken as 4.6 Å$^4$.

The $pK^0$ values of the three amino acids (Table 1$^2$) increase with increasing ethanol content in the solvent mixture. The variation in acidities of the $-\text{NH}_3^+$ group of amino acids determined in the solvent mixture containing 50 mole percent of ethanol and in pure water is not uniform and systematic, while the variation for the second $-\text{COOH}$ group of l-aspartic acid is similar to that generally observed for carboxylic acids like malonic acid$^4$.

Experimentally found deviations of the $\Delta pK$ values were compared with the calculated values by using the Born equation given earlier. Marked deviations from the theoretical line (Fig. 1) is shown for the $-\text{NH}_3^+$ dissociation of l-phenylalanine and l-histidine and $-\text{COOH}$ of l-aspartic acid; on the other hand, $-\text{NH}_3^+$ dissociation of l-aspartic acid exhibits a reasonably satisfactory agreement with the theoretical line until 30 mole percent of ethanol. The only difference in the aspartic acid species and that of l-phenylalanine and l-histidine is that the overall charge of the first is negative while the latter two is zero as these are zwitter ions. These deviations suggest that the effect of the ethanol + water mixtures on the acidity of
<table>
<thead>
<tr>
<th>% of ethanol (v/v)</th>
<th>0</th>
<th>20</th>
<th>40</th>
<th>60</th>
<th>80</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of ethanol (w/w)</td>
<td>0</td>
<td>16.16</td>
<td>33.24</td>
<td>52.03</td>
<td>73.45</td>
</tr>
<tr>
<td>Mole fraction of ethanol</td>
<td>0.00</td>
<td>0.0701</td>
<td>0.1630</td>
<td>0.2979</td>
<td>0.5197</td>
</tr>
<tr>
<td>Dielectric constant of solvent mixture</td>
<td>78.13</td>
<td>70.3</td>
<td>60.4</td>
<td>48.5</td>
<td>37.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>pH values</th>
</tr>
</thead>
<tbody>
<tr>
<td>l-Aspartic acid</td>
</tr>
<tr>
<td>(pK_{COOH(II)})</td>
</tr>
<tr>
<td>(pK_{NH_3^+})</td>
</tr>
<tr>
<td>l-phenyl alanine</td>
</tr>
<tr>
<td>(pK_{NH_3^+})</td>
</tr>
<tr>
<td>l-histidine</td>
</tr>
<tr>
<td>(pK_{NH_3^+})</td>
</tr>
</tbody>
</table>
the amino acids does not depend solely on the electrostatic features, as predicted by Born's theory. It may also be caused by ignoring the variations in dielectric constant of the solvent molecules in the vicinity of an ion.

The contribution of the chemical free energy (Table 1.21) does not exhibit a regular trend with increase in ethanol concentration. The magnitude and sign of the chemical free energy of transfer can provide a rough estimate of the difference in basicity of water and organic solvents. The chemical free energy term includes the medium effect and also the solute-solvent interactions. The irregular nature of \((\Delta G)_{\text{chem}}\) for the three amino acids indicate that the medium effect on the zwitter ion and the negatively charged ion have an important bearing on the deprotonation of zwitter ions, more so at higher ethanol percentages. Understanding of these effects is possible, if the overall effect can be dissected and the contribution of individual species involved in the dissociation equilibria examined.

b) Various terms in the free energy of transfer

The solvent effects on the deprotonation of acids \((A^{Z+})\) of different charge types become easier to understand if we consider the protolytic equilibria of the acid-base system \((A^{Z+} + B^{(Z-1)+})\) in a particular solvent SH with
Table 1.21

Contribution of the electrostatic, $\Delta G^0_{\text{elec}}$, and $\Delta G^0_{\text{fr(chem)}}$ free energies (KJ : mole$^{-1}$) of transfer of dissociation, $\Delta G^0_{\text{diss}}$ from water to water-ethanol mixtures for amino acids at 298 K

<table>
<thead>
<tr>
<th>Vol. % of ethanol</th>
<th>$(\Delta G^0_{\text{elec}})$</th>
<th>l-aspartic acid</th>
<th>l-phenyl alanine</th>
<th>l-histidine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$(\Delta G^0_{\text{fr(diss)}})$</td>
<td>$(\Delta G^0_{\text{chem}})$</td>
<td>$(\Delta G^0_{\text{fr}})$</td>
<td>$(\Delta G^0_{\text{chem}})$</td>
</tr>
<tr>
<td></td>
<td>-COOH (II)</td>
<td>NH$_3^+$</td>
<td>-COOH (II)</td>
<td>NH$_3^+$</td>
</tr>
<tr>
<td>20</td>
<td>0.42</td>
<td>4.58 ; 0.68</td>
<td>4.16 ; 0.26</td>
<td>-1.15</td>
</tr>
<tr>
<td>40</td>
<td>1.12</td>
<td>5.72 ; 1.72</td>
<td>4.60 ; 0.60</td>
<td>1.15</td>
</tr>
<tr>
<td>60</td>
<td>2.36</td>
<td>8.19 ; 2.52</td>
<td>5.83 ; 0.26</td>
<td>1.61</td>
</tr>
<tr>
<td>80</td>
<td>4.68</td>
<td>8.87 ; 2.64</td>
<td>3.81 ; -2.04</td>
<td>0.80</td>
</tr>
</tbody>
</table>
respect to that in the reference solvent water

\[ A^{z+}(s) + B^{(z-1)}(w) + SH(s) + H_2O^+(w) \rightleftharpoons A^{z+}(w) + B^{(z-1)^+}(s) + H_2O(w) + SH_2^+(s) \] ................................. (1)

In process (1), (w) and (s) denote that the respective species are in the standard state in the reference solvent water \((H_2O)\) and the solvent \((SH)\), respectively.

Consequently, the standard free energy change 
\((\Delta G_{\text{diss}}^0)_{A^{z+}}\) accompanying process (1) is given by equation (2) where:

\[ \Delta G_{\text{diss}}^0 = \Delta G^0_{\text{tr}}(H^+) + \Delta G^0_{\text{tr}}(B^{(z-1)^+}) - G^0_{\text{tr}}(A^{2+}) \] ................................. (2)

\(\Delta G^0_{\text{tr}}(H^+)\) stands for the free energy change of the process (3) and

\[ SH(s) + H_2O^+(w) \rightleftharpoons H_2O(w) + SH_2^+(s) \] ................................. (3)

\(\Delta G^0_{\text{tr}}(i)\) is the Gibb's energy change accompanying the transfer of the species \((i)\) from the standard state in the reference solvent water \((w)\) to that in the solvent \((s)\), i.e. of the process \(i(w) \rightarrow i(s)\).

Evidently, solvent effects on the protolytic equilibria of the acids in the solvent with respect to that in water
are related to the deprotonation constant (eq. 4)

\[ \delta (\Delta G^0_{\text{diss}})_{A^+} = 2.303 \, RT \left[ P_{(s)A^+} - P_{(w)A^+} \right] \]  \quad \cdots \quad (4)

Consequently, as equations (2) and (4) suggest, the estimates of individual species are an aid to better understanding of the solvent effects on deprotonation of the acids, provided \( \Delta G^0_t \) of the neutral acid A be determined by a suitable method and that of \( H^+ \) ion concentration in the solvent be known.

The solvent effect on deprotonation constants of the acids relative to that in water \( \delta (\Delta G^0_{\text{diss}}) \), was computed on mole fraction scale using relation (5) where \( M_w \) and \( M_s \) are the molar (or mean molar) mass of water and the mixed solvents respectively

\[ \delta (\Delta G^0_{\text{diss}}) = 2.303 \, RT \left[ P_{(s)K^0} - P_{(w)K^0} \right] + 2.303 \, RT \frac{M_s}{M_w} \]  \quad \cdots \quad (5)

Standard free energies of the neutral acid (\( H_2A \)) from water to mixed solvents can be obtained by the method of Bates et al.\(^\text{27} \) using relation (6) where \( \zeta \) stands for the degree of ionization of the acid in its saturated solution in the respective solvents. \( \zeta \) values are obtained by the use of expression (7):


\[ \Delta g^0_w(H_2A) = 2.3 \text{ RT log} \frac{(1 - \alpha_w)S_w}{(1 - \alpha_s)S_s} + 2.303 \text{ RT log} \frac{M_B}{M_W} \ldots \ldots (6) \]

\[ \alpha = \frac{-(K)_{H_2A}^+ \sqrt{(K)^2_{H_2A} + 4(K)_{H_2A} S Y^2}}{2 S Y^2} \ldots \ldots (7) \]

In these expressions, S stands for solubility and \( \gamma^+ \) the mean activity coefficient.

i) Protonation equilibria for amino acids:

The equilibria may be written as

a) \( H_2A^+ \xrightleftharpoons{pK_{COOH}(I)} H_2A^+ \xrightarrow{pK_{COOH}(II)} HA^- \xrightleftharpoons{pK_{NH_3}^+} A_2^- \) for l-aspartic acid

b) \( H_2A^+ \xrightarrow{pK_{COOH}} HA^+ \xrightarrow{pK_{NH_3}^+} A^- \) for l-phenyl alanine and l-histidine.

Expressions similar to (2) and (4) were written for the various protolytic dissociation. The magnitudes of several free energies of transfer, involved in these processes, were calculated. The data are set out in Tables 1, 2, 2 and 1, 2, 3.

In ethanol-water mixtures, \( \Delta g^0_t(H^+) \) values are found to be increasingly negative indicating increased stabilization of \( H^+ \). The stabilization increased pretty
Table 2.22

Values of $\delta (\Delta G^0)_{H_2A^\pm}$, $\Delta G^0(H_2A^-)$, $\Delta G^0(H^+)$ and $\Delta G^0(HA^-)$ for l-aspartic acid. The $\Delta G$ values are expressed in KJ mole$^{-1}$. The solubilities of l-aspartic acids are expressed in log units.

<table>
<thead>
<tr>
<th>% Ethanol v/v</th>
<th>log S</th>
<th>$\delta (\Delta G^0)_{diss H_2A^\pm}$</th>
<th>$\Delta G^0(H_2A^\pm)$</th>
<th>$\Delta G_t(H^+)$</th>
<th>$\Delta G^0(HA^-)$</th>
<th>$\delta (\Delta G^0)_{diss HA^-}$</th>
<th>$\Delta G^0(A^{2-})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-3.168</td>
<td>0.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>-3.513</td>
<td>4.16</td>
<td>0.90</td>
<td>-5.1</td>
<td>10.16</td>
<td>0.45</td>
<td>15.71</td>
</tr>
<tr>
<td>40</td>
<td>-3.793</td>
<td>4.90</td>
<td>1.55</td>
<td>-12.0</td>
<td>18.45</td>
<td>0.92</td>
<td>32.37</td>
</tr>
<tr>
<td>60</td>
<td>-4.114</td>
<td>6.75</td>
<td>2.02</td>
<td>-20.2</td>
<td>29.04</td>
<td>1.41</td>
<td>50.62</td>
</tr>
<tr>
<td>80</td>
<td>-4.582</td>
<td>8.16</td>
<td>2.83</td>
<td>-21.2</td>
<td>32.18</td>
<td>2.21</td>
<td>53.40</td>
</tr>
</tbody>
</table>
Table 2.23

Values of $\delta (\Delta G^o)_{diss}^{HA^\pm}$, $(\Delta G^o)_{HA^\pm}$, $(\Delta G^o)_{A^-}$ and solubility of l-histidine and phenylalanine

<table>
<thead>
<tr>
<th>% of ethanol v/v</th>
<th>log S</th>
<th>$\delta (\Delta G^o)_{diss}^{HA^\pm}$</th>
<th>$(\Delta G^o)_{HA^\pm}$</th>
<th>$(\Delta G^o)_{A^-}$</th>
<th>log S</th>
<th>$\delta (\Delta G^o)_{diss}^{HA^\pm}$</th>
<th>$(\Delta G^o)_{HA^\pm}$</th>
<th>$(\Delta G^o)_{A^-}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-0.754</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-0.585</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>-0.932</td>
<td>-1.46</td>
<td>0.65</td>
<td>4.36</td>
<td>-0.869</td>
<td>-2.08</td>
<td>1.26</td>
<td>4.28</td>
</tr>
<tr>
<td>40</td>
<td>-1.027</td>
<td>0.39</td>
<td>0.80</td>
<td>13.41</td>
<td>-1.178</td>
<td>-2.29</td>
<td>2.58</td>
<td>13.28</td>
</tr>
<tr>
<td>60</td>
<td>-1.108</td>
<td>0.28</td>
<td>0.95</td>
<td>21.43</td>
<td>-1.522</td>
<td>-3.5</td>
<td>3.98</td>
<td>20.68</td>
</tr>
<tr>
<td>80</td>
<td>-1.420</td>
<td>-0.78</td>
<td>0.77</td>
<td>24.56</td>
<td>-2.188</td>
<td>-4.22</td>
<td>7.04</td>
<td>31.22</td>
</tr>
</tbody>
</table>
fast in the initial stages and then exhibits a slow change after 15 mole percent of ethanol. It is well established that small quantities of monohydric alcohols promote the three-dimensional structure of water\textsuperscript{43, 44} while larger amounts break up this promoted structure, so that there is a region in the water-rich composition where structure promotion is maximum.

ii) Free energy terms for l-aspartic acid:

\[ \Delta G^0_t \text{ vs composition profiles (Fig. 2) for } H_2A^\pm, HA^- \text{ and } A^{2-} \text{ species of l-aspartic acid are increasingly positive showing the increased destabilization of the species with an increased proportion of ethanol. In the case of } HA^- \text{ and } A^{2-} \text{ an initial sharp increase is followed by a slow destabilisation. The change in } \Delta G^0 \text{ for the zwitter ion, } H_2A^\pm, \text{ seems to be very small and is almost 20 fold and 45 fold less than } HA^- \text{ and } A^{2-} \text{ respectively, in the initial stages of ethanol addition. The relative order } A^{2-} \gg HA^- \gg H_2A^\pm \text{ is similar to the basicity order of these species.}

It is known that a general amphiphilic solute molecule M is represented by \( R(X_1, X_2 \ldots) \) where R is the hydrophobic moiety and \( X_1, X_2 \ldots \) are hydrophilic groups capable of undergoing hydrogen bonded interactions with water or any other solvent. In pure water as well as in an ethanol-water mixture, M is enclosed by a hydrophobically related skin.
and $X_1$, $X_2$... enter into H-bonding with water or cosolvent molecules at the skin surface.

In the case of zwitter ion of aspartic acid, the hydrophilic moiety will not be significant as in the case of organic molecules or ions with an aromatic nucleus e.g. nitroaniline. Hence, the dominant field/effect would mainly be due to H-bonding between the carboxylate group and the water molecules surrounding it in the primary hydration. Thus the positively charged $-\text{NH}_2$ group is likely to be solvated preferentially by more 'basic' alcohol molecules and the negatively charged $-\text{COO}^-$ group by the more 'acidic' water molecules. The variation in free energy of transfer for $H_2A^\pm$, therefore, does show weak destabilisation during the alcohol addition. The behaviour exhibited by $HA^-$ and $A_2^-$ is almost similar but is much different than that of $H_2A^\pm$. The two carboxylate ions in $HA^-$ will make the overall charge of the species to be negative, and would accelerate the H-bonding in the initial stages of ethanol addition. The destabilisation for $A^{2-}$ in the initial addition of ethanol shows a steep rise, amounting almost to 30 KJ mole$^{-1}$ when 15 mole % of ethanol is added. The corresponding change for $HA^-$ is 12 KJ mole$^{-1}$. This can be explained as due to the absence of the $\text{NH}_3^+$ group in $A^{2-}$ and also the twice negative charge of the $A^{2-}$ species.
Two negatively charged species, $A^{2-}$ and the absence of any hydrophilic aromatic nucleus in aspartic acid, results in a significant destabilisation of this species on the first addition of ethanol. This behaviour is identical to the behaviour of $Cl^-$ reported by Kundu et al.\textsuperscript{45}. The counter cation i.e. $H^+$ on the other hand shows a prominent stabilisation in the initial stages of addition of ethanol. This observation is in line with the accepted electrolytic nature of zwitter ion.

iii) Free energy terms of l-phenylalanine and l-histidine:

The changes in $\Delta G^0_t(HA^\pm)$ for l-phenylalanine and l-histidine are of the same order and follows the same trend as in $\Delta G^0(H_2A^\pm)$ for l-aspartic acid (Fig. 3). The addition of ethanol produces a destabilisation in the zwitter ion and the maximum value of destabilisation at 80 % ethanol is $\sim 5$ KJ mole$^{-1}$. Further, the destabilisation is continuous and slow. The state of affairs are different in the case of $\Delta G^0(A^{2-})$ for both i.e. l-phenylalanine and l-histidine. In these cases, the change up to 30 mole percent is rapid and levels off beyond this mole percent. The sharp destabilisation of $A^{2-}$ at the initial compositions is the effect of increased basicity of the medium. The levelling of destabilisation at higher compositions is the result of the opposing effects of increased dispersion
interactions and proportionate destabilising Born-type interactions on the doubly charged A\(^{2-}\).

The behaviour of these species should be seen in the light of overall alcohol-water structure and also the solute-solvent and the Born type interactions, the dispersion effects and the hydrophobic effect. It is known that in dilute solutions of alcohol, the alcohol molecule must in some way affect the structure of liquid water, either by being incorporated into the existing structure without changing this a great deal or by depolymerizing the three dimensional clusters. On the other hand, in dilute solutions of water in alcohols the available evidence\(^{46}\) favours the view that water breaks up the alcohol aggregates and water-centered association may take place. Moreover, though both ethanol and water are associated in H-bonding, water is able to form three dimensional clusters while ethanol form only linear or cyclic aggregates\(^{47}, 48\) in spite of the fact that the alcohol oxygen atom has two unshared electron pairs.

The study of thermodynamic properties of alcohol-water mixture has revealed that two competing processes takes place, one involving a water rich solution, the promotion of structures more stable than that of water itself, and in alcohol rich mixtures, the depolymerization of linear and/or
cyclic alcohol aggregates, possibly with the formation of water centered association complexes. The minima in enthalpies of mixing of alcohol-water occurs at ethanol mole fraction of \( \sim 0.2^{44} \). This is also the region after which the levelling effect is seen. In this region \( (\Delta G^0)_{\text{chem}} \) have low values. It seems, therefore, that in this region the overall structure of water is a principal factor, and the solute-solvent interactions are dominant only after a certain percentage of alcohol is added.
References