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Ultrasonic studies on molecular interaction of α-amino acids in aqueous solutions at different *p*H

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A novel method involving ultrasonic velocity measurement has been adopted for the accurate determination of iso-electric point (pI) of eleven α-amino acids at 303 K. The α-amino acids used are aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), phenylalanine (Phe), threonine (Thr), glycine (Gly), alanine (Ala), valine (Val), isoleucine (Ile), proline (Pro) and histidine (His). Allied acoustical properties like adiabatic compressibility (β), acoustical impedance (z), free length (L_f), relative adiabatic compressibility ($\Delta\beta/\beta_0$) apparent molal compressibility (ϕ_κ) available volume (V_a) are computed for aqueous solutions of these amino acids at different *p*H values. Conductance has been measured for aqueous solutions of these amino acids at 303 K at different *p*H values and pI values are evaluated from the *p*H value at which minimum conductance was observed. The pI values determined by ultrasonic and conductivity methods are compared with those obtained by electrometric method. There is found to be good agreement between the values obtained by different methods.

Keywords: Ultrasonic technique, Isoelectric point, Alpha amino acids

1 Introduction

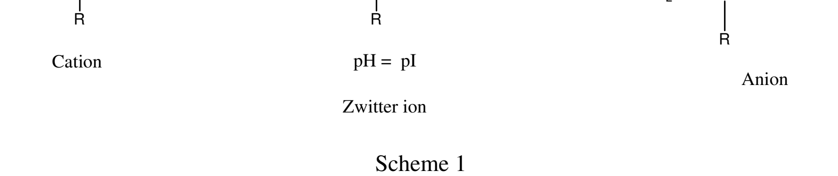
Amino acids are building blocks of proteins and they exhibit both acidic and basic properties. Hydrolysis products of proteins can yield twenty different amino acids, mostly α-amino acids. Besides, over 150 other amino acids are known to occur biologically in free and combined form but never in proteins. Some of the non-protein amino acids are β-alanine, homo cysteine, homo serine, citrulline ornithine and γ-amino butyric acid. They function as precursors or intermediates in metabolism[1-3]. Crystalline amino acids have relatively high melting or decomposition points and are much more soluble in water than in non-polar solvents. Amino acids exist as zwitter ions in neutral aqueous solutions. This is indicated by their high dielectric constants and their large dipole moments which are reflection of both positive and negative charges in the same molecule. Amino acids are ampholytes and they exist in different ionic forms depending upon the *p*H of the medium. At a particular *p*H,characteristic of the amino acid, it can exist in zwitter ion and at this *p*H the net electric charge in the molecule is zero. This *p*H value is called iso-electric point *p*H or iso electric *p*H, designated as pI.

Scheme 1 explains the form in which the amino acid is present at different *p*H.

Iso electric point (pI) is an important and characteristic property of an amino acid and protein. Different amino acids and proteins have different pI values and are used in the separation of these compounds. So, electric focussing[1-3] technique can readily resolve proteins that differ in pI as little as 0.01. The mobility of an ion in the presence of electric field is given by:

$$\upsilon = E\, e/f \qquad \ldots(1)$$

where, f is the frictional coefficient which depends on the size of the particle and viscosity of the medium, e



Scheme 1

the electric charge and υ is the electrophoretic mobility.

At pI value, there is no net charge and hence electrophoretic mobility is zero. Since conductance depends on electrophoretic mobility, the conductance of amino acid solution at pI is expected to be very low. The variation of ultrasonic velocity and absorption in inorganic, organic and organometallic binary systems have been used to access the molecular interactions in these systems[4,5]. Several researchers have used ultrasonic velocity measurements for studying ion-solvent interaction[6]. The propagation of ultrasound is useful in a number of areas such as medical ultrasonic, bio-technology[7], polymers[8-11] and detergents[12,13]. The ultrasonic measurement is used to determine transition temperatures of polymers[14] and related phase transition temperatures. The present work describes the ultrasonic velocity and density measurements of solution containing α-amino acids at 303 K. The main aim of this investigation is to determine the *iso*-electric *p*H values of eleven α-amino acids accurately and compare them with those obtained by electrometric method[18]. Acoustical parameters like adiabatic compressibility (β), acoustical impedance (z), free length (L_f), relative adiabatic compressibility ($\Delta\beta/\beta_0$), apparent molal compressibility (ϕ_κ), available volume (V_a) and equivalent conductivity values are reported in this paper.

2 Experimental Details

Alpha-amino acids used were L-aspartic acid, L-glutamic acid, L-asparagine, L-phenylalanine, L-threonine, glycine, L-alanine, L-valine, L-isoleucine. L-proline and L-histidine are of AnalaR grade Fluka (purum) samples. Acetic acid, sodium acetate, ammonium chloride and ammonium hydroxide used were GR grade samples obtained from SDS Chemicals, India. Water used in the present work was prepared by distilling distilled water over alkaline potassium permanganate. Acidic buffer was prepared by mixing appropriate volumes of 0.2 M sodium acetate and 0.2 M acetic acid solution. Basic buffer was prepared by mixing appropriate volumes of 0.2 M ammonium chloride and 0.2 M ammonium hydroxide solution.

Experimental solutions were prepared fresh and thermo stated before measuring ultrasonic velocity and density. Ultrasonic velocity measurements were made in F-81 model interferometer vibrating at 2 MHz frequency provided by Mittal Enterprises, New Delhi, India. The instrument was calibrated and the accuracy of the instrument is $\pm$ 0.1 ms^{-1}. Densities of the solutions were measured accurately using 10 ml specific gravity bottles in an electronic balance precisely within $\pm$0.1 mg accuracy. The densities of the liquids were determined by relative measurement method. Specific gravity bottle was standardized using double distilled water. The temperature was maintained constant by immersing the bottle in Juloba thermostat for 10 min. The density of the liquid and liquid mixtures can be calculated by using the formula:

$$\rho = (M\,\rho_w / M_w) \qquad \ldots(2)$$

where M is the mass of the liquid or liquid mixtures, M_w is the mass of water and ρ_w is the density of water at the experimental temperature.

Density of water at the experimental temperature was taken from the literature[15].

The conductance measurements were taken in SYSTRONICS (India) conductivity meter (Model 304) using a dip cell of cell constant unity. The conductivity meter was standardized by measuring the conductivity of double distilled water. Direct determination of the conductivity was carried. The *p*H of the solution was determined using ELICO (India) electronic *p*H meter (Model LI 613). The *p*H meter is standardized by using low and high *p*H buffer tablets provided by the manufacturer. The relevant equations used in the computation of acoustical parameters are given below[16,17]:

$$\beta = 1/U^2\rho \qquad \ldots(3)$$

where β is the adiabatic compressibility.

Acoustic impedance can be calculated using the following equation:

$$Z = U\rho \qquad \ldots(4)$$

where U is the ultrasonic velocity, ρ is the density

For computing the free length the equation used is given below:

$$L_f = K/U\rho^{1/2} \qquad \ldots(5)$$

In Eq. (5), K is temperature dependent constant which is given by:

$$K = (93.875+0.345\text{T}) \times 10^{-8}$$

Available volume V_a, apparent molal compressibility Φ_k and relative adiabatic compressibility $\Delta\beta/\beta_0$ were calculated using the following equations:

$$V_a=V_m(1-U/U_0), V_m=M_{eff}/\rho,\ U_0=1600\ ms^{-1} \quad \ldots(6)$$

$$\Phi_k=[1000(\rho_0\beta-\rho\beta_0)/M\rho_0]+(\beta_0 M/\rho_0) \quad \ldots(7)$$

$$\Delta\beta/\beta_0=(\beta-\beta_0)/\beta_0 \quad \ldots(8)$$

In Eqs (6-8), β_0 is the adiabatic compressibility of pure buffer solution.

3 Results and Discussion

Hydrolysis of a protein yields an assortment of α-amino acids, consisting of carboxylic acid (–COOH) and amino (–NH_2) functional groups attached to the same tetrahedral carbon atom. Side chain groups distinguish one amino acid from other. Due to the presence of acidic and basic groups in amino acids, the internal salt formation by a proton transfer occurs resulting into ammonium carboxylate structure. It is commonly referred to as a zwitter ion. Spectroscopic investigation confirms the zwitter ion structure[19]. The predominant molecular species present in an aqueous solution depend on the *p*H of the solution as the amino acids are ampholytes. At the *iso*-electric *p*H, the amino acid exists predominantly in the dipolar ion structure. It may be pointed out that the physical and chemical properties are influenced by the structural changes in amino acids and hence, the *p*H of the medium. Important physical properties which vary with *p*H are melting or decomposition temperature, solubility in water, dielectric constant and dipole moment. Electrical conductance depends on the ionic mobility and there is theoretically zero net charge on amino acid molecular species at pI. Therefore, electrophoretic mobility and conductance are very low at isoelectric *p*H.

Ultrasonic wave propagation through a medium depends on the nature and size of the particles present in its path. The effects of these factors on the acoustical properties of solution containing polymers have been investigated by several researchers[20]. At the *iso* electric point, strong ion-ion interactions are present between adjacent dipolar ions and hence, anomalous behaviour in ultrasonic velocity and related acoustical properties are expected at this *p*H value. Electrometric measurement was used to calculate pI of amino acid which is well supported by theoretical calculation[21].

In present paper, we report the ultrasonic velocity, density values which are useful to find out pI of α-amino acids. The measured ultrasonic velocity and density values for aqueous solutions of 0.005 M amino acids are given in Table 1. The adiabatic compressibility (β), acoustical impedance *z*), free length L_f), relative adiabatic compressibility ($\Delta\beta/\beta_0$), apparent molal compressibility ϕ_κ), available volume (V_a) and equivalent conductance Λ_c) at different *p*H values are also presented in Table 1. The amino acids are so chosen that there is wide range in the pI values. So the ultrasonic velocity measurements were made in buffer solution of *p*H range 2.5-3.4 for the α-amino acids Asp and Glu. For other amino acids, the acoustical properties are determined in the *p*H range 5-8.5.

Table 1 — Ultrasonic velocity, density, acoustical impedance, free length, adiabatic compressibility, relative adiabatic compressibility, apparent molal compressibility, available volume, equivalent conductance values for α-amino acids at different *p*H Temp=303 K
[Amino acid] = 0.005 M

*p*H	U m/s	ρ kg m^{-3}	$Z/10^6$ kg m^{-2} s^{-1}	$Lf/10^{-10}$ M	$\beta/10^{-10}$ M^2/N	$\Delta\beta/\beta_0/10^{-3}$	$\Phi_K/10^{-9}$ m^5/N/mole	$V_a/10^{-6}$ m^3	$\Lambda_c/10^{-3}$ mho m^2/eq
				Aspartic acid					
2.5	1507.2	973.8	1.46	0.422	4.52	27.50	4.350	1.08	3.59
2.6	1508.4	975.8	1.47	0.421	4.50	23.70	3.850	1.06	3.59
2.7	1509.2	989.4	1.49	0.418	4.44	8.60	1.320	1.04	3.19
2.8	1509.6	989.8	1.49	0.418	4.43	7.60	1.190	1.04	3.19
2.9	1512.4	992.4	1.50	0.416	4.41	1.30	0.410	1.00	2.80
3.0	1510.4	996.0	1.50	0.416	4.40	0.35	0.004	1.02	3.19
3.1	1509.8	990.2	1.50	0.418	4.43	7.00	1.100	1.03	3.19
3.2	1508.4	988.4	1.49	0.418	4.45	10.70	1.580	1.05	3.59
3.3	1507.8	976.2	1.47	0.421	4.51	24.10	3.840	1.07	3.59
3.4	1507.6	975.2	1.47	0.421	4.51	25.40	4.050	1.08	3.59

Contd —

Table 1 — Ultrasonic velocity, density, acoustical impedance, free length, adiabatic compressibility, relative adiabatic compressibility, apparent molal compressibility, available volume, equivalent conductance values for α-amino acids at different *p*H Temp=303 K [Amino acid] = 0.005 M

*p*H	U m/s	ρ kg m^{-3}	$Z/10^6$ kg m^{-2} s^{-1}	$Lf/10^{-10}$ M	$\beta/10^{-10}$ M^2/N	$\Delta\beta/\beta_0/10^{-3}$	$\Phi_K/10^{-9}$ m^5/N/mole	$V_a/10^{-6}$ m^3	$\Lambda_c/10^{-3}$ mho m^2/eq
				Glutamic acid					
2.5	1505.2	979.8	1.48	0.421	4.50	23.3	3.51	1.09	6.78
2.6	1505.6	982.6	1.48	0.421	4.49	20.1	2.96	1.09	6.39
2.7	1506.0	988.6	1.49	0.419	4.46	13.5	1.84	1.08	5.99
2.8	1506.6	989.0	1.49	0.419	4.45	12.3	1.69	1.07	5.59
2.9	1507.4	989.4	1.49	0.419	4.45	10.9	1.52	1.06	5.19
3.0	1508.0	989.6	1.49	0.418	4.44	9.9	1.42	1.05	4.78
3.1	1510.8	990.4	1.50	0.417	4.42	5.4	0.95	1.02	3.59
3.2	1509.6	989.6	1.49	0.418	4.43	7.5	1.21	1.03	7.58
3.3	1508.2	988.0	1.49	0.419	4.45	11.2	1.68	1.05	8.38
3.4	1507.0	987.6	1.48	0.419	4.46	13.2	1.90	1.07	8.78
				Asparagine					
5.0	1518.8	966.2	1.47	0.420	4.49	19.4	4.35	0.96	23.9
5.1	1519.0	968.4	1.47	0.420	4.48	16.9	3.93	0.95	23.5
5.2	1519.6	972.6	1.48	0.419	4.45	11.9	3.10	0.94	23.1
5.3	1522.0	977.6	1.49	0.417	4.42	3.7	1.93	0.91	22.7
5.4	1525.6	978.6	1.49	0.416	4.39	2.1	1.33	0.87	21.9
5.5	1524.0	967.4	1.47	0.419	4.45	11.5	3.52	0.89	25.9
5.6	1522.8	968.6	1.47	0.419	4.45	11.8	3.45	0.91	27.1
5.7	1521.6	971.8	1.48	0.418	4.44	10.1	3.01	0.92	28.3
5.8	1521.4	972.8	1.48	0.418	4.44	9.3	2.86	0.92	28.7
5.9	1521.2	973.6	1.48	0.418	4.44	8.8	2.74	0.92	29.5
				Threonine					
5.1	1518.0	978.8	1.49	0.418	4.43	7.7	2.18	0.95	21.5
5.2	1519.0	979.0	1.49	0.418	4.43	6.2	2.02	0.94	21.1
5.3	1520.2	980.0	1.49	0.417	4.42	3.6	1.71	0.93	20.8
5.4	1520.6	980.8	1.49	0.417	4.41	2.3	1.52	0.92	20.4
5.5	1521.0	980.2	1.49	0.417	4.41	2.4	1.58	0.92	20.4
5.6	1524.2	982.0	1.50	0.415	4.38	3.6	0.89	0.88	19.9
5.7	1523.0	981.8	1.50	0.416	4.39	1.9	1.06	0.89	27.5
5.8	1523.2	976.4	1.49	0.417	4.41	3.4	2.00	0.89	27.9
5.9	1522.4	974.6	1.48	0.418	4.43	6.3	2.41	0.91	30.3
6.0	1521.8	973.8	1.48	0.418	4.43	7.9	2.63	0.92	30.7
				Phenylalanine					
5.1	1512.0	988.0	1.49	0.418	4.43	6.3	1.24	1.01	30.7
5.2	1512.6	988.4	1.50	0.417	4.42	5.1	1.10	1.01	30.3
5.3	1513.6	988.0	1.50	0.417	4.42	4.2	1.05	0.99	29.9
5.4	1513.8	987.8	1.50	0.417	4.42	4.1	1.06	0.99	29.5
5.5	1514.0	986.4	1.49	0.417	4.42	5.3	1.29	0.99	29.1
5.6	1514.6	986.2	1.49	0.417	4.42	4.7	1.25	0.98	28.7
5.7	1517.6	990.4	1.50	0.415	4.38	3.5	1.59	0.95	27.5
5.8	1516.0	985.4	1.49	0.417	4.42	3.7	1.23	0.97	31.5
5.9	1515.6	984.8	1.49	0.417	4.42	4.8	1.39	0.98	32.3
6.0	1514.0	983.8	1.49	0.418	4.43	7.9	1.75	0.99	32.7
				Glycine					
5.5	1521.0	1002.4	1.52	0.412	4.31	19.8	-2.34	0.89	26.3
5.6	1521.2	1002.8	1.53	0.412	4.31	20.4	-2.43	0.89	25.9

Contd —

Table 1 — Ultrasonic velocity, density, acoustical impedance, free length, adiabatic compressibility, relative adiabatic compressibility, apparent molal compressibility, available volume, equivalent conductance values for α-amino acids at different *p*H Temp=303 K [Amino acid] = 0.005 M

*p*H	U m/s	ρ kg m^{-3}	$Z/10^6$ kg m^{-2} s^{-1}	$Lf/10^{-10}$ M	$\beta/10^{-10}$ M^2/N	$\Delta\beta/\beta_0/10^{-3}$	$\Phi_K/10^{-9}$ m^5/N/mole	$V_a/10^{-6}$ m^3	$\Lambda_c/10^{-3}$ mho m^2/eq
				Glycine					
5.7	1522.0	1002.2	1.53	0.412	4.30	21.9	-2.59	0.88	25.5
5.8	1523.2	1003.6	1.53	0.411	4.29	23.8	-2.80	0.87	25.1
5.9	1524.0	1004.8	1.53	0.411	4.29	26.0	-3.10	0.86	24.3
6.0	1525.6	1005.6	1.53	0.410	4.27	28.8	-3.40	0.84	23.6
6.1	1524.4	1003.8	1.53	0.411	4.29	25.5	-2.97	0.86	29.5
6.2	1524.2	998.6	1.52	0.412	4.31	20.2	-2.04	0.86	29.9
6.3	1524.0	998.2	1.52	0.412	4.31	19.5	-1.94	0.87	30.7
6.4	1523.8	997.6	1.52	0.412	4.32	18.7	-1.84	0.87	31.1
				Alanine					
5.5	1515.6	1014.2	1.54	0.411	4.29	24.3	-3.80	0.95	23.6
5.6	1516.4	1015.2	1.54	0.411	4.28	26.3	-4.00	0.94	22.0
5.7	1517.6	1013.2	1.54	0.411	4.29	25.9	-3.80	0.93	21.6
5.8	1520.8	1014.8	1.54	0.410	4.26	31.6	-4.50	0.89	20.8
5.9	1524.0	1015.4	1.55	0.408	4.24	36.2	-4.90	0.85	20.4
6.0	1527.6	1021.4	1.56	0.406	4.20	46.3	-6.40	0.81	16.8
6.1	1526.8	1017.8	1.55	0.407	4.21	41.9	-5.70	0.82	21.6
6.2	1525.8	1020.2	1.56	0.407	4.21	42.9	-5.90	0.83	23.6
6.3	1524.0	1021.0	1.56	0.407	4.22	41.4	-5.90	0.85	27.5
6.4	1523.4	1021.2	1.56	0.408	4.22	40.9	-5.90	0.85	29.5
				Valine					
5.5	1503.2	984.2	1.48	0.421	4.50	22.0	2.90	1.12	21.6
5.6	1506.0	984.6	1.48	0.420	4.48	17.8	2.60	1.08	20.8
5.7	1510.8	985.6	1.49	0.418	4.45	10.4	1.80	1.03	20.4
5.8	1514.4	987.8	1.50	0.417	4.41	3.3	0.99	0.98	20.0
5.9	1524.0	990.2	1.51	0.414	4.35	11.7	-5.40	0.87	19.6
6.0	1522.8	987.8	1.50	0.415	4.37	7.7	2.00	0.89	22.4
6.1	1522.4	987.8	1.50	0.415	4.37	7.1	6.60	0.89	23.2
6.2	1522.0	988.0	1.50	0.415	4.37	6.8	7.60	0.90	22.4
6.3	1519.8	988.4	1.50	0.415	4.38	4.3	2.60	0.93	24.0
6.4	1519.6	988.2	1.50	0.415	4.38	3.9	3.20	0.93	25.2
				Isoleucine					
5.5	1511.8	990.0	1.49	0.417	4.42	4.55	0.90	1.01	23.6
5.6	1512.0	989.2	1.50	0.417	4.42	5.09	1.02	1.01	23.1
5.7	1512.8	992.6	1.50	0.416	4.40	0.59	0.32	1.00	22.4
5.8	1514.0	997.4	1.51	0.415	4.37	5.79	-0.66	0.98	21.2
5.9	1516.0	998.0	1.51	0.414	4.36	9.01	-0.99	0.96	20.4
6.0	1522.8	1005.0	1.53	0.411	4.29	2.46	-2.99	0.88	20.0
6.1	1526.0	1003.2	1.53	0.411	4.28	2.70	-3.04	0.84	19.6
6.2	1523.6	1002.0	1.52	0.411	4.30	2.28	-2.56	0.87	27.1
6.3	1522.8	998.8	1.52	0.412	4.32	1.86	-1.91	0.88	27.9
6.4	1522.0	995.4	1.51	0.413	4.34	1.42	-1.22	0.89	29.1
				Proline					
5.7	1513.2	983.6	1.49	0.418	4.44	9.21	1.88	1.01	20.8
5.8	1514.2	984.6	1.49	0.418	4.43	6.86	1.58	0.99	20.4
5.9	1516.0	985.0	1.49	0.417	4.42	4.07	1.30	0.97	19.2
6.0	1517.8	985.2	1.50	0.417	4.41	1.48	1.06	0.95	18.8
6.1	1519.2	986.8	1.50	0.416	4.39	1.98	0.62	0.93	18.4

Contd —

Table 1 — Ultrasonic velocity, density, acoustical impedance, free length, adiabatic compressibility, relative adiabatic compressibility, apparent molal compressibility, available volume, equivalent conductance values for α-amino acids at different *p*H Temp=303 K [Amino acid] = 0.005 M

*p*H	U m/s	ρ kg m^{-3}	$Z/10^{6}$ kg m^{-2} s^{-1}	$Lf/10^{-10}$ M	$\beta/10^{-10}$ M^{2}/N	$\Delta\beta/\beta_0/10^{-3}$	$\Phi_K/10^{-9}$ m^{5}/N/mole	$V_a/10^{-6}$ m^{3}	$\Lambda_c/10^{-3}$ mho m^{2}/eq
				Proline					
6.2	1520.6	988.0	1.50	0.415	4.38	5.03	0.24	0.92	18.0
6.3	1524.8	988.6	1.51	0.414	4.35	11.10	-0.35	0.87	17.6
6.4	1522.4	984.8	1.50	0.415	4.38	4.20	0.59	0.89	18.4
6.5	1522.0	983.6	1.50	0.416	4.39	2.40	0.86	0.90	23.2
6.6	1521.8	982.8	1.50	0.416	4.39	1.30	1.02	0.91	24.0
				Histidine					
7.2	1544.6	986.0	1.52	0.409	4.25	33.70	-2.10	0.64	3.8
7.3	1544.8	987.0	1.52	0.409	4.25	34.90	-2.30	0.63	3.7
7.4	1545.0	990.2	1.53	0.408	4.23	38.30	-2.90	0.63	3.7
7.5	1546.6	992.8	1.54	0.407	4.21	42.80	-3.50	0.61	3.6
7.6	1548.8	999.6	1.55	0.405	4.17	52.10	-4.90	0.58	3.5
7.7	1548.0	998.8	1.55	0.406	4.18	50.30	-4.70	0.59	3.9
7.8	1547.2	998.2	1.54	0.406	4.18	48.70	-4.50	0.60	4.0
7.9	1545.6	998.6	1.54	0.406	4.19	47.10	-4.40	0.62	4.0
8.0	1545.2	998.4	1.54	0.406	4.20	46.40	-4.30	0.62	4.1
8.1	1544.0	998.0	1.54	0.407	4.20	44.60	-4.10	0.64	4.1

The pI values reported by earlier researchers are the *p*H values of the buffer employed or the theoretical values calculated from the pK values. But in the present investigation, the actual *p*H values of aqueous solutions containing α-amino acids at 0.005 M are measured accurately and these values are used in evaluation of pI values. At this concentration, the solvent-amino acid interactions are found to be strong. It is found that in a given system ultrasonic velocity increases with increase in *p*H, reaches maximum at characteristic *p*H and above this *p*H, it decreases. This trend indicates that the molecular interaction increases with *p*H and at pI the interaction is very low due to zwitter ion structure. The interaction decreases above this *p*H. Therefore, the *p*H value at which ultrasonic velocity is the highest may be taken as the *iso*-electric *p*H of the amino acid. Plots of U versus *p*H are given in Fig. 1(a-d). The *p*H value at which ultrasonic velocity is the maximum, can be accurately determined from the differential plots [Fig. 2(a-d)]. It may be noted that at pI, $\Delta U/\Delta p$H is the maximum.

Adiabatic compressibility values are computed (Table 1) from U and ρ values. The decrease in β values with increase in *p*H is indicative of strong interaction between amino acid and solvent molecules and β value is minimum at pI value. Above this *p*H, adiabatic compressibility increases, suggesting that the interaction is less above pI value. It is found that β is minimum at definite *p*H and this can be taken as the *iso* electric point. Instead, the relative adiabatic compressibility can be calculated which should be minimum at *iso*-electric *p*H. These values are given in

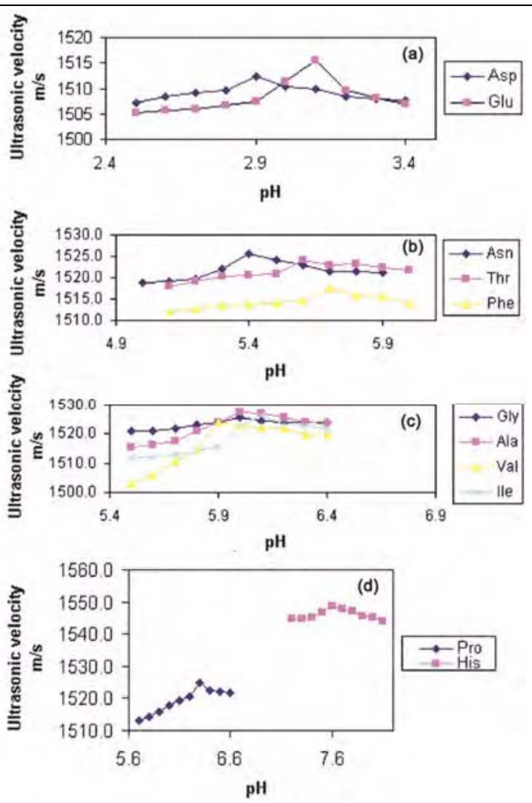


Fig.1 — (a-d) Plots of ultrasonic velocity versus *p*H for different amino acids

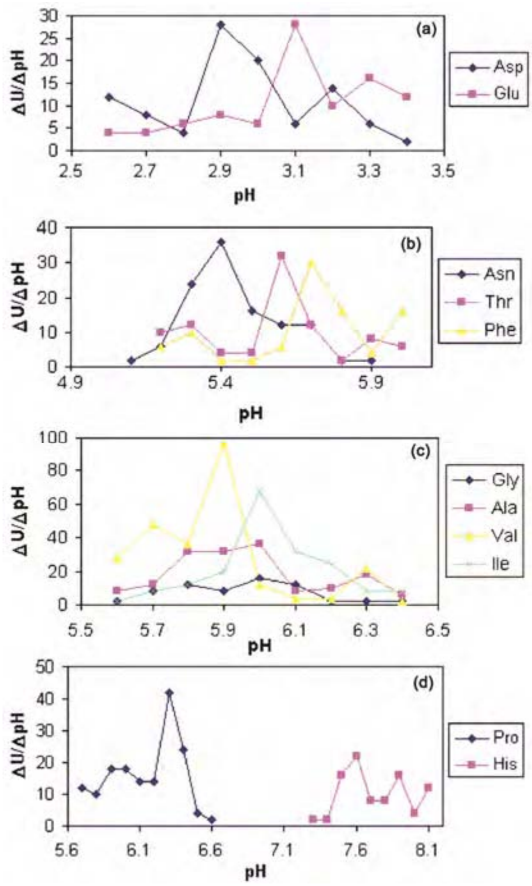


Fig. 2 — (a-d) Plots of $\Delta U/\Delta p$H versus pH for different amino acids

Table 1. It is observed that the relative adiabatic compressibility is minimum at *iso* electric pH. Free length values are computed for these systems at different pH (Table1). It is also the smallest at *iso* electric point suggesting strong interaction at pI value. The acoustical impedance values are calculated for eleven systems at different pH from 2.5-8.5 and they are given in Table 1. The sharp rise in acoustical impedance at *iso* electric point shows the strength of intermolecular attraction which increases suddenly at pI and this is supported by decrease in intermolecular free length at *iso* electric pH.

Since amino acids exist as zwitter ion in aqueous solutions, their volume and compressibility properties should reflect structural interactions with water molecules as in the case of electrolytes. The solute-solvent interaction may be predicted by the study of apparent molal compressibility (ϕ_κ) which is the compressibility of solution of one mole of solute minus the compressibility of the solvent. The $\phi_\kappa$values for aqueous solutions of α-amino acids at 303 K and at different pH values are presented in Table 1. The

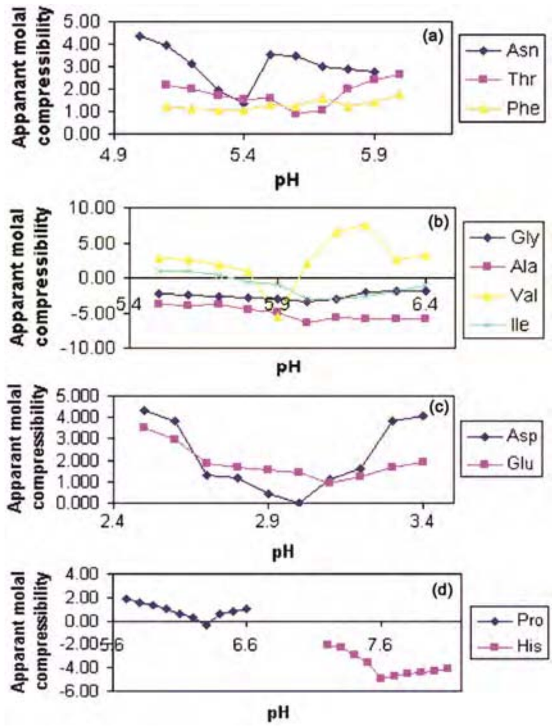


Fig.3 — (a-d) Plots of apparent molal compressibility versus pH for different amino acids

decrease in ϕ_κ value with increase pH may be due to electrostriction and indicates the hydrophobic and hydrophilic interactions in these systems at different pH values. At the *iso* electric pH, the electrostriction effect brings about the shrinkage in the volume of solvent, caused by the zwitter ionic form of the amino acid in solution as compared to that in pure solvent. Thus, ϕ_k value is minimum at pI value of amino acid as evident from the plots in Fig. 3 (a-d).

The conductance of an electrolytic solution at constant temperature depends on the concentration and the type of conducting species[22]. In aqueous solution of α-amino acid at constant temperature and at a particular concentration depends upon the structure of amino acid. Any structural change in the electrolyte should be reflected in the conductance at the same concentration. It is observed that the conductance of electrolyte is minimum at moderate concentrations at which there is ion pair formation[23]. The zwitter ion structure of amino acid may be similar to an ion pair and hence, the minimum conductance may be an indication of dipolar ion structure. The equivalent conductance values are determined for 0.005 M amino acids at 303 K (Table 1) and at different pH values. It is found that Λ is minimum for each amino acid at its pI value. Plots

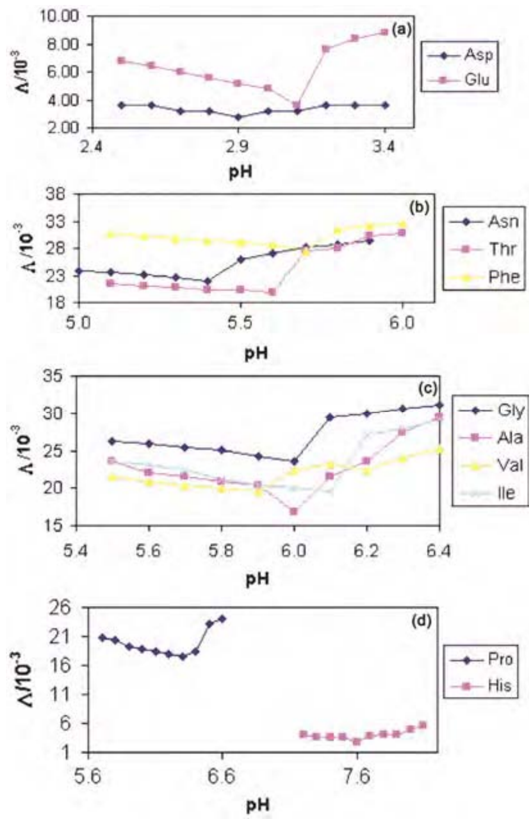


Fig. 4 — (a-d) Plots of equivalent conductance versus *p*H for different amino acids

Table 2 — *Iso*-electric points of α-amino acids obtained by different methods

α-amino acid	Ultrasonic method	Conductivity measurement method	Electrometric measurement[17]	Theoretical Calcuation[20]
Aspartic acid	2.9	2.9	3.0	3.0
Glutamic acid	3.1	3.1	3.1	3.1
Asparagine	5.4	5.4	—	5.4
Phenylalanine	5.7	5.7	5.9	5.9
Threonine	5.6	5.6	—	5.6
Glycine	6.0	6.0	6.1	6.0
Alanine	6.0	6.0	6.1	6.1
Valine	5.9	5.9	6.0	6.0
Isoleucine	6.0	6.0	6.0	6.0
Proline	6.3	6.3	6.3	6.3
Histidine	7.6	7.6	7.6	7.6

of Λ_C versus *p*H are given in Fig. 4(a-d).Thus, *iso* electric points of α-amino acids can also be determined by conductance measurement. These values along with the pI values obtained from the ultrasonic velocity measurement are given in Table 2. These values are comparable and agree well with those determined by electrometric method.

4 Conclusions

Acoustical properties are reported for aqueous solutions of eleven α-amino acids at 303 K and different *p*H values. Abnormal variation in these properties at *iso*-electric *p*H is used to evaluate their *iso* electric points. These values are found to agree satisfactorily with the pI values obtained by electrometric method. The anomalous variation in acoustical property at *iso* electric *p*H establishes the existence of zwitter ion structure at pI value.

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