Electrophoretic Evaluation of Doubtful Dissociation of Some Amino Acids

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A electrophoretic technique evolved in this laboratory has been used to demonstrate the different stages of dissociation of the four amino acids viz. ethylendiamine tetra acetic acid, nitrilotriacetic acid, 2,3 diamino propionic acid and arginine. Dissociation constants of different stages of dissociations have also been calculated. In literature the data on dissociation constants of these acids are ambiguous or incomplete. Our technique thus has solved the ambiguity and enables us to evaluate the unreported dissociation constants.

In our earlier communication (1) electrophoretic technique evolved in this laboratory has been used for the determination of dissociation constants of a few amino acids. This paper is an extension of previous work and demonstrates its advantages over electrometric technique. Four typical amino acids viz. ethylene diamine tetra acetic acid (EDTA), nitrilotriacetic acid (NTA), 2,3-diamino propionic acid and arginine have been selected for investigation. Perusal of literature (24) reveals that the agreement between the constant reported is not good and some dissociation constants are not at all reported. This is so because these values lie in extreme ends of the scale of pH meter. The electrophoretic technique as worked out in this laboratory is not subject to these limitations.

EXPERIMENTAL

The electrophoretic apparatus and procedure are described in refrence 1.

The background electrolyte was 3.0 M with reference to $HClO_4$.

The electrophoretic mobility was calculated at each stage of partial neutralization of background perchloric acid with AR quality sodium carbonate. In each of these cases, till acid strength was 0.1 M, it was determined by titration. Below this hydrogen ion concentration (pH 1-7) Elico pH meter was employed for pH measurements of the background electrolyte at different stage of neutralization. For measuring mobility beyond pH-7 of background electrolyte, the background electrolyte was modified by pipetting out a small amount of the electrolyte and replacing it with an equal aliquot of 3.0 M sodium hydroxide (to keep ionic strength constant). Measurements were thus carried out upto pH 12 stage. Beyond it, hydroxyl ion concentration of background electrolyte was determined titrimetrically with the help of standard hydrochloric acid solution.

The activity of hydrogen ion in highly acid solution (pH< 1) and high alkaline solutions (pH>12) were calculated by multiplying the titrimetric concentration with activity coefficient obtained with the help of Davies equation (5).

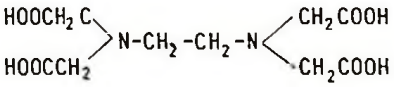
$$-\log f_{\pm} = 0.50\ Z+Z- \left[\frac{I}{\sqrt{I}+1} - 0.20\ I\right]$$

This equation was used by Bell et al.(6) for calculating activity coefficients in highly concetrated solutions.

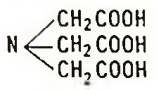
The electrophoretic mobility of different amino acid spots as dependent on pH have been graphically represented in figure-1.

RESULTS AND DISCUSSION

The four amino acids are represented structurally as:



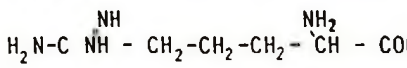
(EDTA)



(NTA)



(2,3-diamino propionic acid)



(Arginine)

They should have 7,5 4 and 4 ionic species respectively (including the protonated amino form). As described earlier (1) mobility curves of these amino acids show as many plateaus as the number of their individual ionic species. Applying the principle of average mobility(1) the dissociation constants of the amino acids have been calculated and are given in Table-1. Literature values have also been given in the

TABLE 1

DISSOCIATION CONSTANTS OF EDTA, 2,3 DIAMINO PROPANOIC ACID & ARGININE.
(Electrophoretic and Reported values)

Amino acid	Temp. 25°C		Literature values						Ref. No.
	μ	Temp. (°C)	Log K_1	Log K_2	Log K_3	Log K_4	Log K_5	Log K_6	
EDTA	3.0	25	-0.18	1.12	2.06	3.06	6.18	10.30	Present study
	?	25	-	1.91	2.21	2.77	6.16	10.26	2
	0.4	20	-	1.91	2.49	-	5.89	9.92	2
	0.1	20	-	1.99	2.67	-	6.16	10.26	2
	0.1	25	-	1.51	-	-	-	-	2
	0.1	30	-	-	2.40	2.86	6.22	10.25	2
	0.02	?	-	-	-	-	6.81	11.33	2
	0.32	25	-	-	-	-	6.29	10.46	2
	0.1	?	-	-	-	2.67	6.22	9.60	2
	0.1	25	-	-	-	-	6.33	9.98	2
	?	33-37	-	-	-	-	6.2	9.8	2
	0.1	20	-	-	-	-	6.16	10.44	2
	0.1	20	-	-	2.40	2.7	6.16	10.23	2
	0.1	20	-	2.2	-	2.7	6.07	10.12	2
	1.0	20	-	-	-	-	6.26	9.95	2
	1.0	20	-	2.3	-	-	6.28	8.85	2
	1.0	20	-	1.4	2.2	-	-	-	2
	-	-	-	-	-	5.8	6.47	10.1	2
	1.2	20	-	-	2.07	2.44	6.21	9.37	2
	0.1	25	0.0	1.5	2.0	2.68	6.17	10.17	3
	0.1	20	-	1.5	2.0	2.66	6.17	10.24	3
	0	-	0.1	1.4	2.2	2.3	6.32	11.014	3
	0.15	25	-	-	-	-	-	10.2	4
	0.1	25	-	-	2.0	2.69	6.18	10.15	4
	1.0	25	-	-	-	-	6.25	9.80	4
	0.1	25	-	-	-	-	6.03	9.38	4
	0.1	25	-	-	2.75	2.75	6.16	10.39	4
	0.1	25	-	-	-	2.7	6.16	-	4
	0.1	25	-	-	-	-	6.18	10.15	4
	?	?	-	-	2.00	2.67	6.16	10.27	4
	6.005	25	-	-	-	2.1	8.5	10.45	4
	0.1	25	-	-	-	2.7	6.16	-	4
	3.0	25	-	1.70	2.13	2.51	7.00	9.04	4
	0.5	25	-	-	-	-	6.12	10.22	4
NTA	3M	25	-0.12	1.60	2.36	9.47	-	-	present study
	0.1	20	-	1.89	2.49	9.73	-	-	
	0.1	25	-	-	4.18	10.11	-	-	2
	1.0	25	-	-	-	8.70	-	-	2
	0.1	20	-	1.75	2.47	9.70	-	-	2
	-	-	-	1.88	2.50	-	-	-	2
	0.1	20	-	-	-	9.87	-	-	2
	0.1	20	-	1.9	2.5	9.73	-	-	2
	1	20	-	1.99	2.27	8.96	-	-	2
	0.2	20	-	1.97	2.60	9.45	-	-	2
	0.1	6.4	-	2.30	3.00	10.76	-	-	2
	0.1	25	-	1.8	2.48	9.65	-	-	3
	0.1	20	0.8	1.8	2.48	2.71	-	-	3
	0	20	1.1	1.65	2.94	10.334	-	-	3
	0.15	25	-	-	-	9.81	-	-	4
	0.1	25	-	1.68	2.67	9.49	-	-	4

Amino acid	Temp. 25°C μ	Temp. (°C)	Literature values Log K_1	Log K_2	Log K_3	Log K_4	Log K_5	Log K_6	Ref. No.
	0.1	25	-	2.06	2.95	9.49	-	-	4
	0.5	25	-	1.70	2.28	8.95	-	-	4
	0.1	25	-	1.98	2.38	9.95	-	-	4
	0.1	25-30	-	-	2.88	9.58	-	-	4
2, 3-diamino propionic acid	3.0	25	1.84	6.84	9.84	-	-	-	Present study
	0.1	25	-	6.69	9.95	-	-	-	2
	0.1	25	-	6.67	9.40	-	-	-	3
	0.5	25	-	6.55	9.35	-	-	-	3
	0.5	25	-	6.70	9.50	-	-	-	4
	0.1	25-50	-	6.67	9.62	-	-	-	4
Arginine	30M	25	2.18	2.18	13.30	13.30	-	-	Present study
	0	19	-	9.11	-	-	-	-	2
	0.1	25	2.05	9.01	-	-	-	-	3
	0.1	25	-	9.36	-	-	-	-	4

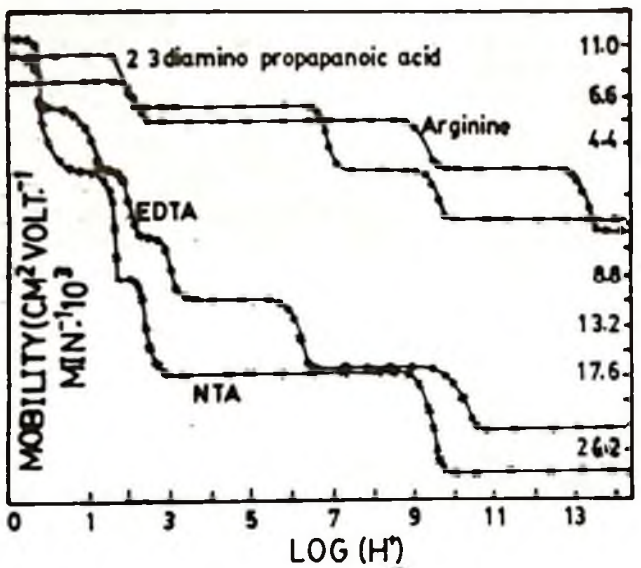


Fig. 1 Mobility Curves

Table for the sake of comparison. The literature does not give any information regarding first dissociation constant (K_1) of 2,3 - diaminopropanoic acid and the last dissociation constant of arginine (K_3). The values of the first dissociation constants of nitrilotriacetic acid and ethylene diamine tetraacetic acid given in literature, are ambiguous. The electrophoretic studies give a precise indication of these hitherto doubtful and unreported dissociations. These dissociations are clearly elucidated in the curves and their K values have been calucu-lated and given in Table -1.

In general our calculated values agree with literature values; the small difference being ascribed to the different ionic strength. It is to be emphasized that the electrophoretic technique has great advantage over the common electrometric method in as mech as it gives a clear picture of different stages of dissociation and is helpful in calculating their dissociation constants even of those stages which occur beyond the mesurable range of pH meter.

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