Influence of Dielectric constant on Protonation Equilibria of L-Methionine in aqueous solutions of propylene glycol and acetonitrile

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ABSTRACT

The protonation constants of L-methionine have been studied pH metrically in various concentrations (0.0-60.0% v/v) of 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 MINIQUAD75 and the best fit chemical models are selected based on statistical parameters. Linear variation of step-wise protonation constants (log K) with reciprocal of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces.

Keywords: Protonation constants, L-methionine, propylene glycol, acetonitrile, MINIQUAD75.
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INTRODUCTION

All plants and some forms of bacteria can synthesize L-methionine (Met) by two different sequences [1]. Mammalian tissues and many bacteria synthesize it from cobalamine enzyme where as all the plants and some bacteria synthesize it from homocysteine. Cerebrospinal fluid levels of Met, homocysteine and cystathionine were studied in patients with psychotic disorders [2]. Met is synthesized from cysteine and *o*-phosphohomoserine involving three enzymes, cystathionine γ-synthase, cystathionine *ß*-lyase and methionine synthase [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, this medium 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]. The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex

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formed by the metal ion and (iii) the bonding behavior of the protein residues with the metal ion. The species refined and their relative concentrations under the present experimental conditions represent the possible forms of these amino acids in the biological fluids.

Acetonitrile (AN) is a polar solvent widely employed for industrial and research application both as a pure solvent and in mixtures with water over the whole composition range. In particular, density measurements at 298.15 K have been reported by several authors [6, 7]. Therefore cations and especially anions have lower solvation energies in acetonitrile than in water, except in those cases where there is specific interaction with the solvent. Thus cations are reduced at considerably more positive potential [8] in acetonitrile than in water. It is a protophobic dipolar aprotic solvent and it does not form any hydrogen bond with solute species. The protophobic character of AN may arise from the possible formation of dimers which are shown to exist from IR studies [9]. Very few studies have been reported in the literature [10-12]. Hence, the protonation constants of L-methionine in PG- and AN- water mixtures are reported in this communication.

MATERIALS AND METHODS

2.1 Materials
0.05 mol L^{-1} solution of L-methionine (GR, E-Merck, Germany) was prepared in triple distilled water by maintaining 0.05 mol L^{-1} acid (HNO_3) concentration to increase the solubility. Propylene glycol (AR, E-Merck) and Acetonitrile (AR, Qualigen) 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 Met, 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 were carried out in a medium containing varying concentrations of solvent (0.0-60.0% v/v), 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

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check whether complete equilibration was achieved or not. Typical alkalimetric titrations are given in Fig. 1.
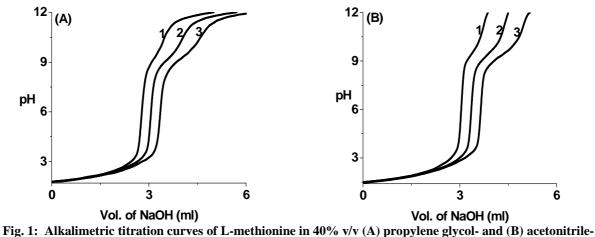

Fig. 1: Alkalimetric titration curves of L-methionine in 40% v/v (A) propylene glycol- and (B) acetonitrile-water mixtures; 1, 2 and 3 indicate 0.25, 0.375 and 0.50 mmol of ligand, respectively.

RESULTS AND DISCUSSION

3.1 Modeling strategy
The approximate protonation constants of L-methionine were calculated with the computer program SCPHD [15] and they were refined using non-linear least-squares computer program MINIQUAD75 [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 squares of deviations in the 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 -1.02 and 1.20. 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 L-methionine in PG- and AN- water mixtures.

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

Solvent % v/v	log β (SD) LH	log β (SD) LH$_2$	NP	U$_{corr}$ x10^8	χ^2	Skewness	Kurtosis	R-factor	pH-range
				PG					
0.0	9.10(2)	11.29(2)	84	4.9	11.05	-0.11	2.42	0.0136	1.9-9.20
10.0	8.84(2)	10.88(2)	112	7.56	11.93	1.20	16.20	0.0133	1.7-9.20
20.0	9.23(3)	11.75(4)	121	3.18	3.69	-0.30	2.93	0.0278	1.7-10.0
30.0	9.11(2)	11.68(3)	94	7.14	5.79	-0.43	3.44	0.0158	1.8-9.50
40.0	9.13(2)	11.71(2)	96	9.01	5.67	-0.65	3.17	0.0160	1.8-9.80
50.0	9.07(2)	11.60(2)	85	6.83	8.45	0.00	2.53	0.0181	2.0-10.4
60.0	9.23(1)	12.10(2)	85	4.12	9.39	-1.02	3.47	0.0126	2.0-10.2
				AN					
10.0	9.52(2)	11.87(2)	121	5.77	6.07	-0.39	3.07	0.0107	1.8-10.3
20.0	9.64(2)	12.03(3)	73	1.54	3.81	0.32	2.95	0.0262	2.0-10.3
30.0	9.66(2)	12.49(2)	108	7.14	6.44	-0.43	2.86	0.0134	1.8-10.3
40.0	9.69(2)	12.73(2)	109	3.87	20.90	-0.22	2.54	0.0099	1.8-10.3
50.0	10.23(3)	13.66(3)	80	8.48	2.60	0.00	3.80	0.0168	2.0-10.3
60.0	10.07(4)	13.43(6)	94	2.79	18.21	-1.02	3.37	0.0281	2.0-10.3

U$_{corr}$ = U/ (NP-m); where m = number of species; NP = Number of experimental points;   SD= Standard deviation.

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

Ingredient	% Error	PG log β$_1$ (SD)	PG log β$_2$ (SD)	AN log β$_1$ (SD)	AN log β$_2$ (SD)
	0	9.11(2)	11.68(3)	9.66(2)	12.49(2)
Alkali	-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)

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

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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. The log K values in present study are linearly increasing (Fig. 2) with decreasing dielectric constant of the medium in both the media (PG and AN).
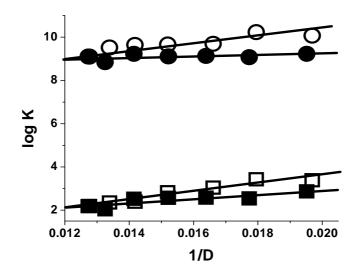

Fig. 2: Variation of step-wise protonation constants (log K) with reciprocal of dielectric constant (1/D) of L-methionine in PG (solid symbol)- and AN (hollow symbol)- water mixtures: Squares (log K_1), Circles (log K_2).

L-methionine exists as anion (L^-), zwitterion (LH) and cation (LH_2^+) (Fig. 3) at different pH values. 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.
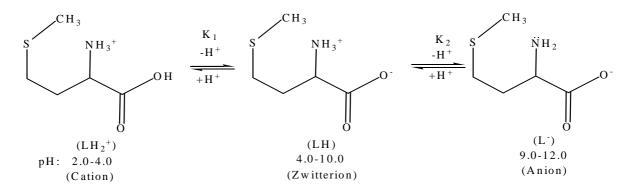

Fig. 3: Protonation-deprotonation equilibria of L-methionine

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3.4 Distribution diagrams

Secondary formation function ($\overline{n_H}$) average number of moles of protons bound per mole of ligand is useful to detect the number of protonation equilibria. The formation functions (Fig. 4) indicate that Met has two protonation constants. The pH values at half integrals of n_H correspond to the log K values of the ligands. The distribution plots (Fig. 4) of L-methionine indicate that LH 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.
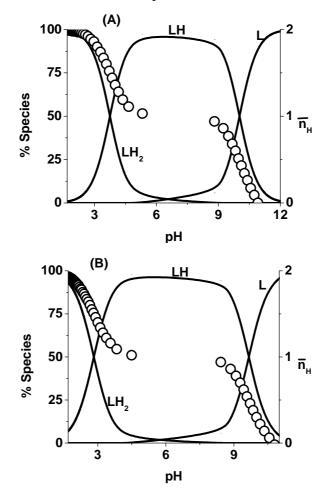

Fig. 4: Formation functions (o) and species distribution diagrams (solid line) of L-methionine in 30% v/v (A) PG- and (B) AN- water mixture.

CONCLUSION

1. L-methionine forms LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- successively with increase in pH.
2. 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 the non-electrostatic forces in the protonation-deprotonation equilibria.

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3. 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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