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THE EFFECT OF SOLVENT AND TEMPERATURE ON PROTONATION CONSTANTS OF DL-PHENYLALANINE IN DIFFERENT AQUEOUS SOLUTIONS OF METHANOL AT DIFFERENT TEMPERATURES

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ABSTRACT. This research work has two sections. In first section, the equilibrium constant for protonation processes of DL-phenylalanine (K_1, and K_2) were determined in binary mixed solvents of water–methanol, containing 0, 10, 20, 30, 40, 50, 60, 70, and 80 % (v/v) methanol, at T = 298.15 K and constant ionic strength (0.1 mol.dm^{-3}NaCl). The obtained data (K_1, and K_2) were analyzed using Kamlet, Abboud, and Taft parameters. In second section, for DL-phenylalanine in aqueous solution, the values K_1, and K_2 were determined at T = (298.15, 303.15, 308.15, 313.15, and 318.15) K and constant ionic strength (0.1 mol.dm^{-3}NaCl). Using these K_1, and K_2, the thermodynamic properties (changes of enthalpy, ΔH, changes of entropy, ΔS, and changes of Gibbs free energy, ΔG) were calculated for DL-phenylalanine in aqueous solution. In both sections, the values of K_1, and K_2 were determined using the spectrophotometric and potentiometric methods.

Keywords: *protonation constants, DL-Phenylalanine, potentiometric, spectrophotometric, changes of enthalpy, changes of entropy, and changes of Gibbs free energy.*

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INTRODUCTION

Amino acids are classified as biologically significant organic compounds, which are made from amine (-NH_2) and carboxylic acid (-COOH) functional groups, along with a side chain specific to each amino acid [1-2].

The attachment of the amine and the carboxylic acid group together to the first (alpha-) carbon atom is very important in biochemistry of amino acids [3].

Amino acids exist as zwitterions at the isoelectric point, in which both the amino and carboxylic acid groups are almost totally ionized; the physical properties of the amino acids are largely governed by the degree of ionization at different pHs. The side chain of an amino acid can alter its physical properties by modifying: the net charge at a given pH, the relative affinity for water, and the pH at which there is no net charge (the isoelectric point). Of course, peptides are themselves composed of covalently bonded amino acids; their properties will therefore be dominated by the nature of the side chains on the constituent amino acids [4].

Phenylalanine is an essential α-amino acid with the formula $C_9H_{11}NO_2$. It can be viewed as a benzyl group substituted for the methyl group of alanine, or a phenyl group in place of terminal hydrogen of alanine [5].

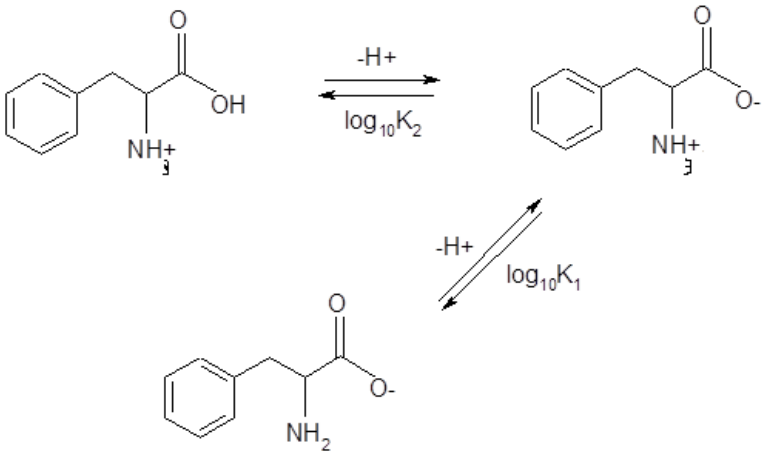
Phenylalanine exists in two forms: L-phenylalanine and D-phenylalanine. They are nearly identical but have slightly different molecular structure. The L-form is found in foods and used to produce proteins in human body while, D-form can be synthesized to use in certain medical applications [6].

Protonation constant of different species of amino acids and dipeptides were studied in different research works [7-8]. The protonation constant is used for determining solubility and permeability of solutions in the environmental and pharmaceutical fields [9–10]. There are various experimental methods to measure acid dissociation constant, such as HPLC, potentiometer, and spectrophotometry [11-12]. Solving is the first step to determine the physiochemical properties of a substance. Therefore, it is essential to measure the solubility of a substance in special solvent at different temperatures and various ionic strengths which can describe the thermodynamic system of solution, such as enthalpy and entropy changes of dissolving processes.

In modern food industry, amino acids are important to improve quality of food products. Among them D-L Phenylalanine is used as a food additive and it is essential to determine their thermodynamic properties and protonation constants.

In the present study, the protonation constants (K_1 and K_2) were determined for DL- phenylalanine in mixed solvent of water and methanol at constant temperature. These data were analyzed using Kamlet, Abboud, and

Taft parameters. Also, K_1 and K_2 were determined in aqueous solution at temperatures T = (298.15, 303.15, 308.15, 313.15, and 318.15) K. using these data, values of ΔH, ΔS, and, ΔG were calculated for DL-phenylalanine in aqueous solution.



Scheme 1. Chemical structure of DL-Phenylalanine

RESULTS AND DISCUSSION

Determination of protonation constant for D-L- phenylalanine

The protonation constants of DL-Phenylalanine were determined spectrophotometrically based on the relation A = f (pH) [13].

The computer program Squad was used to calculate the measured absorbance, A [(200–400) nm in the interval of 0.5 nm], and p_cH from the spectrophotometric titration [14, 15]. To fit the data in the computer program, the error square sum of the difference in the experimental absorbance and the calculated ones were minimized. The protonation constants with different stoichiometries can be determined by the program. Each titration had an experimental point (absorbance against p_cH) above 35 (maximum 50). The solutions were used without change during the experiments, and the absorbance values remained unchanged. Table 1 shows the results of spectrophotometric and potentiometric pH titrations for the acidity constants of the proton donors of the DL-Phenylalanine, Eq. 5, in various aqueous solutions of methanol and under various temperatures at aqueous solution accompanied by the values presented in the literature for comparison [16].

$H_nL^{n-1} + H^+ \rightleftarrows H_{1+n}L^n$; $K_{1+n} = \frac{[H_{1+n}L^n]}{[H_nL^{n-1}][H^+]}$ (1)

Where L shows DL-Phenylalanine molecule and n (charge of the ion) can be 0, 1, or 2 for the different protonation equilibria of the base.

Amino acids exist as zwitterions at the isoelectric point, in which both the amino and carboxylic acid groups are almost totally ionized; the physical properties of the amino acids are largely governed by the degree of ionization at different pHs. It is obvious that for a dibasic acid the first ionization constant K_1 is the sum $k_1 + k_2$ and the second ionization constant K_2 is $(k_{12} \cdot k_{21})/(k_{12} + k_{21})$, where the subscript 12 denotes loss of proton 2 following loss of proton 1 and subscript 21 denotes loss of proton 1 following loss of proton 2. The chemical interpretation of the changes is not straightforward, even though from model compounds the carboxyl proton is predicted to be the most acidic. The calculations involving the microscopic constants indicate that the first and second K correspond to removal of the carbonyl proton and the ammonium proton respectively. It can be determined by NMR spectroscopy exactly [17- 20].

Depending on the acidity of the medium (solution), hydrogen ions can add to DL-Phenylalanine molecule (Scheme 1) in two steps. In first and second step of protonation of DL-Phenylalanine, proton is added to carboxyl group and amine group, respectively. The second (Eq. 2), and first (Eq. 3) steps of protonation of DL-Phenylalanine occur according Eqs. 6 and 7:

$L^- + H^+ \rightleftarrows HL$ (2)

$HL + H^+ \rightleftarrows H_2L^+$ (3)

The protonation constants achieved in this examination are in fine agreement with those reported previously [21]. There are a few differences due to the various experimental methods that have been employed to determine the values. In Figure 1, the equilibrium distribution of various species of DL-Phenylalanine in water is indicated as a function of p_cH.

Figure 1 helps us find out the values of K,s for DL-Phenylalanine in water at various aqueous solutions of methanol and various temperatures. In Figure 1, the points a and b are the isoelectric points. In these points, the concentrations of the acid and the base are equal to each other. For an acid (HA), the below equation shows the relationship between $\log_{10}K$ and pH:

$\log_{10}K = pH + \log [A^-]/[HA]$ (4)

In Eq. 4, $[A^-]$ and [HA] are the concentrations of acid HA and base A-, respectively. At isoelectric points (a and b), $[A^-]$ = [HA] and pH = $\log_{10}K_a$. The calculations are based on the protonation constant values in various aqueous solutions of methanol and various temperatures given in Table 1 and Table 3, respectively.

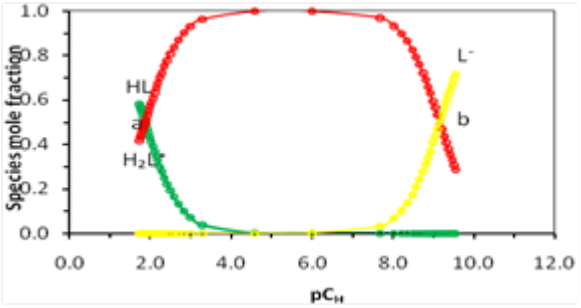


Figure 1. Distribution diagram of the different species of DL-Phenylalanine in water at 298.15 K and an ionic strength of 0.1 mol·dm^{-3} (NaCl).

Investigation of solvent effect

This research work shows that the different steps of protonation of DL-Phenylalanine, in water-alcohol mixed solvents, have different behaviors. The value of first protonation constant, K_1, increase and also the values of second protonation constant, K_2, decrease with increasing mole fraction of alcohol in water-alcohol mixed solvents (Table 1). It is very difficult to interpret the variation of the protonation constant values of DL-Phenylalanine regarding to the volume percentage of the methanol, in water-methanol solvent, using the dielectric constant of the solutions as the only parameter.

Table 1. Protonation constant of DL-Phenylalanine in aqueous solutions of methanol at 298.15 K and in NaCl 0.1 M

Alcohol V %	H_2O+Methanol		
	Log_{10} K_2	Log_{10} K_1	Ref.
0	1.86±0.03	9.15±0.02	21
10	2.02±0.03	8.96±0.01	This work
20	2.08±0.02	8.86±0.04	This work
30	2.12±0.04	8.75±0.03	This work
40	2.16±0.04	8.61±0.02	This work
50	2.22±0.03	8.47±0.03	This work
60	2.29±0.02	8.34±0.01	This work
70	2.36±0.04	8.226±0.03	This work
80	2.42±0.01	8.13±0.02	This work

In general, the standard Gibbs energy of protonation equilibria includes two terms: an electrostatic term, which can be estimated by the Born equation [22, 23], and a nonelectrostatic term, which includes specific solute-solvent interaction. When the electrostatic effects predominate on nonelectrostatic effects, according to the Born equation (Eq. 5), the plot of $\log_{10}K$ versus the reciprocal of dielectric constant of the media, ε, should be linear diagram as the below:

$$\Delta \log K = (121.6n/r)(1/\varepsilon - 0.0128) \quad (5)$$

where *r* is the common radius of the ions and n is the square summation of the charges involved in the protonation equilibria.

For example, n = 0, or 2 for the charge types $HL + H^+ \rightleftarrows H_2L^+$, $L^- + H^+ \rightleftarrows HL$, respectively. As it can be seen in Fig. 2, the correlation between $\log_{10}K_1$, and $\log_{10}K_2$ with reciprocal of the dielectric constant of the water–methanol mixtures are almost linear (with correlation coefficient between 0.91-0.93). It indicates that the protonation constants depend not only on the electrostatic forces but also strongly depend on the solute-solvent interactions of the different species in the mixtures [23]. Therefore, it is necessary to clarify the nature of solute-solvent interactions for a better understanding of the solvent effects.

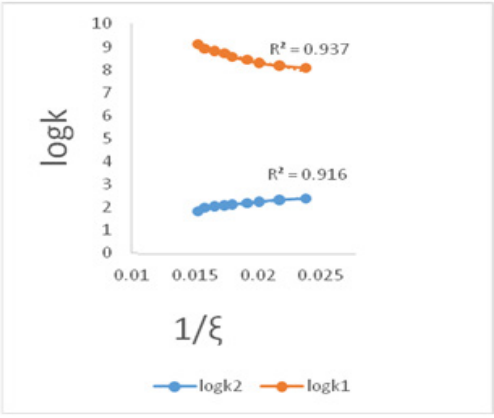


Figure 2. Plots of the experimental values of $\log_{10}K_1$, $\log_{10}K_2$ versus the reciprocal of the dielectric constant of different mixed solvents (water-methanol) at 298.15 K and an ionic strength of 0.1 mol.dm^{-3} (NaCl).

To study on the effect of solute-solvent interaction in the protonation or other equilibrium constants, a multi-parametric equation was used. This equation is based on the linear solvation energy relationship (LSER) theory and was developed by Kamlet, Abboud, and Taft (KAT) [24, 25]. The KAT equation contains non-specific as well as specific solute-solvent interactions, separately. These interactions can be subdivided into solvent Lewis-acidity interactions (hydrogen-bond acceptor, HBA solute, and hydrogen-bond donor, HBD solvent) and solvent Lewis-basicity interactions (HBD solute-HBA solvent). In general, all of these parameters constitute more comprehensive measures of solvent polarity than the dielectric constant or any other single physical characteristic, because they reflect more reliably the complete picture of all intermolecular forces acting between solute and solvent molecules. This approach has been widely and successfully applied in the correlation analysis of all kinds of solvent-dependent processes [26]. Using the solvatochromic solvent parameters (α, β,and π^*), a multiparametric equation, Eq. 6, has been proposed. This equation has been previously introduced [27,28].

$$\log K = A_o + a\alpha + b\beta + p\pi^* \quad (6)$$

In Eq. 6, A_o represents the regression value and π^* is the index of the solvent dipolarity/polarizability which is a measure of the ability of a solvent to stabilize a charge or a dipole by its own dielectric effects. The coefficient α represents the solvent hydrogen-bond donor (HBD) acidity. In a solvent, this coefficient (α) describes the ability of a solvent to donate a proton to a solute and generate hydrogen bond. The coefficient β is the solvent hydrogen-bond acceptor (HBA) basicity. This coefficient (β) describes the ability of a solvent to accept a proton, from a solute, and generate hydrogen bond. The regression coefficients (a, b, and p) can show the relative susceptibilities of the solvent dependence of $\log_{10}K$,s to the solvent parameters. In order to explain the $\log_{10}K$ values through the KAT solvent parameters, the protonation constants were correlated with the solvent properties by means of single, dual, and multiple regression analysis by a suitable computer program (Microsoft Excel Solver and Linest) [29,30]. We used the Gauss–Newton non-linear least-squares method, in the computer program, to refine the $\log_{10}K$ by minimizing the error squares sum from Eq. 7.

$$U=\sum(\log_{10}K_{exp}-\log_{10}K_{calc})^2 \quad (7)$$

The procedure that is used in the regression analysis involves a rigorous statistical treatment to find out which parameter, in Eq. 6, is the best suited for water-methanol mixed solvents. So, a stepwise procedure and least-squares analysis were applied to select the significant solvent properties to be

influenced in the model and to obtain the final expression for the protonation constants. Therefore, the KAT equation, Eq. 6, was reduced to single, dual, and multiparameters for correlation analysis of log10K in various solvent mixtures. The used computer program can calculate the values of A_o, a, b, p, and some statistical parameters including the r^2 regression coefficient, f-test (f), the residual sum of squares (rss), standard deviation of any parameter and the overall standard error (ose) for $\log_{10}K$. For all water-methanol mixtures that were used in this work, the KAT parameters and the dielectric constants values were obtained from the plot of each property versus the mole fraction of methanol (in various methanol- water solutions). Some other percentages of aqueous solutions of methanol are listed in Table 3 [31, 32].

Table 2. KAT solvatochromic and the dielectric constants of different aqueous methanol.

Methanol % (v/v)	α^a	β^a	π^{*a}	ε^b
0	1.17	0.47	1.09	78.60
10	1.15	0.49	1.04	74.83
20	1.13	0.51	0.99	70.86
30	1.11	0.53	0.94	66.67
40	1.09	0.55	0.89	62.26
50	1.08	0.57	0.85	57.60
60	1.06	0.58	0.80	52.70
70	1.04	0.60	0.75	47.54
80	1.02	0.62	0.70	42.15

a: Ref. [31], b: Ref. [32]

Although the solvent polarity is known as the main reason of the variation of $\log_{10}K$ values in water-methanol mixtures, the single-parameter and multiparameter correlations of $\log_{10}K_1$, and $\log_{10}K_2$ with π^*, α, and β didn't give us good results in all cases. However, the correlation analysis of $\log_{10}K_1$, and $\log_{10}K_2$ values with dual parameter equations show the more significant results compared to the single and/or multiparameter models. The obtained expressions of the KAT equation for each property, as dual-parameters, are shown below:

$$\log_{10}K_2 = 1.21(0.82)+2.04(0.65)\beta-0.22(0.44)\pi^*$$
$$r^2=0.997 \quad OSE=1.21*10^{-2} \quad rss=8.9*10^{-4} \quad F=1373.54 \quad (8)$$

$$\log_{10}K_1 = 10.89(0.71)-4.39(0.56)\beta+0.32(0.38)\pi^*$$
$$r^2=0.998 \quad OSE=1.17*10^{-2} \quad rss=8.2*10^{-4} \quad F=1735.35 \quad (9)$$

We obtained the KAT equations (eqs. 8 and 9) for DL-Phenylalanine, in both equations the correlation analysis of the dual parameter of the KAT equation, the value of coefficient β is greater than value of coefficient π^*($\beta > \pi^*$). It can be seen in Eq. 8, for the first ionization process the value of coefficient β parameter has positive value in this system, the hydrogen- bond acceptor basicity parameter plays a major role and the polarity parameter of the solvent has less significance. Therefore, $\log_{10}K_2$ values increase with increasing of the hydrogen bond basicity parameter. In Eq. 9 the second ionization process the value of coefficient β parameter is negative. This indicates the hydrogen- bond acceptor basicity parameter plays a major role. So, $\log_{10}K_1$ values decrease with an increase in the hydrogen bond basicity parameter [38, 39].

Investigation of temperature effect: thermodynamic analysis

The changes in Gibbs energy (ΔG), enthalpy (ΔH), and entropy (ΔS) are important thermodynamic parameters. The ΔG is the key parameter, because its value under a particular set of reactant concentrations dictates the direction of biomolecules equilibria in solutions. If its sign is negative, the binding reaction or conformational transition will proceed spontaneously to an extent governed by the magnitude of ΔG. If its sign is positive, the magnitude of ΔG specifies the energy needed to drive the reaction to form a product. The free energy is a balance between enthalpy and entropy [38, 39].

Table 3. protonation constant of DL-Phenylalanine in aqueous solution at temperatures 298.15 K to 318.15 K and in NaCl 0.1 M

Specie	*T (K)*	$\log K_2$	$\log K_1$	Ref
	298.15	1.86±0.02	9.15±0.02	21-25
	303.15	1.91±0.03	9.06±0.01	This work
DL-Phenylalanine	308.15	2.03±0.01	8.95±0.02	This work
	313.15	2.08±0.03	8.83±0.03	This work
	318.15	2.13±0.02	8.69±0.04	This work

The change in Gibbs energy in gas or solution phases can be calculated from Eq. 10:

$$\Delta G^{\circ} = -RT\ln K_a \approx 2.303\ RT\ pK_a \qquad (10)$$

In Eq. 10, R is the universal gas constant (8.314 J. K^{-1}.mol^{-1}), T is the temperature (K), and K_a is the equilibrium constant. The values of ΔH and ΔS can be determined using the van't Hoff equation and by plotting $\ln K_a$ vs. 1/T [35]:

$$pK_a = \Delta H/2.303RT - \Delta S/2.303\ R \quad (11)$$

The signs ΔG, ΔH, and ΔS can show the state of chemical reactions. The Chemical reactions can be spontaneous at each temperature when ΔH is negative and ΔS have positive values. Other states are observable in the references 35 to 39 [35- 39]. The values of temperature can affect the state of the chemical reactions when ΔH and ΔS have the same sign. As a result, the first and second ionization reactions of DL-Phenylalanine are spontaneous at low temperature.

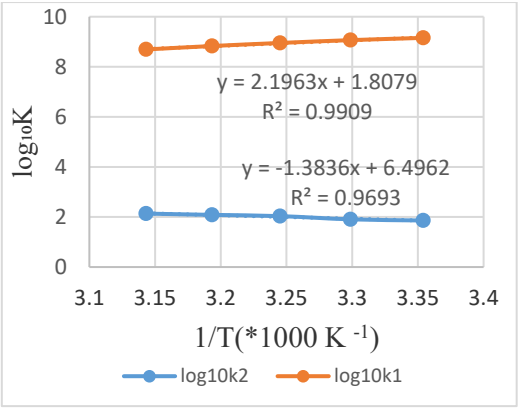


Figure 3. Curve - $\log_{10} K_a$ values vs. 1/T for DL-Phenylalanine.

It can be seen in Table 3 that in the protonation process of DL-Phenylalanine, $\log_{10}K_2$ increases with increasing temperature and also, $\log_{10}K_1$ decreases with increasing temperature.

Finally, we plotted $\log_{10}K$ versus 1/T for DL-Phenylalanine (Fig. 3). Using the slope and intercept of Eq. 11, the values of ΔH and ΔS were obtained.

Table 4. Thermodynamic properties for DL-Phenylalanine solved in water in constant ionic strength.

Specie DL-Phenylalanine	ΔH (kJ.mol^{-1})	ΔS (J.mol^{-1}.K^{1})	298.15 K	303.15 K	ΔG (kJ.mol^{-1}) 308.15 K	313.15 K	318.15
First ionization process	-26.5	-124.6	10.6	11.2	11.9	12.5	13.1
Second ionization process	42.1	-34.6	52.4	52.6	52.7	52.9	53.1

In addition, the values of ΔG for the deprotonation processes of DL-Phenylalanine were calculated using the pK_a values (Eq. 10) at each temperature. As shown in Table 4, the values of ΔG increase with increasing temperature; ΔS and ΔH are negative for the first ionization process and ΔS is negative for the second ionization process and ΔH is positive for the second ionization process. It is well known that $\Delta G = \Delta H - T\Delta S$. In this research work, the absolute value of $T\Delta S$ is more than the absolute value of ΔH. Therefore, the value of ΔG will be positive (see Table 4).

CONCLUSIONS

In this research work, the equilibrium constants of protonation process for DL-Phenylalanine were experimentally determined at the various temperatures and the different aqueous solutions of methanol. The results show that the value of $\log_{10}K_2$ increases and the value of $\log_{10}K_1$ decreases with increasing alcohol content of mixtures. In addition, the same results were observed when the temperatures increased in water solvent. The correlations of the constants with either a macroscopic parameter, such as dielectric constant, or a microscopic parameter, such as the Kamlet, Abboud, and Taft (KAT) solvatochromic parameters, were determined. The results showed the correlation between $\log_{10}K_2$ and $\log_{10}K_1$ with reciprocal of the dielectric constant of the water–methanol mixtures are almost linear and the KAT dual-parameter correlations show important improvements with respect to single- or multi-parameter models. The obtained results by examination of thermodynamic properties show that ΔG increases when the temperatures increase, ΔS, and ΔH are negative during the first ionization and ΔH is positive during the second ionization whereas ΔS is negative during the second ionization.

EXPERIMENTAL SECTION

Chemicals

DL-Phenylalanine, Scheme 1, was purchased from Sigma-Aldrich Company as reagent grade materials. Sodium hydroxide (NaOH) and hydrochloric acid (HCl) solutions were prepared from a titrisol solution (Merck Company). Sodium chloride (Merck Company) was dried using the vacuum at room temperature for at least 72 h before using. All dilute solutions were prepared using double-distilled water with a conductance equal to 1.2 ± 0.1 μS.

Apparatus

A Metrohm model 781 pH ion-meter was applied to determine the electromotive force (E). A 80-cm^3 thermostated double-walled glass vessel was used for all titrations. A UV-Vis Shimadzu 2100 spectrophotometer, with a Pentium 4 computer, as well as thermostat, matched at 10 mm quartz cells, were used for spectrophotometric and potentiometric measurements at the same time. To have data and put them in an excel program to calculate protonation constants. In addition, a flow type measurement cell was used. A circular thermostat was used to keep the temperature constant at the range ± 0.1 K. To measure the absorbance and the emf (electromotive force) of the solution at the same time, a peristaltic pump was used. This apparatus facilitates the circulation of the solution from the potentiometric cell to the spectrophotometric cell. To prevent carbon dioxide from staying in the system, a flow of purified nitrogen was allowed to pass through a sodium hydroxide solution and then it bubbled slowly through the reaction solution.

Procedure

In this study, the measurements were achieved at 298.15 K and a constant ionic strength of 0.1 $mol.dm^{-3}$ sodium chloride (NaCl) and with 0.1 $mol.dm^{-3}$ sodium hydroxide solution both with the same ionic strength and mole fraction of organic solvent [(0–80) % methanol v/v]. Also in this work under various temperatures (298.15 K to 318.15 K), the procedure was repeated at an ionic strength of 0.1 mol/dm^3. The protonation constants were evaluated from the measurements of absorbance against emf by titration of 25 mL of DL-Phenylalanine [(1.0×10^{-5} to 5.0×10^{-5} $mol.dm^{-3}$)].

In the first phase, the electrode system calibration was performed by Gran's method [40]. To this aim, a specified amount of an acidic solution (0.01 $mol.dm^{-3}$HCl), at the same temperature, solvent composition and constant ionic strength that were going to be used in subsequent experiments, was placed in the double-wall thermostated vessel. Next phase was the electrode immersion in the solution in the vessel and titration of the acidic solution with a strong base (0.1 $mol.dm^{-3}$NaOH, each addition 50 µL). Following each addition of the titrant, the potential effect was allowed to become stable. Next, to calculate the cell parameter (E°), and electrode calibration slope, Nernstian parameter (k), the reported emf values were used. This method was continued to pH ≅1.5.

Secondly, a sodium hydroxide solution (0.1 $mol.dm^{-3}$) was used to titrate 25 mL of an acidic solution (0.01 $mol.dm^{-3}$HCl) of DL-Phenylalanine (1.0×10^{-5} to 5.0×10^{-5} $mol.dm^{-3}$) at the same temperature, ionic strength, and solvent composition. Then, the emf and the absorbance values (in the

range of 200 to 400 nm and interval of 0.5 nm) were determined. The procedure was repeated in various compositions of the mixed solvent in ionic strength 0.1 mol/l and various temperatures. Following the method explained in the literature [41, 42], the reported emf values were then converted to p_cH (−log $[H^+]$).

In a solution, the measured potential of the cell could be expressed as Eq. 12:

$$E_{cell} = E^{\circ}_{cell} + k\log_{10}[H^+] + k\log_{10}\gamma H^+ + E_{LJ} \quad (12)$$

Where E°_{cell} represents the standard potential of the cell, E_{LJ} denotes the liquid junction potential, k = 2.303RT/F in which R, T and F have the usual meaning, and γH^+ shows the activity coefficient of hydrogen ion. Measurement of emf (electromotive force) versus H^+ concentration, in solution, is very useful because the computing of activity coefficients of hydrogen ion, in various aqueous mixtures of organic solvents, is very difficult. In this research work, the ionic strength of the solution is kept constant therefore the activity coefficient of hydrogen ion is also constant. The non-ideality of solutions is then included in E'_a (the specific constant of the potentiometric cell in the acidic region), therefore:

$$E_{cell} = E'_a + k\log_{10}[H^+] \quad (13)$$

Where E'_a is $E^{\circ}_{cell} + k\log_{10}\gamma[H^+] + E_{LJ}$. In calibration step, the values of E'_a and k were readily calculated from linear regression between E and $\log_{10}$ $[H^+]$ [43, 44].

In the acidic region, the concentration of hydrogen ion can be expressed as:

$$[H^+] = (M_{HCl}V_o - M_{NaOH}V1)/(V_\circ + V_1) \quad (14)$$

Where, M_{HCl} and M_{NaOH} are the molarities of hydrochloric acid and sodium hydroxide, respectively. $V_\circ$ and V_1 are the volumes of hydrochloric acid and sodium hydroxide, respectively, which were added to solution. Finally:

$$p_cH = (E'_a - E_{cell})/k \quad (15)$$

In equation 4, P_cH (or pH) is $-\log[H^+]$.

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