

Molal Solubility, Dissociation, Association and Solvation Parameters for Saturated Phenylalanine Solutions in Various Solvents at 298.15 K

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Abstract The molal solubilities for saturated solutions of phenylalanine at 298.15 K in various solvents were determined. The solvents used are, water (W), ethanol (Et), dimethylsulphoxide (DMSO), acetonitrile (AN), methanol (Me), acetone (Ac), 1-4, dioxane (Di) and N, N-dimethylformamide (DMF). From the experimental data for solubilities, pH and densities, the different volumes, molar: electrostriction and Van der Waals and apparent molar volumes were estimated. The free energies of dissociation (ΔG_d), free energies of association (ΔG_A), difference free energies ($\Delta\Delta G$) and free energies of solvation (ΔG_s) for phenylalanine saturated solutions in various solvents were also calculated. The solvation numbers were also estimated. It was concluded that the solute-solvent interaction increased by increasing the solvation free energies due mainly to the greater of the association parameters in the organic solvents.

Keywords Solubility, Association Constants, Dissociation Constants, Densities, Different Volumes, Free Energies, Solvation Number, Phenylalanine

1. Introduction

Knowledge of the existence of amino acids dates back over a century in many cases, as does knowledge of their extended in proteins. When amino acids were discovered, their identity was established by isolating and purifying the individual compounds and obtaining elemental analysis. After the advent of paper chromatography, this technique was used with a variety of different solvents to identify elution characteristic and demonstrate the purity of isolated compounds. Amino acids were located by the use of a reagent that produced colour with the compound. The most common reagent used for locating amino acids is ninhydrin. Which produces a purple colour with imines, a pink or yellow color with amino acids, and various intermediate colors, with compounds containing an amino group and a sulfonid group and so on. It also reacts with small peptides such as glutathione. The techniques of paper chromatography were applied to the separation of mixtures of amino acids, such as the components of the protein after hydrolysis, and then to the separation of free amino acids in physiologic fluids and tissues.

It was extended by the use of two dimensional chromatography, in which a different solvent was used in each

direction[1]. Later electrophoresis was employed as one of the separating techniques. After locating the amino acids by reaction with ninhydrin, the spots could be cut out, eluted with alcohol or acetone and measured spectrophotometrically[2]. In order to measure the free amino acids in biological tissues and fluids, all proteins must be removed. Various methods have been employed to accomplish this, such as ultra filtration, equilibrium dialysis, and proton precipitation. A variety of protein precipitation has been employed for this purpose, the most common being picric acid. For extracting tissues the most common methods employ 10% trichloroacetic acid, usually by blending or homogenizing with 510 vol of acid followed by a high speed centrifugation[2]. Plasma, cerebrospinal fluid and urine are most commonly deproteinized using 5 vol 3% sulphosalicylic acid as protein precipitated, again followed by centrifugation. The precipitated proteins can be purified by washing with hot trichloroacetic acid to remove nucleic acids and with a variety of organic solvents to remove lipids. The proteins can then be hydrolysed, usually with 6N HCl under vacuum at 120°C for 24 h or more, and their amino acid composition determined[3].

Many publications have done on the behaviour of weak acids in anhydrous solvents. Interesting work has been done by Kolthoff et al.[4,5]. Aleksandrov et al.[6,7] studied the dissociation of salicylic acid in butane-2-one. Kreshkov et al.[8] studied the dissociation of amino acids (as weak) acids in mixtures of formic and ethylmethylketone and in mixtures of acetic acid-ethylmethylketone.

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Gomaa et al.[9] studied association, dissociation and hydrogen bonding of salicylic acid in water-N, N- dimethylformamide mixtures from solubility measurements.

The aim of this work is to evaluate the solubility of phenylalanine in different solvents and discuss in details the solvation parameters for the solubility process. Study the effect of different solvents of the solubility and the physical behaviour accompanied is very important for the biochemical analysers. Also apply a theoretical model for evaluating different microscopic interactions of phenylalanine is necessary.

The aim of this work is to evaluate the solubilities of phenylalanine in different solvents and discuss in details the solvation parameters for the solubility processes for phenylalanine which is one of the most essential amino acids.

2. Experimental

The phenylalanine and solvents used, ethanol (Et), dimethylsulphoxide (DMSO), acetonitrile (AN), methanol (Me), acetone (Ac), 1-4 dioxane (Di) and dimethylformamide (DMF) were supplied from Merck. The saturated solutions of phenylalanine were prepared by dissolving it in the solvents used. The solutions were saturated with N₂ gas in closed test tubes. The tubes were placed in a shaking water bath of the type assistant for a period of four days, followed by another two days without shaking to reach the necessary equilibrium.

The solubility of phenylalanine in each solution was determined gravimetrically by taking 1 ml of the saturated solution and subjecting it to complete evaporation using small aluminium disks heated by an infrared lamp.

The pH readings of the saturated solutions were measured using a pH-meter of the type Tacussel /Minis 5000. The densities were measured by using weighing bottle 1 ml and analytical balance (4 digits) of the type Mettler Toledo DA.

3. Results and Discussion

Table 1. Molal solubilities (m), densities (d) and pH values for saturated phenylalanine solution in different solvents at 298.15 K

Solvent	m/(g.mol/kg solvent)	d	pH
H ₂ O	0.1535	1.0246	6.760
Et (Ethanol)	2.4470x10 ⁻³	0.8255	7.352
DMSO (Dimethyl sulphoxide)	0.0071	1.1136	9.496
AN (Acetonitrile)	7.0530x10 ⁻⁵	0.7785	10.816
Me (Methanol)	2.3210x10 ⁻²	0.8167	7.240
Di (Dioxane)	1.08780x10 ⁻³	1.0296	5.616
Ac (Acetone)	1.4490x10 ⁻³	0.8008	8.210
DMF (Dimethylformamide)	2.6581x10 ⁻³	0.9529	10.048

Amino acid contents have been studied in a variety of biological samples. Of these, plasma, urine, cerebrospinal fluid, which are accessible and clinically significant, have received most attention of these samples investigated include amniotic fluid, blood cells, fees, sweat, saliva, hair, finger-

nails and fingerprints. Even small amounts of protein in biological samples interfere in chromatographic analysis and have been removed. Therefore solubility measurements are very important to solve this problem[1].

The calculated molal solubilities (m) phenylalanine saturated solution was given in Table (1) from at least three average measurements. Also the measured densities and pH values of the amino acid used are also listed in Table (1).

The molar volumes (V_M) of phenylalanine were obtained by dividing the molar mass by the densities and their values are listed in Table (2). The packing density as reported by Kim et al.[10] and El-Khouly et. al.[11], i.e. the relation between Van der Waals volume (V_W) and the molar volume (V_M) of relatively large molecules (above 40) was found to be a constant value and equal to 0.661.

$$P = \frac{V_W}{V_M} = 0.661 \pm 0.017 \quad (1)$$

The electrostriction volume (V_e) which is the volume compressed by the solvent, was calculated by using equation (2) after El-Khouly[12].

$$V_e = V_W - V_M \quad (2)$$

The molar, Van der Waals and electrostriction volumes of phenylalanine in various solvents at 298.15 K were tabulated in Tables (2).

The different association, dissociation parameters were calculated using model explained before by us concentrating phenylalanine zwitter ion has effective hydrogen atom and that is clear from the pH values which are in weak organic acid range. Various analytical methods have been described in literature, only a few were developed for clinical use. For a good route analytical procedure, several factors have to be considered. Cost (both time and money), choice of simple reagent, simplicity of the procedure, and chromatographic properties of derivatives[2]. In this work simple and cheap methods for analysis can be used.

Table 2. Molar volumes (V_M), Van der Waals volumes (V_W), electrostriction volumes (V_e) and apparent molar volumes (V_φ) for saturated solutions of phenylalanine in different solvents at 298.15 K (in cm³/mole)

Solvent	V _M	V _W	-V _e	V _φ
H ₂ O	161.2191	106.5658	54.6530	161.0436
Et	200.0993	132.2650	67.8337	174.6008
DMSO	148.3373	98.0510	50.2863	146.2907
AN	212.1846	140.2540	71.9300	171.8580
Me	202.2670	133.6980	68.5690	200.2480
Di	160.4360	106.0490	54.3880	158.0711
Ac	206.2780	136.3500	68.9280	188.9220
DMF	173.3550	114.5870	58.7680	169.7592

The apparent molar volumes V_φ[13-15] were calculated using equation (3)[16]

$$V_\phi = M/d_o - (d - d_o/dd_o) 1000/m \quad (3)$$

Where M is the molar mass of benzoic acid, m is the concentration; d and d_o are the densities of saturated solution and pure solvents, respectively.

The values of V_φ for phenylalanine in various solvents at 298.15 K are presented in Table (2).

The activity coefficient was calculated by using the relation $\log \gamma_{\pm} = -0.5062 \sqrt{m}$ [17,18].

K_{ass} values were calculated[14] from the ratios of association constant to dissociation constant (i.e., K_1/K_2) for the dimers of phenylalanine which form a complex ion ((HA_2^-)) and hydrogen ion (H^+), and the values of K' (where K is the dissociation constant of the associated acid complex, H_2A_2) are given by the following equations

$$K' = a^2 H^+ / m^2 \quad (4)$$

$$p_a H^+ = \frac{1}{2} \log \frac{K_1}{K_2} - \log m = pH - \log \gamma_{\pm} \quad (5)$$

$$\frac{K_1}{K_2} = K' K_{ass} \quad (6)$$

Where a is the activity. The values obtained K_1/K_2 , K' and K_{ass} are reported in Table (3). The maximum value of K_{ass} was found to be by using DMF where water is the least association parameters.

The free energies of dissociation (ΔG_d), free energies of association (ΔG_A), difference free energies ($\Delta\Delta G$) and free energies of solvation (ΔG_s) for phenylalanine saturated solutions in various solvents were calculated by using the following equations and tabulated in Table (4).

$$\Delta G_d = -RT \ln K' \quad (7)$$

$$\Delta G_{ass} = -RT \ln K_{ass} \quad (8)$$

$$\Delta\Delta G = \Delta G_{ass} - \Delta G_d \quad (9)$$

$$\Delta G_s = -RT \ln K_{sp} \quad (10)$$

$$pK_{sp} = -2 \log m - 2 \log \gamma_{\pm} \quad (11)$$

Table 3. Log activity coefficients ($\log \gamma_{\pm}$), dissociation constants (K) and association constants for phenylalanine saturated solutions in different solvents at 298.15 K

Solvent	$\log \gamma_{\pm}$	$p_a H^+$	K'	$\frac{K_1}{K_2}$	K_{ass}
H ₂ O	-0.1980	6.9586	2.0610×10^3	7.8521×10^{12}	3.8091×10^9
Et	-0.0282	7.3802	9.1000×10^6	3.4101×10^8	37.3622
DMSO	-0.0041	9.5001	1.7112×10^5	5.0800×10^{14}	2.9900×10^7
AN	-4.8100×10^{-4}	10.8160	2.3501×10^{10}	2.1321×10^{13}	907.2601
Me	-0.0860	5.6320	1.3002×10^3	3.6001×10^{-4}	2.7801×10^{-7}
Di	-0.0164	7.3260	2.6002×10^7	2.1010×10^5	8.0711×10^{-3}
Ac	-0.0217	7.3737	2.5801×10^6	1.1702×10^9	453.4880
DMF	-0.0268	10.0748	14.3001×10^5	9.9300×10^{14}	6.9100×10^7

Table 4. Free energies of dissociation (ΔG_d), free energies of association (ΔG_A), difference free energies ($\Delta\Delta G$) and free energies of solvation (ΔG_s) for phenylalanine saturated solutions in different solvents at 298.15 K (in kJoule/mole)

Solvent	ΔG_d	ΔG_A	$\Delta\Delta G$	ΔG_s
H ₂ O	-18.919	-54.694	-35.775	-10.163
Et	-39.727	-9.000	-12.817	-26.507
DMSO	-29.859	-42.676	-5.56464	-21.312
AN	-59.205	-16.884	42.321	-58.740
Me	-17.776	37.426	55.202	-17.279
Di	-42.330	11.949	54.279	-29.927
Ac	-36.602	-15.165	21.437	-28.931
DMF	-35.139	-44.749	-9.610	-26.134

Table 5. Molar volumes of solvents (V_s), difference in different Van der Waals volumes (ΔV_w) and solvation numbers (n_s) for phenylalanine a saturated solutions in different solvents at 298.15 K

Solvent	V_s	ΔV_w	n_s
H ₂ O	18.0724	116.083	6.423
Et	58.6804	90.383	1.540
DMSO	71.2995	124.597	1.747
AN	52.8450	82.394	1.559
MeOH	40.7322	88.980	2.184
Di	86.3552	116.599	1.350
Ac	175.411	86.298	0.491
DMF	77.4118	108.061	1.396

The solvation volumes for phenylalanine were evaluated from the difference between Van der Waals of phenylalanine in absence and given before in various solvents. The Van der Waals volume of phenylalanine was calculated from Bondi method[19] and found to be $222.648 \text{ cm}^3/\text{mole}$. Subtracting this value from V_w in solvents and divide the results by the molar volumes of the solvents taken from ref.[18], n (the solvation number) was obtained and given in Table (5).

It was concluded that the solute-solvent interaction increased by increasing $\Delta\Delta G$ and ΔG_s due mainly to the greatest of the association parameters in the corresponding solvents.

Also it was observed that the association, dissociation and microscopic interactions between solute and solvent is important for any solvent individually, whether it is polar, aprotic or amphiprotic. Increasing of the solvation numbers favour more solute-solvent interactions between phenylalanine and solvents. Also small solvation numbers favour more solute-solute interaction or ion pair formation resulting in the decrease of the solute-solvent interactions in the solvent under discussion. Big positive values for $\Delta\Delta G$ and big negative. This work gives a lot of data which help any analyst to use suitable solvent for phenylalanine in chromatographic analysis.

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