

Influence of Dielectric constant on Protonation Equilibria of Glutathione in aqueous solutions of propylene glycol and acetonitrile

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Abstract:

The protonation constants of glutathione have been determined pH metrically in 0.0-60.0% v/v propylene glycol- and acetonitrile- water mixtures maintaining an ionic strength of 0.16 mol L^{-1} at 303.0 K. The protonation constants have been calculated with the computer program MINQUAD75 and the best fit chemical models are selected based on statistical parameters. Linear variation of step-wise protonation constants with reciprocal of dielectric constant of the solvent mixture has been attributed to the dominance of electrostatic forces.

Keywords: Protonation constants, glutathione, propylene glycol, acetonitrile, MINQUAD75.

Introduction

Glutathione (GSH) is a tripeptide which is synthesized in the body from the amino acids L-cysteine, L-glutamic acid, and glycine. The sulfhydryl group (SH) of cysteine serves as a proton donor and is responsible for its biological activity. GSH is the most abundant non-protein thiol in biological cells [1]. GSH is an important antioxidant in plants, animals, fungi, and some bacteria, preventing damage to cellular components caused by reactive oxygen species [2]. Its intracellular concentration is around 5 mmol per gram of tissue [3].

1,2-Propanediol, also called propylene glycol (PG), is a clear, viscous, colorless and odorless liquid with a dielectric constant of 30.2 [4]. The dielectric constant of the medium decreases with increasing mole

fraction of the PG. Hence, PG is chosen to study the acido-basic equilibria to mimic the physiological conditions where the concept of equivalent solution dielectric constant for protein cavities is applicable [5].

Acetonitrile (AN) is a protophobic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. It is a weak base [6] and a much weaker acid [7] than water. Therefore cations and anions have lower solvation energies in acetonitrile than in water [8]. The protophobic character of AN is due to formation of dimers [9]. Very few protonation studies of GSH have been reported in the proposed media in literature [10-12]; hence, the authors are reporting the protonation constants of GSH in PG- and AN- water mixtures.

2. Experimental

2.1 Materials

0.05 mol L⁻¹ solution of Glutathione (GR, E-Merck, Germany) was prepared in triple distilled water by maintaining 0.05 mol L⁻¹ acid (HNO₃) concentration to increase the solubility. Propylene glycol (AR, E-Merck) and Acetonitrile (AR, Qualigens) were used as received. Sodium nitrate was prepared to maintain the ionic strength in the titrand. Sodium hydroxide of 0.4 mol L⁻¹ was prepared. The strengths of alkali and mineral acid were determined using the Gran plot method [13, 14].

2.2 Procedure

The titrimetric data were obtained by using calibrated ELICO (Model LI-120) pH-meter (readability 0.01). The glass electrode was equilibrated in a well stirred solvent solution containing inert electrolyte. The effects of variations in asymmetry, liquid junction potential, activity coefficient, sodium ion error and dissolved carbon dioxide on the response of glass electrode were accounted for in the form of correction factor [10]. For the determination of protonation constants of GSH, initially titrations of strong acid with alkali were carried out at regular intervals to check whether complete equilibration was achieved. Then the calomel electrode was refilled with solvent solution of equivalent composition as that of the titrand. All the titrations have been carried out in a medium containing varying concentrations of solvent (0.0-60.0% v/v) and maintaining an ionic strength of 0.16 mol L⁻¹ with sodium nitrate at 303.0 K. In each of the titrations, the titrand consisted of approximately 1 mmol mineral acid in a total volume of 50 ml. Titrations with different volumes (2.5, 3.75 and 5.0 ml) of ligand were carried out with 0.4 mol L⁻¹ sodium hydroxide. Other experimental details are given elsewhere [11].

2.3 Alkalimetric Titration Assembly

The glass electrode was equilibrated in well-stirred PG- and AN- water mixtures containing inert electrolyte for several days. At regular intervals titration of acid with alkali was carried out to check whether complete equilibration was achieved or not. Typical alkalimetric titrations are given in Figure 1.

3. Results and Discussion

3.1 Modeling strategy

The approximate protonation constants of glutathione were calculated with the computer program SCPHD [15] and they were refined using non-linear least-square computer program MINQUAD75 [16]. The variation of overall protonation constants were analyzed on electrostatic grounds on the basis of solute-solute and solute-solvent interactions. The best fit models that contain the type of species and overall protonation constants (log β) along with some of the important statistical parameters are given in Table 1.

A very low standard deviation in log β values indicates the precision of these parameters. The small values of U_{corr} (sum of the squares of deviations in concentrations of ligand and hydrogen ion at all experimental points corrected for degree of freedom) indicate that the experimental data can be represented by the models. Small values of mean, standard deviation and mean deviation for the systems corroborate 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 leptokurtic as well as platykurtic patterns. The values of skewness are between -1.41 and 1.55. These data evince that the residuals from 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. The statistical parameters thus show that the best fit models portray the acido-basic equilibria of glutathione in PG- and AN- water mixtures.

3.2 Effect of systematic errors

In order to rely upon the best fit 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 alkali, mineral acid and the ligand. 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 those in the ligand and log F.

3.3 Effect of solvent

Effect of solvent on protonation constant depends upon electrostatic and non-electrostatic factors. Born's classical treatment holds good in accounting for the electrostatic contribution [17] which is related to dielectric constant. Hence, the logarithm of step-wise protonation constant (log K) should vary linearly as a function of the reciprocal of dielectric constant (1/D) of the medium. In present study the log K values are linearly increasing (Figure 2) with decreasing dielectric constant of both the media (PG and AN). The cation stabilizing nature of co-solvent, specific solvent-water interactions, charge dispersion and specific interactions of co-solvent with solute account for the linear relationship of log K with 1/D. Glutathione exists as anions (L³⁻, LH²⁻, and LH₂⁻), zwitterion (LH₃) and cation (LH₄⁺) (Figure 3) at different pH values.

3.4 Distribution diagrams

Secondary formation function (n_H) - average number —

of moles of protons bound per mole of ligand- is useful to detect the number of protonation equilibria. The formation functions (Figure 4) indicate that GSH has four protonation constants. The pH values at half

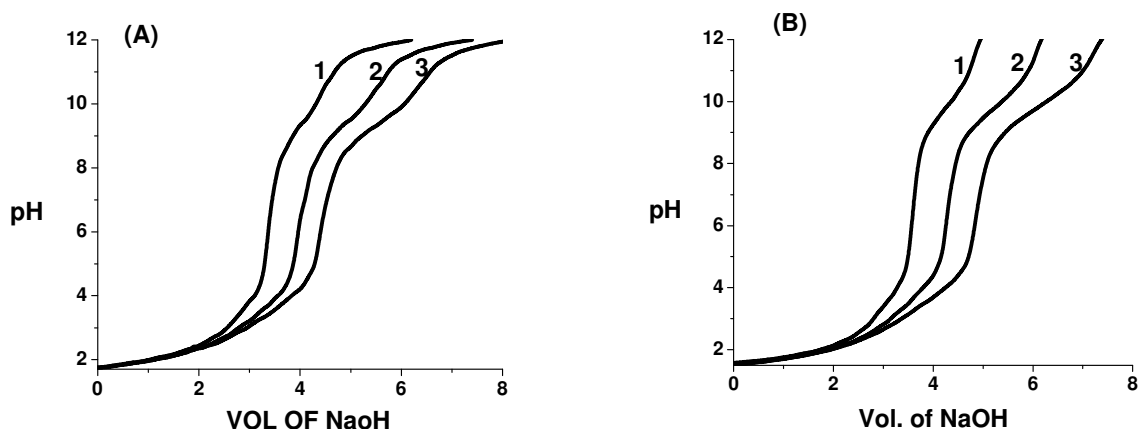


Figure 1: Alkalimetric titration curves of GSH in 30% v/v (A) PG- and (B) AN- water mixtures; 1, 2 and 3 indicate 0.25, 0.375 and 0.50 mmol of ligand, respectively.

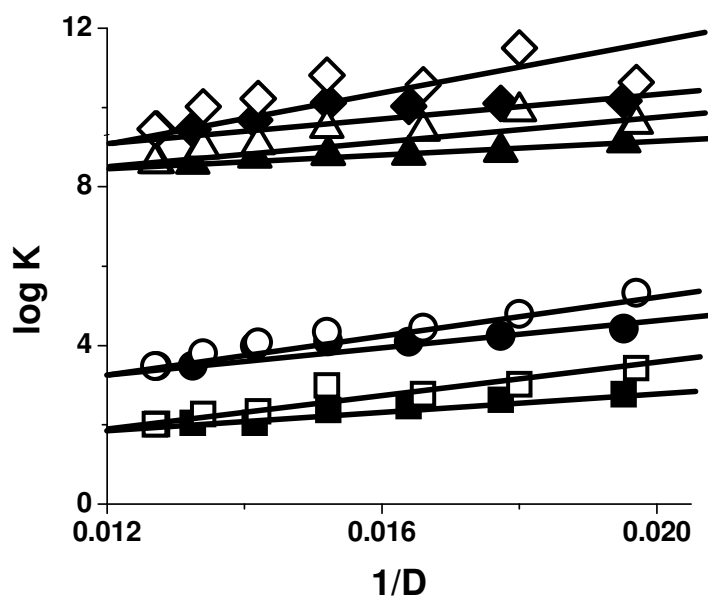


Figure 2: Variation of step-wise protonation constants ($\log K$) with reciprocal of dielectric constants ($1/D$) of Glutathione in PG (solid symbol)- and AN (hollow symbol)- water mixtures: Squares ($\log K_1$), Circles ($\log K_2$), Triangles ($\log K_3$) and Rhombus ($\log K_4$).

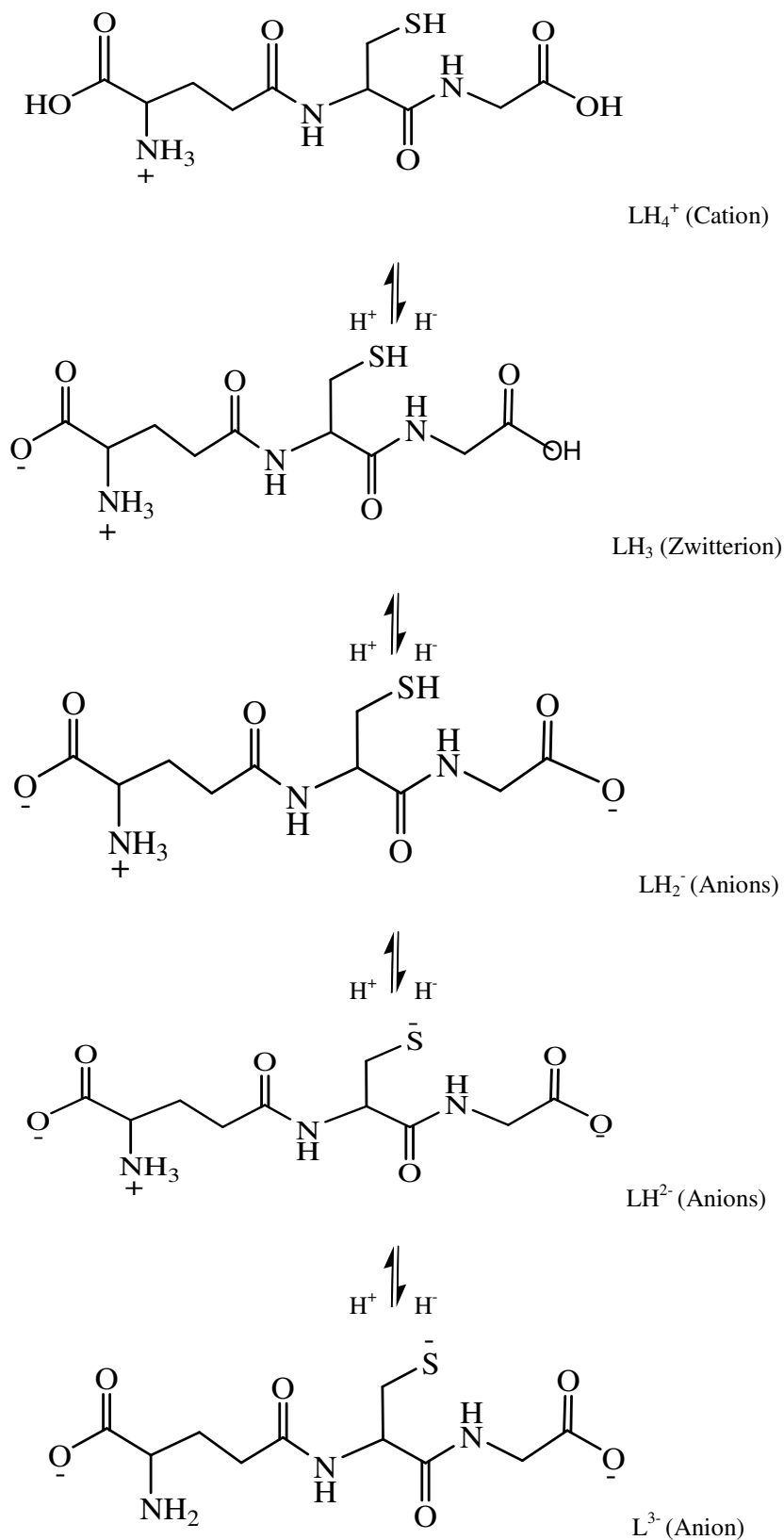


Figure 3: Protonation-deprotonation equilibria of Glutathione

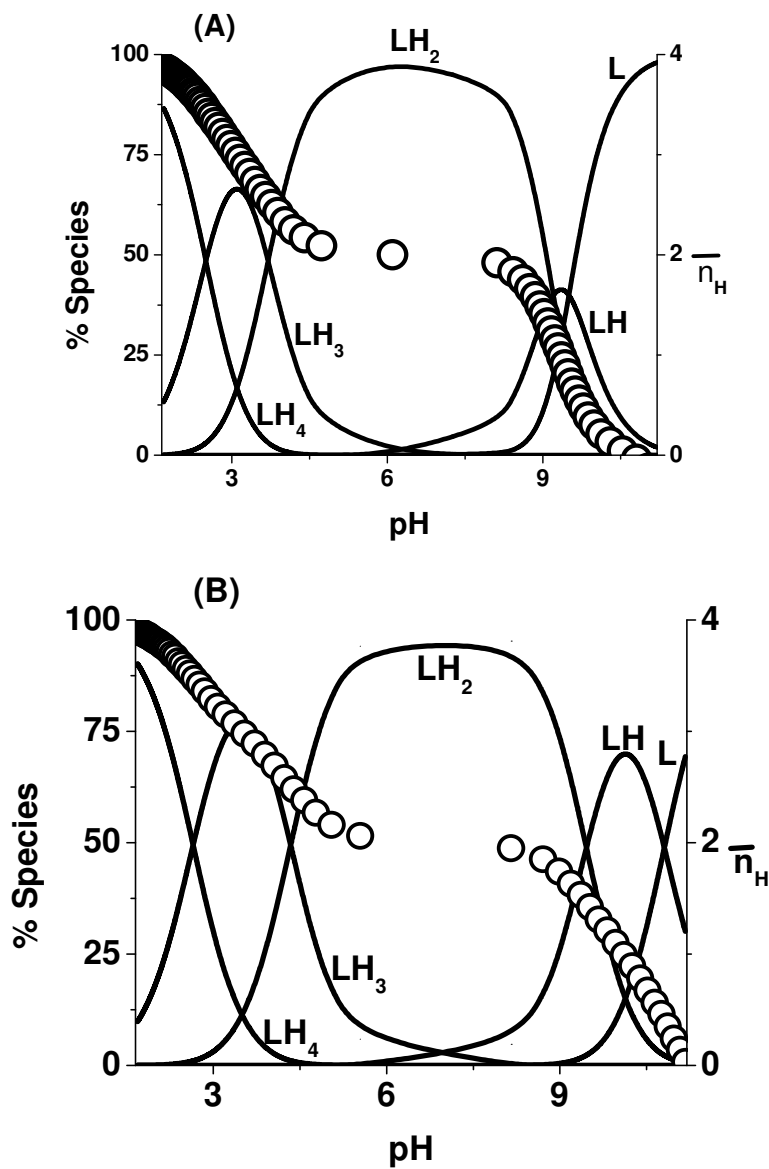


Figure 4: Formation functions (o) Species distribution diagrams (solid line) of Glutathione in (A) 30% v/v propylene glycol- and (B) 30% v/v acetonitrile- water mixture.

Table 1: Parameters of the best fit chemical models of protonation equilibria of GSH in PG- and AN- water mixtures at 303.0 K and ionic strength, $\mu = 0.16 \text{ mol L}^{-1}$

% v/v	Log β (SD)				U_{corr}	NP	Skewness	Kurtosis	χ^2	R-Factor	pH-Range
	LH	LH ₂	LH ₃	LH ₄							
PG											
0	9.46(2)	18.07(1)	21.55(3)	23.56(4)	1.50	105	0.23	5.15	50.0	0.0109	2.3-9.7
10	9.45(1)	18.03(1)	21.52(2)	23.57(2)	3.71	181	0.48	6.59	79.37	0.0053	1.6-9.7
20	9.68(2)	18.41(1)	22.40(3)	24.45(3)	1.63	180	-0.53	4.45	19.25	0.0070	1.6-9.7
30	10.12(6)	18.91(4)	23.01(6)	25.39(7)	1.10	132	0.18	4.67	39.06	0.0106	1.8-9.7
40	10.03(2)	18.84(2)	22.93(3)	25.40(3)	1.63	135	-0.09	4.35	18.89	0.0058	1.8-9.7
50	10.10(2)	18.97(2)	23.22(2)	25.85(3)	2.50	116	0.00	4.21	15.59	0.0054	2.0-9.7
60	10.17(3)	19.29(2)	23.71(3)	26.49(3)	4.26	117	0.77	3.92	11.74	0.0052	2.0-9.7
AN											
10	10.02(1)	18.99(1)	22.79(2)	25.06(3)	2.00	147	-0.16	3.28	19.09	0.0050	1.8-10.3
20	10.22(1)	19.29(1)	23.35(3)	25.69(3)	1.97	141	0.08	5.26	23.53	0.0050	1.8-10.3
30	10.81(4)	20.27(3)	24.61(4)	27.26(4)	2.28	146	0.47	4.03	22.40	0.0050	1.8-10.3
40	10.57(2)	19.98(2)	24.43(3)	27.20(3)	2.12	145	-1.41	7.19	38.72	0.0051	1.8-10.3
50	11.50(2)	21.40(2)	26.19(4)	29.21(6)	0.15	135	-0.67	11.75	59.19	0.0090	1.8-10.3
60	10.63(6)	20.22(4)	25.55(7)	28.98(8)	0.77	157	1.55	5.35	59.96	0.0138	2.0-10.3

$U_{\text{corr}} = U/(NP-m) \times 10^8$, where, m = number of species; NP = number of experimental points

Table 2: Effect of systematic errors in influential parameters on the protonation constants of Glutathione in 30% v/v PG-water mixtures.

Ingredient	% Error	$\log \beta_1(\text{SD})$	$\log \beta_2(\text{SD})$	$\log \beta_3(\text{SD})$	$\log \beta_4(\text{SD})$
Alkali	0	10.12(6)	18.91(4)	23.01(6)	25.39(7)
	-5	9.52(5)	12.38(6)	10.08(4)	13.22(5)
	-2	9.27(3)	11.94(4)	9.83(2)	12.77(3)
	+2	8.96(2)	11.43(3)	9.50(1)	12.22(2)
	+5	8.73(2)	11.07(3)	9.25(2)	11.83(3)
Acid	-5	8.77(3)	10.96(3)	9.30(2)	11.76(3)
	-2	8.98(2)	11.39(3)	9.52(1)	12.19(2)
	+2	9.24(3)	11.97(4)	9.81(2)	12.79(3)
	+5	9.47(4)	12.47(6)	10.02(4)	13.26(6)
Ligand	-5	9.07(2)	11.70(3)	9.61(2)	12.50(2)
	-2	9.10(2)	11.68(3)	9.66(2)	12.49(2)
	+2	9.13(3)	11.66(3)	9.66(2)	12.49(2)
	+5	9.15(3)	11.66(3)	9.71(2)	12.48(2)
log F	-5	9.11(3)	11.65(3)	9.65(2)	12.46(2)
	-2	9.11(2)	11.67(3)	9.66(2)	12.46(2)
	+2	9.11(2)	11.69(3)	9.66(2)	12.50(2)
	+5	9.12(2)	11.71(3)	9.67(2)	12.52(2)

integrals of n_H correspond to the log K values of the ligands. The distribution plots (Figure 4) of GSH indicate that LH_2^- is present to an extent of 90% in the pH range 4.0-10.0 and successive deprotonation takes place with increase in the pH.

4. Conclusions

- i) Glutathione forms LH_4^+ at low pH and gets deprotonated with the formation of LH_3 , LH_2^- , LH^- and L^{3-} successively with increase in pH.
- ii) The log K values of protonation constants increase linearly with decreasing dielectric constant of PG- and AN- water mixtures. This trend indicates the dominance of electrostatic forces over than non-

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electrostatic forces in the protonation-deprotonation equilibria.

- iii) The effect of systematic errors in the influential parameters shows that the errors in the concentrations of alkali and mineral acid affect the protonation constants more than that of the ligand and log F.

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