Scientific paper

Determination of Protonation Constants of L-Glutamic Acid and L-Methionine in 1,2-Propanediol-Water Mixtures

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Abstract

The solute-solvent interactions of L-glutamic acid and L-methionine have been studied in 0–60% V/V 1,2-propanediol-water media using the pH-metric method. The protonation constants have been calculated with the computer program MINIQUAD75. Selection of the best fit chemical model of the acid-base equilibria is based on standard deviation in protonation constants and residual analysis using crystallographic R-factor and sum of squares of residuals in all mass-balance equations. Linear variation of protonation constants with inverse of dielectric constant of the solvent mixture has been attributed to the dominance of the electrostatic forces. Distributions of species, protonation equilibria and effect of influential parameters on the protonation constants have also been presented.

Keywords: acid-base equilibria, L-glutamic acid, L-methionine, 1,2-propanediol

1. Introduction

L-Glutamic acid (Glu) is a ubiquitous amino acid present in many foods either in free form or in peptides and proteins.[1] Glutamate impairs neuronal calcium extrusion while reducing sodium gradient.[2–5] Glutamate-induced intracellular calcium changes and neurotoxicity in cortical neurons in vivo was studied.[6] Because of its capacity to induce depolarizing effects, it acts as an excitatory transmitter in cerebral cortex. In brain, glutamate is continuously released from nerve terminals during neuronal activity and has to be removed rapidly from the synaptic cleft to ensure uninhibited operation of the central nervous system (CNS). Glutamate is also the neurotransmitter at the synapse, between the photoreceptors and bipolar cells of the vertebrate retina.[7] Glutamate receptors in the vertebrate CNS are classified depending on the antagonistic action of N-methyl-D-aspartate (NMDA), kinate or quisqualate.[8] NMDA channels are permeable to calcium ions but are blocked by magnesium ions.[9–12] Blood plasma samples from HIV-1-infected persons contained elevated glutamic acid concentration up to 6-fold compared to normal levels.[13]

All plants and some forms of bacteria can synthesize methionine (Met) by two different sequences.[14] 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.[15] Met is synthesized from cysteine and *o*-phosphohomoserine involving three enzymes, cystathionine γ-synthase, cystathionine β-lyase and methionine synthase.[16]

1,2-Propanediol, also called propylene glycol (PG), is a clear, viscous, colorless and odorless liquid with a dielectric constant of 30.2.[17] The dielectric constant of the medium decreases with increase in the mole fraction of 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.[18] The present study is useful to understand (i) the role played by the active site cavities in biological molecules, (ii) the type of complex 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.

Effect of *N*,*N*'-dimethyl formamide,[19,20] urea and sodium lauryl sulphate mixtures on the protonation equilibria of L-glutamic acid was reported earlier.[21,22]

2. Experimental

2.1. Materials

Solutions (0.05 mol L^{-1}) of L-glutamic acid (G.R, Merck, Germany) and L-methionine (Himedia, India) were prepared in triple-distilled deionized water by maintai-

ning 0.05 mol L^{-1} nitric acid concentration to increase the solubility. 1,2-Propanediol was obtained from Merck, India, and used as received. Nitric acid (Merck, India) of 0.2 mol L^{-1} was prepared. Sodium nitrate of 2 mol L^{-1} was prepared to maintain the ionic strength in the titrand. Sodium hydroxide of 0.4 mol L^{-1} was prepared. All the solutions were standardized by standard methods. 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).[23] The strengths of alkali and mineral acid were determined using the Gran plot method.[24]

2.2. Alkalimetric Titrations

The glass electrode was equilibrated in a well stirred PG-water mixture containing inert electrolyte for several days. At regular intervals titration of strong acid with alkali was carried out to check the complete equilibration of the glass electrode. The calomel electrode was refilled with PG-water mixture of equivalent composition as that of the titrand. Alkalimetric titrations were performed in media containing 0–60% volume/volume (v/v) PG-water mixtures pH-metrically. The details of experimental procedure and titration assembly have been detailed elsewhere.[25]

2.3. Modeling Strategy

The approximate protonation constants of L-glutamic acid were calculated with the computer program SCPHD.[26] The best fit chemical model for each system investigated was arrived at using non-linear least-squares computer program, MINIQUAD75,[27] which exploits the advantage of constrained least-squares method in the initial refinement and reliable convergence of Marquardt algorithm. The variation of stepwise protonation constants (log K) with the dielectric constant of the medium was analyzed on electrostatic grounds for the solute-solute and solute-solvent interactions.

3. Results and Discussion

The best fit models containing the type of species and log values of overall formation constants ($\log \beta$) along with some of the important statistical parameters are given in Table 1. A very low standard deviation (SD) 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 systems corroborate that the residuals are around a zero mean with little dispersion.

3.1. Residual Analysis[28]

In data analysis with least squares methods, the residuals (the differences between the experimental data and the data simulated based on model parameters) are assumed to follow Gaussian or normal distribution. When the data are fit into the models, the residuals should be ideally equal to zero. If the statistical measures of the residuals and the errors assumed in the model are not significantly different from each other, the model is said to be adequate. Further, a model is considered adequate only if the residuals do not show any trend. Respecting the hypothesis that the errors are random following normal distribution in the least squares analysis, the residuals are tested for normal distribution. Such tests are χ^2, skewness, kurtosis and R-factor. These statistical parameters, show that the best fit models portray the acido-basic equilibria of Glu and Met in PG-water mixtures, as discussed below.

χ^2test

χ^2 is a special case of gamma distribution whose probability density function is an unsymmetrical function. This distribution measures the probability of residuals forming a part of standard normal distribution with zero mean and unit standard deviation. If the χ^2 calculated is less than the table value, the model is accepted.

Crystallographic R-test

Hamilton's R factor ratio test is applied in complex equilibria to decide whether inclusion of more species in the model is necessary or not. In pH-metric method the readability of pH meter is taken as the R_{limit}, which represents the upper boundary of R beyond which the model bears no significance. When different values are obtained for models containing different numbers of species, models whose values are greater than R-table are rejected. The low crystallographic R-values given in Table 1 indicate the sufficiency of the model.

Skewness

It is a dimensionless quantity indicating the shape of the error distribution profile. A value of zero for skewness indicates that the underlying distribution is symmetrical. If the skewness is greater than zero, the peak of the error distribution curve is to the left of the mean and the peak is to the right of the mean if skewness is less than zero. The values of skewness recorded in Table 1 are between –0.50 and 0.89. These data evince that the residuals form a part of normal distribution; hence, least–squares method can be applied to the present data.

Kurtosis

It is a measure of the peakedness of the error distribution near a modal value. For an ideal normal distribution kurtosis value should be three (mesokurtic). If the calculated kurtosis is less than three, the peak of the error distribution curve is flat (platykurtic) and if the kurtosis is

Table 1. Parameters of the best fit chemical models of acido-basic equilibria of L-glutamic acid and L-methionine in PG-water mixtures at 303 K and ionic strength, I = 0.16 mol L^{-1}.

% v/v PG	log ß$_1$(SD)	log ß$_2$(SD)	log ß$_3$(SD)	NP	U_{corr}	Skewness	χ^2	R-factor	Kurtosis	pH-Range
L-glutamic acid										
00.00	9.30(1)	13.46(1)	15.52(1)	141	0.110	0.89	13.38	0.00438	6.84	1.74–10.0
10.00	9.52(1)	13.80(1)	16.11(1)	100	0.478	0.11	25.76	0.00346	5.68	2.0–9.8
20.00	9.48(1)	13.82(1)	16.09(1)	89	1.093	0.22	9.96	0.00620	3.23	2.2–10.2
30.00	9.56(1)	14.02(1)	16.48(1)	93	0.799	0.22	18.90	0.00525	3.88	2.2–10.6
40.00	9.60(1)	14.18(1)	16.75(1)	85	0.507	0.19	19.65	0.00420	5.67	2.3–10.2
50.00	9.63(1)	14.33(1)	17.05(1)	112	1.151	0.20	12.43	0.00665	4.19	2.4–10.4
60.00	9.81(1)	14.73(1)	17.56(2)	102	2.010	0.01	18.20	0.00810	5.87	2.2–10.8
L-methionine										
00.00	8.86(1)	11.06(7)	–	100	0.076	0.23	1.44	0.00459	3.62	1.8–9.2
10.00	9.03(1)	11.38(1)	–	73	0.371	0.05	12.47	0.00422	4.10	2.1–10.0
20.00	8.90(1)	11.05(1)	–	70	3.607	0.09	12.69	0.01397	2.67	2.1–10.0
30.00	9.01(1)	11.39(1)	–	65	1.569	0.50	1.64	0.00967	3.34	2.2–10.5
40.00	8.97(1)	11.51(1)	–	58	0.775	0.06	12.69	0.00728	3.01	2.4–10.4
50.00	8.95(1)	11.66(1)	–	62	2.331	0.80	18.45	0.01240	5.85	2.4–10.5
60.00	8.99(1)	11.82(1)	–	63	3.123	0.00	5.81	0.01311	2.51	2.3–9.6

greater than three, the distribution shall have sharp peak (leptokurtic). The kurtosis values in the present study indicate that the residuals form leptokurtic pattern.

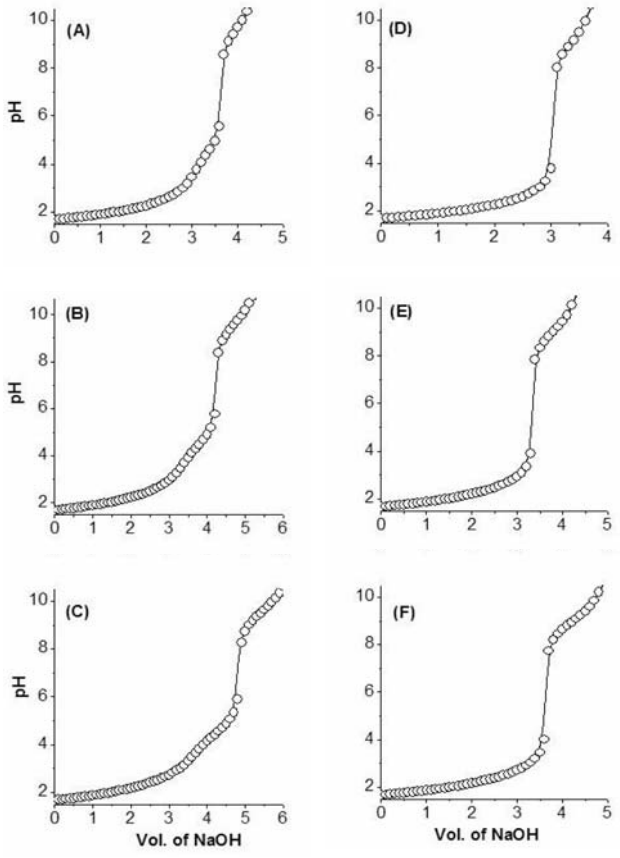

Figure 1. Simulated (o) and experimental (solid line) alkalimetric titration curves in 30% (v/v) PG-water mixture; (A), (B) and (C) for 0.25, 0.375 and 0.50 mmol Glu, and (D), (E) and (F) for 0.25, 0.375 and 0.50 mmol Met, respectively.

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

3.2. Effect of Solvent

The variation of protonation constant or change in free energy with co-solvent content depends upon two

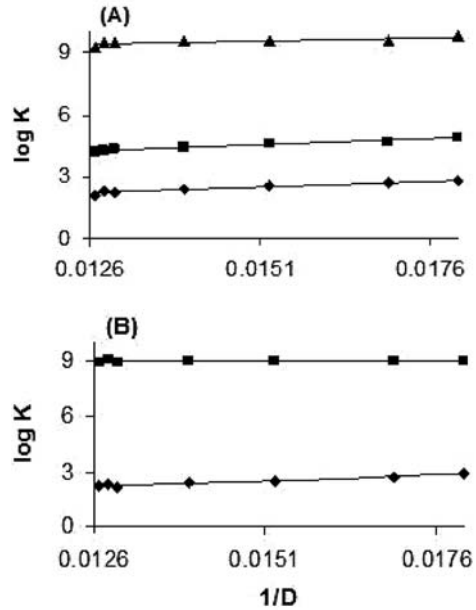

Figure 2. Variation of step-wise protonation constant (log K) with reciprocal of dielectric constant (1/D) in PG- water mixtures. (A) glutamic acid, B) methionine (♦) log K_1 (■) log K_2 (▲)log K_3.

factors, viz., electrostatic and non-electrostatic. Born's classical treatment holds good in accounting for the electrostatic contribution to the free energy change.[29] According to this treatment, the energy of electrostatic interaction 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 the dielectric constant (1/*D*) of the medium. These plots (Figure 2) in PG-water mixtures show that the log *K* values are linearly increasing with decreasing dielectric constant values.

In the case of some mono- and di- carboxylic acids and simple phenolic ligands, electrostatic (long-range, non specific or universal) solute-solvent interactions are predominant in binary mixtures of water with methanol, ethanol, dioxane or acetone as co-solvent. Only in such cases the data fit into Born model. Many workers were of the opinion that both electrostatic and non-electrostatic effects should be considered even in the case of simple acido-basic equilibria; one dominates the other, depending upon the nature of solute and solvent.[30] These amino acids can exist in anionic, zwitterionic and cationic forms (Figure 3) in different equilibria investigated. The cation stabilizing nature of co-solvents, specific solvent-water interactions, charge dispersion, and specific interactions of co-solvent with solute (indicated by the changes in the solubility of different species in the aquo-organic mixtures) account for the deviation of classical linear relationship of log *K* with 1/*D*. K_1, K_2 and K_3 are step-wise protonation constants for the reactions mentioned in Figure 3.

3.3. Distribution Diagrams

The distribution plots (Figure 4) produced using the protonation constants from the best fit models (Table 1) show the formation of LH_3^+, LH_2, LH^- and L^{2-} in the case of Glu and LH_2^+, LH and L^- in the case of Met. In both the cases, LH is present to an extent of 90% in the pH range 4.0–9.0. The monoprotonated species (LH_3 in the case of Glu and LH_2 in the case of Met) exist in the pH ranges 2.0–4.0 and 2.0–6.0, respectively. Deprotonation takes place with increase in the pH and it leads to the formation

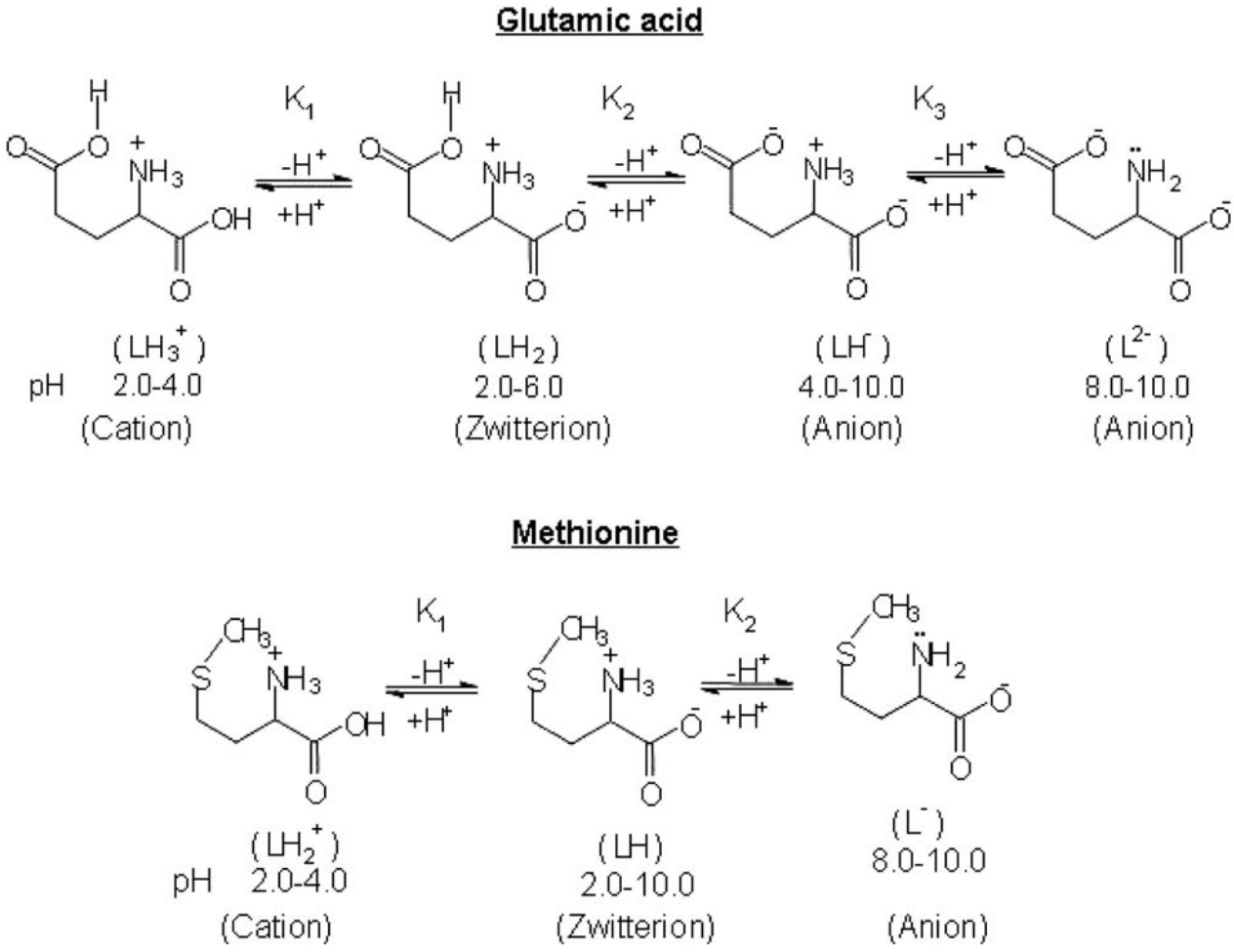

Figure 3. Protonation-deprotonation equilibria of L-glutamic acid and L-methionine.

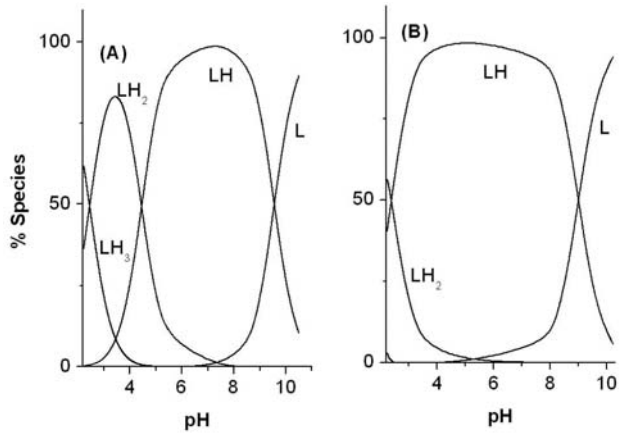

Figure 4. Species distribution diagrams of (A) Glu, (B) Met in 30% v/v PG-water mixture.

of LH^- and L^{2-} in the pH-ranges 4.0–9.0 and 8.0–10.0, respectively, in the case of Glu and L^- in the case of Met in the pH range 6.0–10.0.

3.4. Effect of Systematic Errors in the Best Fit Model

Any variations in the parameters like concentrations of ingredients affect the magnitudes of equilibrium constants. Such parameters are called influential or dangerous parameters. 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 alkali, mineral acid and 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 that of the ligand.

4. Conclusions

1. L-Glutamic acid forms LH_3^+ at low pH and gets deprotonated with the formation of LH_2, LH^- and L^{2-} successively with increase in pH.
2. L-Methionine forms LH_2^+ at low pH and gets deprotonated with the formation of LH and L^- with increase in pH.
3. The log values of protonation constants increase linearly with decreasing dielectric constant of PG-water mixtures. This trend indicates the dominance of electrostatic forces in the protonation-deprotonation equilibria.
4. 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.

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

Ingredient	% Error	log ß(SD)		
		011	012	013
L-glutamic acid				
Alkali	0	9.56(1)	14.02(1)	16.48(1)
	–5	10.11(2)	15.01(5)	17.73(7)
	–2	9.78(1)	14.40(2)	16.96(3)
	+2	9.36(1)	13.65(1)	16.02(2)
	+5	9.05(2)	13.11(4)	15.35(6)
Acid	–5	9.20(1)	13.31(3)	15.45(4)
	–2	9.42(1)	13.74(1)	16.07(1)
	+2	9.71(1)	14.31(2)	16.90(2)
	+5	9.93(2)	14.75(4)	17.54(5)
Ligand	–5	9.40(1)	13.80(1)	16.30(2)
	–2	9.50(1)	13.93(1)	16.41(1)
	+2	9.63(1)	14.11(1)	16.55(1)
	+5	9.72(1)	14.24(1)	16.65(2)
L-methionine				
Alkali	0	9.01(1)	11.39(1)	–
	–5	9.47(3)	12.16(5)	–
	–2	9.19(1)	11.69(2)	–
	+2	8.84(1)	11.10(1)	–
	+5	8.59(3)	10.68(5)	–
Acid	–5	8.64(2)	10.63(4)	–
	–2	8.86(1)	11.09(1)	–
	+2	9.16(1)	11.69(2)	–
	+5	9.40(3)	12.15(4)	–
Ligand	–5	8.95(1)	11.37(1)	–
	–2	8.99(1)	11.38(1)	–
	+2	9.03(1)	11.39(1)	–
	+5	9.07(1)	11.41(1)	–

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Povzetek

S pH-metrijo smo proučevali interakcije topljenec-topilo L-glutaminske kisline in L-metionina v mešanicah 1,2-propandiola in vode v območju sestave med 0 in 60 utežnimi %.
S pomočjo programa MINIQUAD75 smo izračunali konstante protonacije. Na osnovi standardnih deviacij, stabilnostnih constant, rezidualne analize z uporabo kristalografskega R-faktorja in vsote najmanjših kvadratov v zvezah za snovno bilanco smo se odločili za najboljši kemijski model za ravnotežje kislina-baza. Linearna odvisnost dobljenih konstant protonacije od racipročne vrednosti dielektrične konstante kaže na prevlado elektrostatičnih sil. Prikazana je tudi porazdelitev prisotnih species v sistemu, protonacijska ravnotežja in vpliv različnih parametrov na vrednost konstanto protonacije.