

## A Continuous Titration Technique for Determining pK Values of Amino Acids in Mixed Solvents

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The dissociation constants of amino acids have been determined pH-metrically in different mixtures of varying compositions with the help of a new technique, viz., continuous titration technique. This method is quick and gives reasonably good practical pK values.

### INTRODUCTION

A continuous titration technique developed in this laboratory has been utilized to investigate proton ligand formation constants of DL-serine, DL-threonine, glutamic acid, L-asparagine, histidine and arginine in various compositions of aquo-organic media. The solvents used for the study were dimethyl formamide (DMF), dimethyl sulfoxide (DMSO) and acetone. The method proved<sup>1</sup> to be quite convenient and accurate in determining pK values of the amino acids in a wide range of solvent composition and involves no tedious calculations.

### EXPERIMENTAL

All the chemicals used were of AR grade (Merck or Fluka). DMF was purified by the method described by Ruhoff<sup>2</sup>. DMSO and acetone were purified as described previously<sup>3</sup>. Deionized water was used for preparing the solutions. pH-measurements were made at 25°C in 0.1 M (NaClO<sub>4</sub>) ionic strength on an Elico pH-meter, model L1-10. The reproducibility was within 0.02 pH units. The continuous titration technique<sup>4</sup> has been used in the present work for the determination of pK values of different amino acids in a number of mixed solvents such as 10-80% DMF-water (v/v), 10-80% DMSO-water (v/v) and 10-80% acetone-water (v/v) mixtures.

#### Continuous Titration Technique

The method involves the following procedure: Prethe and Mali<sup>4</sup> applied this method to the substituted benzoic acids in the medium of dioxane-water and could get appreciably good agreement of pK values compared to those obtained by Irving-Rossotti method<sup>5</sup>

In the present investigation the amino acids were subjected to this comparably

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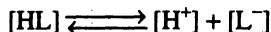
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quicker technique of getting  $pK$  values in the mixed solvent media as DMF-Water, DMSO-Water and acetone-water at 25°C.

## RESULTS AND DISCUSSION

The experimental results are reported in Tables 1-3 for DMF-water, DMSO-water and acetone-water media respectively.

During the conventional titration of ligand with base Irving-Rossotti technique<sup>5</sup> in 0/100 and 80/20 dioxane-water media the following equilibrium (if the initial starting concentration of HL were  $2 \times 10^{-a}$ ) at half-neutralisation could be written.



$$[10^{-a}] = [10^{-pK_0}] + [10^{-a}] \text{ in 0/100 dioxane-water, and}$$

$$[10^{-a}] = [10^{-pK_{80}}] + [10^{-a}] \text{ in 80/20 dioxane-water}$$

where  $pK_0$  and  $pK_{80}$  are the values in 0 and 80% dioxane-water medium respectively.

In the method of continuous titration pH is adjusted to  $pK_0$  initially which changes to the same  $pK_{80}$  obtained by conventional method, on the addition of dioxane indicating that  $H^+$  has changed from  $10^{-pK_0}$  to  $10^{-pK_{80}}$ .

On the addition of dioxane, the dielectric constant of the medium decreases which facilitates the combination of  $H^+$  and  $L^-$  forming undissociated HL. The new concentration of the HL and  $L^-$  in 80/20 dioxane-water solution will thus be

$$[HL] = 10^{-a} + (10^{-pK_0} - 10^{-pK_{80}})$$

and

$$[L^-] = 10^{-a} - (10^{-pK_0} - 10^{-pK_{80}})$$

where  $a < pK_0 < pK_{80}$ .

The new ratio would thus be

$$\frac{[HL]}{[L^-]} = \frac{10^{-a} + (10^{-pK_0} - 10^{-pK_{80}})}{10^{-a} - (10^{-pK_0} - 10^{-pK_{80}})} \neq 1$$

The above ratio could still be brought very close to unity by making  $10^{-pK_0} - 10^{-pK_{80}}$  very small (as is possible in the case of  $10^{-pK_0} - 10^{-pK_{20}}$ ). The dissociation constants are, however, beyond our control. If  $pK_{80}$  is large,  $10^{-pK_{80}}$  will be small as compared to  $10^{-a}$  and  $10^{-pK_a}$  which may, therefore, be neglected. Thus,

$$\frac{[HL]}{[L^-]} = \frac{10^{-a} + 10^{-pK_0}}{10^{-a} - 10^{-pK_0}} \neq 1$$

Therefore

$$1 + \frac{2 \times 10^{-pK_0}}{10^{-a} - 10^{-pK_0}} \neq 1$$

The ratio  $[HL]/[L^-]$  deviates from unity by this amount which may be put equal to C.

TABLE-1  
DISSOCIATION CONSTANTS OF AMINO ACIDS IN DMF-WATER MIXTURE

DMF % (v/v)	Method	DL-serine		DL-Threonine		Glutamic acid		L-Asparagine		Histadine		Arginine	
		pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>
0	CT	2.20	9.05	2.25	8.98	4.07	9.38	2.17	8.78	6.07	9.06	2.10	9.03
	AS	2.20	9.05	2.25	8.98	4.07	9.38	2.17	8.78	6.07	9.06	2.10	9.03
10	CT	2.44	9.15	2.39	8.96	4.19	9.36	2.31	8.61	6.06	9.05	2.30	8.88
	AS	2.46	9.15	2.41	8.99	4.21	9.35	2.32	8.52	6.08	9.00	3.32	8.92
20	CT	2.61	9.21	2.57	9.07	4.45	9.47	2.49	8.83	5.97	9.12	2.44	9.05
	AS	2.60	9.19	2.58	9.10	4.47	9.46	2.51	8.85	5.99	9.21	2.46	9.09
40	CT	3.05	9.39	3.06	9.28	5.09	9.76	3.03	9.04	5.92	9.28	2.96	9.21
	AS	3.06	9.37	3.09	9.30	5.12	9.76	3.05	9.08	5.90	9.30	3.00	9.28
50	CT	3.35	9.50	3.37	9.39	5.46	9.91	3.38	9.12	5.92	9.42	3.29	9.34
	AS	3.37	9.49	3.37	9.40	5.48	9.87	3.40	9.14	5.94	9.44	3.30	9.36
60	CT	3.74	9.71	3.76	9.56	6.00	10.19	3.80	9.39	5.92	9.63	3.74	9.56
	AS	3.72	9.61	3.78	9.56	6.01	10.21	3.82	9.38	5.96	9.65	3.76	9.58
80	CT	4.46	9.89	4.19	9.69	7.38	10.39	—	9.57	5.92	9.83	4.76	9.76
	AS	ppt	9.96	4.54	9.74	7.40	10.43	5.05	9.59	5.90	9.82	4.77	9.80

CT: Continuous titration, AS = Albert Sargeant

TABLE-2  
DISSOCIATION CONSTANTS OF AMINO ACIDS IN DMSO-WATER MIXTURE

DMSO % (v/v)	Method	DL-Serine		DL-Threonine		Glutamic acid		L-Aspergine		Histadine		Arginine	
		pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>
0	CT	2.20	9.05	2.25	9.89	4.07	9.38	2.17	8.78	6.07	9.06	2.10	9.03
	AS	2.20	9.05	2.25	9.98	4.07	9.38	2.17	8.78	6.07	9.06	2.10	9.03
10	CT	2.40	9.12	2.43	9.02	4.53	9.46	2.36	8.80	6.05	9.09	2.26	9.08
	AS	2.42	9.17	2.45	9.04	4.55	9.45	2.39	8.82	6.04	9.17	2.25	9.10
20	CT	2.61	9.21	2.62	9.12	4.76	9.62	2.61	8.95	6.04	9.13	2.45	9.19
	AS	2.66	9.26	2.64	9.18	4.79	9.63	2.64	8.97	6.04	9.27	2.50	9.21
40	CT	3.17	9.52	3.26	9.43	5.24	9.95	3.31	9.36	6.07	9.43	3.03	9.48
	AS	3.25	9.52	3.22	9.46	5.25	9.97	3.35	9.38	6.07	9.44	3.04	9.50
50	CT	3.59	9.71	3.70	9.60	5.73	10.20	3.74	9.50	6.10	9.58	3.45	9.68
	AS	3.57	9.72	3.71	9.62	5.75	10.25	3.78	9.54	6.11	9.60	3.47	9.69
60	CT	4.07	10.15	4.27	9.99	6.40	10.53	4.29	9.50	6.16	9.99	3.95	10.00
	AS	4.15	10.08	4.18	9.94	6.42	10.55	4.32	9.84	6.08	9.97	3.97	10.01
80	CT	5.65	10.45	5.70	10.28	8.07	10.52	5.51	10.98	6.30	10.24	5.28	10.75
	AS	5.69	10.50	5.51	10.28	8.10	10.48	5.53	10.96	ppt	—	5.23	10.72

CT: Continuous titration, AS: Albert Sargeant

TABLE-3  
DISSOCIATION CONSTANTS OF AMINO ACIDS IN ACETONE-WATER MIXTURE

Acetone % (v/v)	Method	DL-serine		DL-threonine		Glutamic acid		L-Asparagine		Histidine		Arginine	
		pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>	pK <sub>1</sub>	pK <sub>2</sub>
0	CT	2.20	9.05	2.25	8.98	4.07	9.38	2.17	8.78	6.06	9.06	2.10	9.03
	AS	2.20	9.05	2.25	8.98	4.07	9.38	2.17	8.78	6.06	9.06	2.10	9.03
10	CT	2.35	8.96	2.40	8.71	4.25	9.32	2.28	8.74	5.98	8.91	2.20	8.97
	AS	2.37	9.02	2.39	8.75	4.28	9.31	2.30	8.76	5.78	9.07	2.23	8.89
20	CT	2.45	8.87	2.49	8.43	4.42	8.31	2.42	8.74	6.00	8.86	2.39	8.97
	AS	2.50	8.96	2.46	8.44	4.40	9.29	2.44	8.78	5.78	8.97	2.44	8.85
40	CT	2.82	8.73	2.87	8.14	4.80	9.47	2.77	8.76	5.94	8.72	2.81	8.91
	AS	2.70	8.82	2.80	8.16	4.72	9.48	2.78	8.84	5.64	8.83	2.88	8.81
50	CT	3.04	8.60	3.10	8.08	5.01	9.50	2.99	8.79	6.02	8.67	3.00	8.91
	AS	3.08	8.69	3.09	8.10	5.04	9.52	3.00	8.80	3.61	8.60	3.18	8.81
60	CT	3.29	8.55	3.32	7.98	5.28	9.51	3.00	8.80	5.99	8.37	3.30	8.90
	AS	—	8.69	3.23	7.97	5.32	9.54	3.20	8.32	5.52	8.67	3.42	—
80	CT	3.82	8.47	3.86	7.85	5.75	9.44	ppt	8.80	5.80	8.50	3.86	8.90
	AS	—	8.46	ppt	7.70	6.88	9.48	ppt	9.39	5.84	8.55	3.89	—

CT: Continuous Titration, AS: Albert Sargeant

On solving,

$$10^{-pK_0 - a} = \frac{2 + C}{C} = \frac{2}{C} + 1$$

Obviously as the difference between  $pK_0$  and  $a$  increases, deviation of  $C$  from unity becomes smaller and smaller. Thus the difference in the starting concentration of HL and  $pK_0$  (the initially adjusted dissociation constant) should be higher for the usefulness of continuous titration. In order to check this the continuous titrations have been carried out with initial ligand concentration =  $6 \times 10^{-3}$ ,  $2 \times 10^{-3}$  and  $0.75 \times 10^{-3}$  M by Prethe and Mali<sup>4</sup>. The results agree well in the case of solutions with higher initial ligand concentration and show deviation at lower initial concentration, *i.e.*,  $0.75 \times 10^{-3}$  M.

We have, therefore, chosen the concentration of the ligands as  $5.0 \times 10^{-3}$  M. The agreement is very much satisfactory between the values obtained by present method and those by Albert Sergeant method<sup>6</sup> in the entire range of aquo-organic composition. The new method is advantageous because it gives  $pK$  values of any ligand at any desired organic solvent percentage in the aquo-organic medium within the smallest possible variation.

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