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Effect of Dielectric constant on Protonation Equilibria of Glycylglycine in Aqueous Solutions of propylene glycol and Dioxan

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ABSTRACT

Knowledge of the protonation constants of dipeptides is important and necessary for complete understanding of their physicochemical behaviour. In this study, the protonation constants of glycylglycine were studied in propylene glycol and dioxan-water mixtures (0 - 60% v/v) at 303.0 ± 0.1 K at an ionic strength of 0.16 M using pH-metric technique. The protonation constants were calculated with the computer program MINIQUAD75 and selection of the best fit chemical models is based on the statistical parameters. The effect of solvent on protonation constants was discussed based on electrostatic forces operating on the protonation equilibria. Distribution of species, protonation equilibria and effect of influential parameters on the protonation constants have also been presented.

Keywords: Glycylglycine, Propylene glycol, Dioxan; Protonation constants, MINIQUAD75, Dielectric constant.

INTRODUCTION

Peptides are an amazing class of compounds constructed from relatively simple building blocks, the amino acids. They are components of tissues [1] exhibiting a remarkable range of biological properties acting as antibiotics, hormones, food additives, poisons or pain-killers [2]. Investigations involving the peptides in acid-base reactions [3-6], particularly, small peptides have attracted great attention in relation to the bioinorganic chemistry because these compounds are usually considered as good model systems to attain a better insight into the characteristics of naturally occurring metalloproteins [7, 8]. On the other hand, several metal complexes containing peptide groups have displayed diverse pharmacological activities. For instance, copper complexes with amino acids and peptides as ligands show anti-inflammatory and cytostatic activities [9, 10].

Vast data are available on the protonation and stability constants of the amino acids and simple peptides in water and organic solvents [11-18]. However, the protonation constants of amino acids and peptides in these organic solvents are often different from those in water, as these media tend to be lipophilic rather than hydrophilic [19, 20]. Little is known about the chemistry of amino acids and simple peptides in mixed solvents, in regard to their protonation stability constants [21] and experiments shown that one solvent alone is not an ideal model for *in vivo* reactions [22]. It has been suggested that mixture of solvents such as organic solvent-water mixtures provide a better model for *in vivo* reactions. The hydrophobic and hydrophilic properties of a solute peptide may be reflected by preferential solvation in such mixed solvents. Therefore, the influence which the solvent exerts on the protonation constant (K) values depends upon the extent and nature of the solute-solvent interaction, which involves species participating in the acid-base equilibrium [23]. Hence, studies in media made up of organic solvent-water mixtures should provide some understanding of the chemistry of peptides in living systems.

Glycylglycine (GG) is the simplest dipeptide made from the residues of two glycine molecules. Besides an important component of the tissue, glycylglycine is a potentially useful zwitterionic buffer in the physiological pH range (6.0 - 8.5) [24]. In this paper, the protonation constants of glycylglycine have been determined pH-metrically in aqua-organic mixtures containing propylene glycol (PG) and dioxan (DOX) frequently used as media in biochemistry and biology laboratories.

MATERIALS AND METHODS

1. Experimental

1.1 Chemicals and Standard Solutions

All the chemicals used in this investigation were of analytical reagent grade purity. Solution (0.05 M) of glycylglycine (Acros Organic, USA) was prepared by maintaining 0.05 M nitric acid concentration to increase the solubility. 1, 2-propanediol and 1,4-dioxan (Merck, India) were used as received. Nitric acid (Merck, India) of 0.2 M was prepared. Sodium nitrate (Merck, India) of 2.0 M was prepared to maintain the ionic strength in the titrand. 0.4 M of Sodium hydroxide (Merck, India) was prepared. Triple-distilled deionized water was used for preparation of all the solutions.

The acid and base solutions were standardized by standard methods. The concentration of the alkali was determined by titrating it with standard oxalic acid and potassium hydrogen phthalate solutions, while the normality of nitric acid was determined using the standardized sodium hydroxide and the primary standard borax solutions. So as to assess the errors that might have crept into the determination of the concentrations, the data were subjected to analysis of variance of one way classification (ANOVA) using the computer program COST [25]. The strength of the prepared carbonate-free sodium hydroxide solution was determined by titrating it against nitric acid solution using the Gran plot method [26].

1.2 Alkalimetric Titrations

The pH measurements of proton-ligand system were carried out in aqueous media containing varying compositions of organic solvent (propylene glycol or dioxan) in the range of 0 - 60% v/v maintaining an ionic strength of 0.16 M with sodium nitrate at 303.0 ± 0.1 K using a Digital pH

meter MK-VI type (readability 0.01). Potassium hydrogen phthalate (0.05 M) and borax (0.01 M) solutions were used to calibrate the pH meter. In each titration, the titrand consisted approximately 1 mmol of nitric acid. The amounts of the glycylglycine (ligand) in the titrands ranged between 0.25 and 0.50 mmols. The glass electrode was equilibrated in a well stirred organic solvent-water mixture containing inert electrolyte for several days. At regular intervals, the strong acid was titrated against alkali to check the complete equilibration of the glass electrode. The details of experimental procedure and titration assembly used in our laboratory have been given elsewhere [27].

RESULTS AND DISCUSSION

The correction factor to be applied to pH meter dial reading was calculated with the computer program SCPHD [28]. The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINQUAD75 which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm [29]. The variation of stepwise protonation constants was analyzed mainly on electrostatic grounds on the basis of solute-solute and solute-solvent interactions.

Table 1. Parameters of the best fit chemical models of protonation equilibria of GG in PG and DOX-water mixtures at 303.0 K and ionic strength, $\mu = 0.16$ M

% v/v Organic solvent	$\log \beta_1(\text{SD})$	$\log \beta_2(\text{SD})$	NP	U_{corr}	χ^2	Skewness	R-factor	Kurtosis	pH-Range
	LH	LH ₂							
PG									
00.0	7.89(16)	11.12(22)	67	3.261	12.15	0.13	0.0116	2.15	2.2 - 8.3
10.0	7.84(33)	11.20(34)	41	0.510	2.68	0.84	0.0047	3.48	2.4 - 8.2
20.0	7.83(22)	11.14(29)	57	5.145	49.93	0.78	0.0161	6.96	2.4 - 8.2
30.0	7.98(9)	11.33(15)	55	1.596	1.85	0.06	0.0096	3.22	2.1 - 8.2
40.0	7.95(7)	11.42(11)	88	0.985	13.82	-0.30	0.0059	4.11	2.0 - 8.5
50.0	7.96(9)	11.48(15)	75	1.988	7.76	-0.79	0.0097	3.15	2.2 - 8.6
60.0	7.99(9)	11.66(16)	62	1.899	5.03	-0.66	0.0099	3.86	2.4 - 8.7
DOX									
00.0	7.89(16)	11.12(22)	67	3.261	12.15	0.13	0.0116	2.15	2.2 - 8.3
10.0	8.01(7)	11.29(11)	77	1.084	18.16	-0.06	0.0074	9.30	2.2 - 9.6
20.0	8.16 (8)	11.57(14)	80	1.526	20.50	-0.91	0.0078	4.70	2.1- 9.6
30.0	8.11(10)	11.71(16)	79	1.896	23.16	-0.45	0.0085	10.56	2.1 - 9.4
40.0	8.16(8)	12.02(14)	90	1.489	72.98	-1.26	0.0072	4.63	2.0 - 9.6
50.0	8.12(8)	12.24(12)	82	0.855	13.07	-0.90	0.0053	3.58	2.0 - 9.0
60.0	7.94(11)	12.31(18)	83	1.998	8.84	-0.32	0.0082	3.45	2.0 - 9.0

SD = Standard deviation; $U_{\text{corr}} = U/(NP-m) \times 10^8$; NP = Number of points; m = number of protonation constants.

The results of best fit models which contain the type of species and overall protonation constants of glycylglycine along with some important statistical parameters are given in Table 1. A very low standard deviation in $\log \beta$ values indicates the precision of these parameters. The small values of U_{corr} (the sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points) corrected for degrees of freedom, indicate that the experimental data can be represented by the model. Small values of mean, standard deviation and mean deviation for the system confirm that the residuals are around a zero mean with little dispersion. For an

ideal normal distribution, the values of kurtosis and skewness should be three and zero, respectively.

The kurtosis values in the present study indicate that the residuals form nearer to mesokurtic as well as very few are platykurtic and leptokurtic patterns. The values of skewness recorded in the table are between -1.12 and 0.84. These data evince that the residuals form a part of normal distribution; hence, least squares method can be applied to the present data. The sufficiency of the model is further evident from the low crystallographic R values (0.0047 to 0.0116). Thus, the statistical parameters show that the best fit models represent the acid-base equilibria of glycylglycine in both PG and DOX-water mixtures.

The primary alkalimetric titration data are simulated and compared with the experimental alkalimetric titration data, to verify the sufficiency of the model. The overlap of the typical experimental and simulated titrations data given in Figure 1 indicates that the proposed models signify the experimental data.

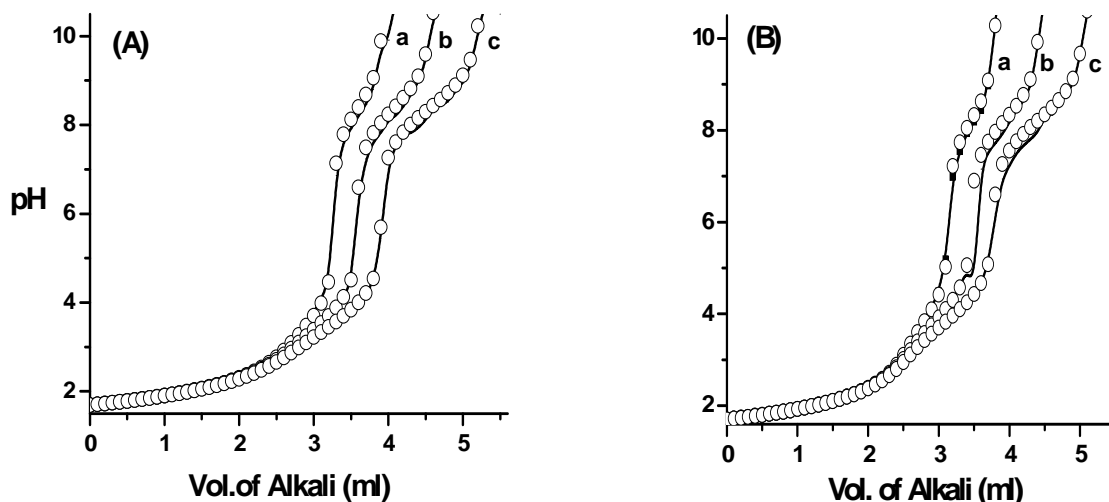


Figure 1: Simulated (o) and experimental (solid line) alkalimetric titration curves in 40% v/v organic solvent; (A) PG and (B) DOX : (a) 0.25, (b) 0.375 and (c) 0.50 mmol of GG

1.3 Protonation equilibria

Glycylglycine has three functional groups (carboxyl, amino and amido); however, only the carboxyl and amino groups involve in the acid-base equilibria. The typical distribution plots (Figure 2) produced using the protonation constants from the best fit models (Table 1) show the existence of LH_2^+ , LH and L^- species. The LH_2^+ species is predominant at low pH and its concentration decreases exponentially and becomes almost zero around pH 7.0. The most predominant species is LH (zwitterionic) form of glycylglycine present to an extent of 90% in the pH range 4.0 - 8.0. Around pH 5.0 formation of the free ligand (L^-) observed while its concentration progressively increases and attains its maximum at higher pH in both media.

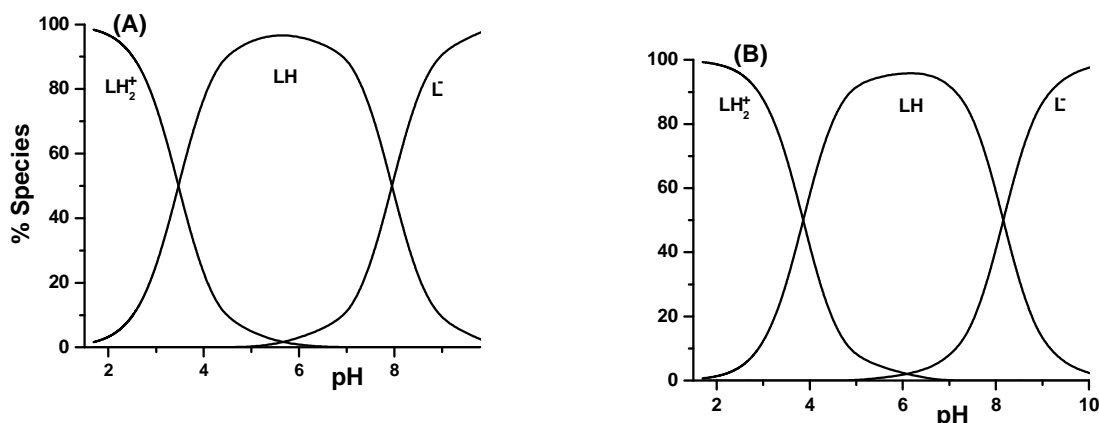


Figure 2: Distribution diagrams of GG species in 40% v/v of organic solvent: (A) PG-water and (B) DOX-water mixtures

The corresponding protonation-deprotonation equilibria and pH ranges of existence of the species are shown in Figure 3 in which, the most protonated glycyglycine loses carboxylic and amino protons successively as the amount of alkali added increases.

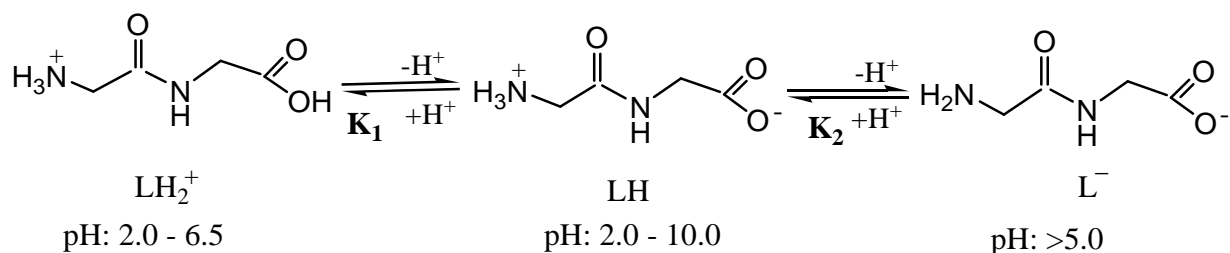


Figure 3: Protonation-deprotonation equilibria of glycyglycine

The protonation constants and number of equilibria could be determined from the secondary formation functions like average number of protons bound per mole of ligand (\bar{n}_H) and number of moles of alkali consumed per mole of ligand (**a**). Plots of \bar{n}_H versus pH for different concentrations of the ligand should overlap if there is no formation of polymeric species. The overlapping of formation curves for glycyglycine (Figure 4A) rule out the polymerization of the ligand molecules. The pH values at half integral values of \bar{n}_H correspond to the protonation constants of the ligand which can also be used to detect the number of equilibria. Thus, the two half integrals (0.5 and 1.5) in Figure 4A confirm the presence of two protonation-deprotonation equilibria in the pH range of the present study. The maximum value of \bar{n}_H is two, which clearly infers that each glycyglycine molecule has two bound protons.

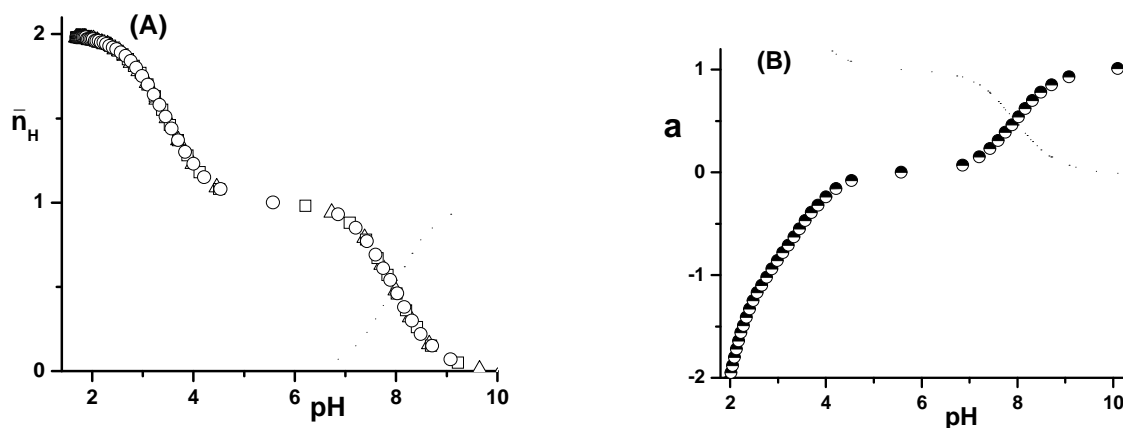


Figure 4: Plots of \bar{n}_H versus pH (A) and a versus pH (B) of GG: (∇) 0.25, (\square) 0.375, and (\circ) 0.50 mmol of GG

A typical plot of a versus pH is given in Figure 4B. The negative values of a correspond to the number of moles of free acid present in the titrand and the number of associable protons. The positive values of a indicate the number of dissociable protons in the ligand molecules.

1.4 Effect of Solvent

One of the most important factors determining the equilibrium constants is the reaction medium, so, the solvent effect on protonation constants could be explained on the basis of dielectric constant of the medium, solvent structure, preferential solvation, and microscopic parameters (as Kamlet-Taft solvatochromic parameters) [30-33]. This variation of protonation constant or change in free energy with the organic solvent content depends up on two factors: an electrostatic one, which can be estimated by the Born equation [34] and a non-electrostatic one, which includes specific solute-solvent interaction. When the electrostatic effects predominate, according to Born's equation, the energy of electrostatic interaction is related inversely to dielectric constant [35]. Hence, the logarithm of step-wise protonation constant ($\log K$) should vary linearly as a function of the reciprocal of the dielectric constant ($1/D$) of the medium.

It was observed that in both the media the $\log K_1$ values corresponding to the formation equilibria of cationic form increase as the content of organic solvent increases. However, $\log K_2$ values relating to the protonation equilibria of the anionic form are almost unaltered (show less variation) by the change in solvent composition. The results are in agreement with those reported in the literature for the media containing pure water as solvent and dioxan-water mixtures [1, 24] but the small differences are possibly due to the different experimental procedures, temperature, the various solvent mixtures and different background electrolytes used.

In this study the linear variation of $\log K$ values of glycylglycine as function of $1/D$ (Figure 5) in both PG-water and DOX-water mixtures shows the dominance of electrostatic interactions. Thus, the observed variation in $\log K$ (significant for $\log K_1$ but less in $\log K_2$) values when the amount of the solvent PG or DOX in the medium is increased can be mainly attributed to ions association reaction, solute-solvent interaction, proton-solvent interaction and solvent basicity (acidity) effects.

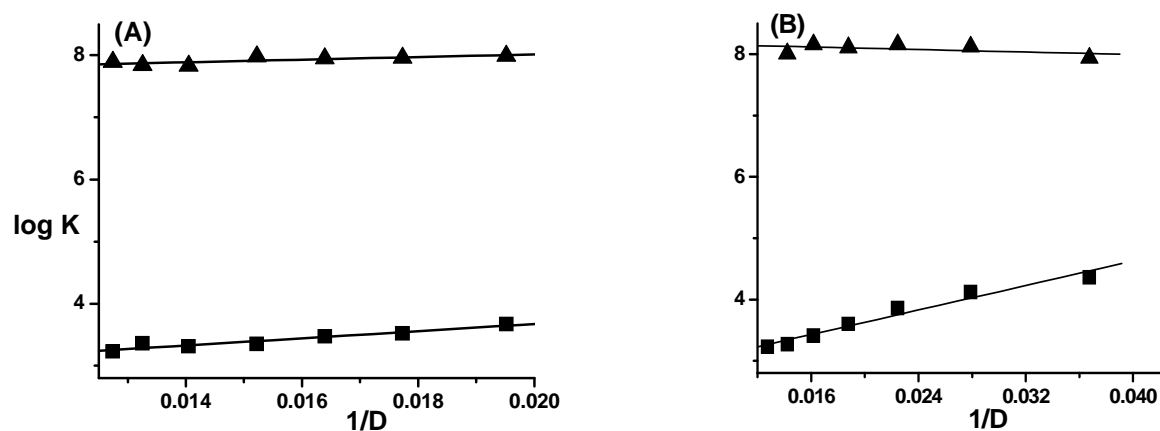


Figure 5: Variation of step-wise protonation constants (log K) of GG with reciprocal of dielectric constant (1/D) in PG-water (A) and DOX-water mixture (B): (■) log K₁ (▲) log K₂

Table 2: Effect of errors in influential parameters on the protonation constants of GG in 40% v/v organic solvent-water mixtures

Ingredient	% Error	PG		DOX	
		log β ₁ (SD)	log β ₂ (SD)	log β ₁ (SD)	log β ₂ (SD)
Acid	0	7.95(7)	11.42(11)	8.16(8)	12.02(14)
	-5	7.90(26)	11.33(41)	8.13(24)	11.93(41)
	-2	7.91(12)	11.38(19)	8.15(12)	11.98(20)
	+2	7.96(12)	11.46(20)	8.18(13)	12.06(23)
	+5	7.99(27)	11.54(43)	8.21(25)	12.13(45)
Alkali	-5	7.99(24)	11.53(34)	8.21(24)	12.12(41)
	-2	7.96(12)	11.46(20)	8.18(12)	12.06(22)
	+2	7.93(12)	11.37(18)	8.14(11)	11.99(19)
	+5	7.90(23)	11.33(37)	8.12(22)	11.94(35)
Ligand	-5	7.94(8)	11.42(12)	8.16(9)	12.02(15)
	-2	7.94(7)	11.42(11)	8.16(8)	12.02(14)
	+2	7.94(7)	11.42(11)	8.17(8)	12.02(14)
	+5	7.95(8)	11.42(12)	8.17(8)	12.02(14)
log F	-5	7.94(12)	11.41(18)	8.16(8)	12.02(14)
	-2	7.95(7)	11.42(11)	8.16(8)	12.02(14)
	+2	7.94(7)	11.43(11)	8.16(8)	12.02(14)
	+5	7.94(7)	11.43(11)	8.16(8)	12.02(14)

1.5 Effect of systematic errors on best fit model

MINIQUAD75 does not have provision to study the effect of systematic errors in the influential parameters like concentrations of ingredients and electrode calibration on the magnitude of protonation constants. In order to rely upon the best chemical model for critical evaluation and application under varied experimental conditions with different accuracies of data acquisition, an investigation was made by introducing pessimistic errors in the concentrations of mineral acid, alkali, ligand and value of correction factor (log F). The results of a typical system given in

Table 2 emphasize that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand and correction factor.

CONCLUSION

In this work, species and the protonation constants of glycylglycine have been determined in PG-water and DOX-water mixtures of varying compositions (0 - 60% organic solvent by volume). Glycylglycine exists in the form of LH_2^+ at low pH and get deprotonated with the formation of LH and L^- successively with increase in pH. The linear variation of log values of stepwise protonation constants with decreasing dielectric constant of the media indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria of glycylglycine. The effect of systematic errors in the influential parameters on the protonation constants shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than those in the ligand and correction factor.

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