

### 630. *The Titration and the Apparent Dissociation Constants of Weak Acids in Mixed Aqueous Solvents.*

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The effect of acetone, ethanol, dioxan, and formaldehyde on the pH-titration curves of weak acids in aqueous solution is recorded. The apparent dissociation constants of alanine in these solvents, determined from the titration curves, are remarkably consistent. The application of corrections for the effect of the non-aqueous component on the electrode system reduces this agreement. It is suggested that amino-acids may buffer the electrode system as between different solvents.

THE behaviour of the optical rotatory power of alanine in aqueous acetone and dioxan<sup>1</sup> suggested that loosely bound compounds might be formed in neutral as well as in alkaline<sup>2</sup> solutions. We have examined the effect of dioxan and acetone on the titration of aqueous alanine with sodium hydroxide solution and compared it with the effect on the titration curves of boric acid and phenol. The results of previous work<sup>3</sup> on the titration in aqueous formaldehyde and 20% dioxan are confirmed.

TABLE 1. *Apparent dissociation constants of weak acids (0.025N) in aqueous solvents at 20°.*

Acid	Solvent (%. w/w)	Curve in Fig.	$pK_a$	$pK_a'$	$pK_a$	$pK_a'$	$pK_a/pK_a'$	$pK_h$
Alanine	Water	A	10.03	10.03	14.00	14.00	1.40	3.97
	8.1% Acetone	B	10.03	10.02	14.30	14.28	1.43	4.27
	20.8% Acetone	C	10.03	10.00	14.74	14.67	1.47	4.71
	44.2% Acetone	D	10.04	9.94	15.60	15.40	1.55	5.56
	70.4% Acetone	E	10.19	9.95	16.52	16.04	1.62	6.33
	78.5% Acetone	F	10.37	10.04	16.74	16.08	1.61	6.37
	12% Formaldehyde		7.31	7.28	12.06	11.99	1.65	4.75
	25.7% Dioxan	G	10.15	10.12	14.66	14.60	1.44	4.51
	50.9% Dioxan	H	10.35	10.27	15.32	15.15	1.48	4.97
	75.6% Dioxan	I	10.50	10.29	16.72	16.29	1.59	6.22
	20.8% Ethanol		9.94	9.89	14.50	14.41	1.46	4.56
	44.1% Ethanol		10.00	9.88	14.90	14.67	1.49	4.90
	70.0% Ethanol		10.15	9.88	15.88	15.32	1.56	5.63
Glycine	Water		9.88	9.88	14.14	14.14	1.43	4.26
	42.3% Acetone		9.84	9.75	15.74	15.56	1.60	5.90
	67.1% Acetone		9.89	9.68	16.87	16.45	1.71	6.98
	25.7% Dioxan		9.98	9.95	14.36	14.30	1.44	4.38
	50.9% Dioxan		10.16	10.08	14.94	14.77	1.47	4.78
	75.6% Dioxan		10.38	10.17	16.56	16.13	1.60	6.18
Boric acid	Water	J	9.22	9.22	14.24	14.24	1.54	5.02
	44.2% Acetone	K	11.00	10.90	15.40	15.30	1.40	4.40
	50.9% Dioxan	L	11.28	11.20	15.34	15.17	1.36	4.06
	12% Formaldehyde	M	8.61	8.58	12.06	11.99	1.40	3.45
Phenol	Water	N	9.95	9.95	13.96	13.96	1.40	3.01
	44.2% Acetone	O	11.62	11.53	15.24	15.04	1.31	3.62
	50.9% Dioxan	P	11.82	11.74	15.24	15.07	1.29	3.42
	12% Formaldehyde	Q	9.60	9.57	12.28	12.21	1.28	2.68
Sodium hydrogen carbonate	Water		10.16	10.16	14.12	14.12	1.39	3.96
	44.2% Acetone		11.44	11.34	15.30	15.10	1.34	3.86
	12% Formaldehyde		9.76	9.73	12.38	12.31	1.27	2.52

The dissociation constant of alanine is sensibly unaffected by the presence of acetone even in quite high aqueous concentrations. Thus, although acetone facilitates the titration of amino-acids to much the same extent as formaldehyde, it must be for a different reason, since formaldehyde markedly increases the dissociation constant (Table 1). Though

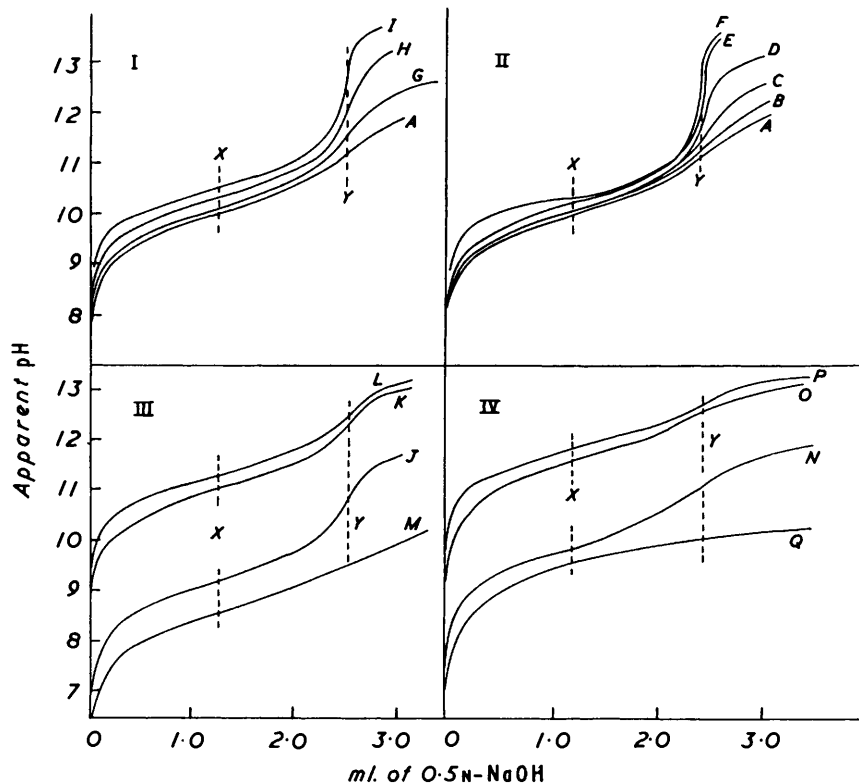
<sup>1</sup> Hargreaves and Richardson, *J.*, 1957, 3823.

<sup>2</sup> Levy, *J. Biol. Chem.*, 1932—33, **99**, 767.

<sup>3</sup> Dunn and Loshakoff, *J. Biol. Chem.*, 1936, **113**, 691; Duggan and Schmidt, *Arch. Biochem.*, 1943, **1**, 453.

the behaviour of the rotatory power in these solvents is probably best explained in terms of the formation of compounds<sup>1</sup> between the solute and the solvent, the fact that the apparent dissociation constant of alanine is virtually unaltered by the presence of the organic component suggests that this compound formation must be extremely tenuous. Since acetone affects the titration curves through its effect on the dissociation of the solvent it possibly affects the rotatory power of alanine through its effect on the solvating properties of the solvent rather than direct interaction with the alanine. Other organic components could act similarly.

pH-Titration curves of (I) alanine in dioxan solutions, (II) alanine in acetone solutions, (III) boric acid and (IV) phenol against sodium hydroxide.



For key to curves, see Table 1. X = half-equivalence point, Y = equivalence point.

*Form of the Titration Curves.*—Potentiometric titration curves of boric acid and of phenol (Fig., III and IV) show that the end-point is not improved on addition of the organic component to the aqueous solvent. Table 1 and the Figure show that a reasonable end-point may be expected when  $pK_s/pK_a$  exceeds *ca.* 1.44 for alanine. Solution of the quadratic equation governing this type of equilibrium<sup>4</sup> gives  $[H^+] = L^{\frac{1}{2}} - \frac{1}{2}N$ . Differentiation of this yields

$$d \text{ pH}/db = -1/(L^{\frac{1}{2}} + \frac{1}{2}N) \cdot [\frac{1}{2}L^{\frac{1}{2}}(NS - K_a K_s/b^2) - S]$$

where  $L = \frac{1}{4}N^2 + K_a K_s/b$ ,  $N = K_a(1 - a/b) - K_s/b$ ,  $S = (K_a a + K_s)/2b^2$ . The roots of  $L$  can be positive or negative.  $K_a$  is the dissociation constant of the acid,  $K_s$  the ionic product of the solvent, and  $a$  and  $b$  the molar concentrations of acid and base respectively

<sup>4</sup> Glasstone, "An Introduction to Electrochemistry," van Nostrand, New York, 1942, p. 393.

With our values of  $a = b = 0.025$ ,  $K_a = 10^{-10}$ , and  $K_s = 10^{-14}$ , we obtain approximately, at the end-point

$$d \text{pH}/db = \pm \sqrt{(K_a/4_a K_s)}$$

This expression provides an alternative method of estimating the sensitivity of the end-point, but it does not take into account deviations from ideality and the actual slope differs from that derived by a factor varying between 1 and 4 so that neither  $\text{p}K_b$  nor  $\text{p}K_s/\text{p}K_a$  has a definite critical value as between different acids. The slope of the neutralisation curve at the end-point should be inversely proportional to the square-root of the hydrolysis constant,  $K_h = K_s/K_a$ . Thus a decrease in the ratio  $K_a : K_s$  will be accompanied by an increase in the hydrolysis of the salt and also by a reduction in the sensitivity of the end-point.

In the expressions used for  $K_s$  the concentration of the solvent is usually taken as constant, but in these mixed solvents it is desirable to allow for the varying proportions of the water. A full allowance for the solvating properties of water ( $\text{HOH} + \text{HOH} \rightleftharpoons \text{H}_3\text{O}^+ + \text{OH}^-$ ) leads to the expression  $K_s' = K_s/N^2$  where  $N$  is the mole-fraction of water in the solvent, whereas only a single exponent for  $N$  is required if the organic component takes part in the solvation preferentially. The use of the mole fraction instead of molar concentration follows Gutbezahl and Grunwald,<sup>5</sup>  $\text{p}K_s'$  thus having the same numerical value and the same dimensions as  $\text{p}K_w$  in pure water.

Similarly the equilibrium constant of the reaction  $\text{HA} + \text{HOH} \rightleftharpoons \text{H}_3\text{O}^+ + \text{A}^-$  is given by  $K_a' = K_a/N$ . Table 1 shows that  $\text{p}K_a'$  varies rather less than  $\text{p}K_a$ , as expected, but it seems likely that the mole-fraction term over-corrects the expression since solvolysis may also occur with the organic component.

To the nearest unit in the second decimal place of pH units the Henderson equation  $\text{p}K = \text{pH} + \log[\text{HA}]/[\text{A}^-]$  can be substituted for the equation governing this type of equilibrium so that  $\text{p}K$  can be determined as the pH at half-neutralisation.  $\text{p}K_s$  can similarly be determined from the pH at the neutral point from  $\text{pH} = \frac{1}{2}\text{p}K_a + \frac{1}{2}\text{p}K_s + \frac{1}{2}\log c$ , where  $c$  is the concentration of the salt produced.

*Variation of the Apparent Dissociation Constant with Solvent.*—The effectiveness of acetone and dioxan in improving the end-point in the titration of amino-acids is due to their effect on  $K_s$  rather than on the dissociation constant of the amino-acid. With boric acid and phenol the acid dissociation constants are moved nearer to the dissociation constant of the solvent by the organic component so that the end-point is worse. Table 1 shows how  $\text{p}K_a$  and  $\text{p}K_s$  are affected. It is noteworthy that formaldehyde affects the  $\text{p}K_a$  for alanine much more than does acetone or dioxan; its effect is also opposite in sign to that of the other organic components. This seems to be attributable to compound formation.

The contrasting behaviour of the amino-acids and that of the other acids studied may be related to the effect of the dielectric constant of the solution on the dissociation of acids of ammonium-ion type and of acids where positive and negative charges are separated in the process. The effect of dielectric constant may in turn be linked with the state of solvation of the respective ions or molecules. The hydrogen ion, being common to both types of acid, cannot be involved in the difference in their behaviour. The small change observed in the apparent  $\text{p}K_a$  of the amino-acids as the aqueous solution is diluted with the organic component suggests that the difference between the solvation energies in water and in the organic liquid of the acid must be similar to that of its dissociated parts.

Harned and Birdsall's<sup>6</sup> results for glycine (as calculated by Robinson and Stokes<sup>7</sup>) do not show this constancy; their dissociation constants, which were extrapolated to zero ionic strength, decrease more markedly with the proportion of dioxan. On the other

<sup>5</sup> Gutbezahl and Grunwald, *J. Amer. Chem. Soc.*, 1953, **75**, 565.

<sup>6</sup> Harned and Birdsall, *ibid.*, 1943, **65**, 1117.

<sup>7</sup> Robinson and Stokes, "Electrolyte Solutions," Butterworths, London, 1955, p. 504.

hand Lonning and Treadwell's<sup>8</sup> results for glycine in acetone are more in agreement with this work.

With aliphatic carboxylic acids  $pK_h$  varies much less in aqueous dioxan than does  $pK_h$  of alanine (Table 2; calculated from Harned and Owen's results<sup>9</sup>).  $pK_a$  increases with  $pK_s$  as the organic component is added, and  $pK_s/pK_a$  is thus decreased and the end-

TABLE 2.  $pK_h$  of carboxylic acids in aqueous dioxan solution.

Dioxan (% w/w)	Formic acid	Acetic acid	Propionic acid
0	10.41	9.41	9.24
20	10.61	9.50	9.32
45	10.83	9.60	9.36
70	11.02	9.70	9.40

point rendered less satisfactory. The fact that carboxylic acids dissociate less readily in aqueous dioxan than in water<sup>9</sup> may mean that the ions are less extensively solvated than in water. The molecular and ionic species of the amino-acids, on the other hand, appear to be solvated to about the same relative extent in all solvents studied. At all concentrations used here there was always sufficient water present to provide a sheath around the acid species.

Table 1 shows that the apparent dissociation constant of alanine and of glycine in aqueous acetone varies very little up to a concentration of 70% of acetone, agreeing very well with those obtained in pure water. Alcohol-water mixtures show a similar constancy and dioxan-water mixtures produce a slightly greater variation.

*Correction for the Effect of the Solvent on the Liquid Junction.*—The theoretical difficulties of treating such systems absolutely made us attempt to determine an empirical correction to enable these results to be compared with those obtained in pure water. Such a correction was suggested by van Uitert and Haas,<sup>10</sup> and in a different form by Gutbezahl and Grunwald:<sup>5</sup>

$$pH_A = pH_A^s + C, \quad pH_B = pH_B^s + C$$

where  $C$  is a term correcting for the effect of the organic component in the solvent. Then  $pH_A = pH_A^s + pH_B - pH_B^s$ . The subscripts A and B refer to the test solution and to a standard solution of hydrochloric acid respectively, and the superscript denotes a mixed aqueous solvent. The values are given in Table 3. This correction is only strictly valid

TABLE 3. Empirical electrode corrections for mixed aqueous solvents.

(%, w/w) *	$A \dagger$	$M_A - M_A^s$	Sum ‡	(%, w/w) *	$A \dagger$	$M_A - M_A^s$	Sum ‡	(%, w/w) *	$A \dagger$	$M_A - M_A^s$	Sum ‡
Acetone				Ethanol				Dioxan			
10.2	0.996	0.06	0.06	9.9	0.977	0.02	0.02	12.9	0.998	0.04	0.05
20.8	0.993	0.10	0.10	20.8	0.958	-0.01	0.00	25.7	0.996	0.03	0.04
32.2	0.989	0.10	0.11	32.1	0.949	-0.05	-0.04	38.3	0.989	0.04	0.05
44.2	0.986	0.14	0.15	39.2	0.942	-0.11	-0.09	50.9	0.968	0.13	0.16
56.7	—	0.24	—	44.1	0.932	-0.18	-0.16	63.1	0.904	0.27	0.31
70.4	—	0.49	—	56.8	0.924	-0.25	-0.23	75.6	0.815	0.74	0.87
				70.0	0.906	-0.33	-0.29				

\* Of component shown in centre heading.

†  $A$  = estimated mean activities (from references 11 and 12) used to calculate column 4.

‡ Sum =  $M_A - M_A^s + \log(a_A/a_A^s)$ .

if the protonic activities are identical in the test and in the standard solution, but using estimated values for the activities of the hydrogen ion<sup>11,12</sup> we can derive a correction

<sup>8</sup> Lonning and Treadwell, *Helv. Chim. Acta*, 1945, **28**, 1037.

<sup>9</sup> Harned and Owen, "Physical Chemistry of Electrolytic Solutions," Reinhold, New York, 1950, p. 581.

<sup>10</sup> van Uitert and Haas, *J. Amer. Chem. Soc.*, 1953, **75**, 451.

<sup>11</sup> Feakins and French, *J.*, 1956, 3168.

<sup>12</sup> Ref. 9, pp. 548, 550.

which involves only the junction potential by standardising the meter with two buffer solutions so that the meter readings  $M_{s,1} = -\log a_{s,1}$  and  $M_{s,2} = -\log a_{s,2}$ . But the meter measures e.m.f.,  $E$ , which is given by:

$$\begin{aligned} E_{s,1} &= -\phi \log a_{s,1} + E^1 \\ E_{s,2} &= -\phi \log a_{s,2} + E^1 \end{aligned}$$

Similarly for the test solutions:

$$E_A = -\phi \log a_A + E^1 ; E_B = -\phi \log a_B + E^1$$

and in mixed solvents

$$E_A^s = -\phi \log a_A^s + E^{1s} ; E_B^s = -\phi \log a_B^s + E^{1s}$$

thus  $\log a_B^s$  can be evaluated \* if the values for solution A are known and on the assumption that the liquid junctions are similar for A and B.

Then, since  $M_B = -\log a_B$ :

$$-\log a_B^s = M_B^s + (M_A - M_A^s) + \log (a_A/a_A^s)$$

which is reasonable where a saturated potassium chloride bridge is employed. An uncertainty is, however, introduced since the anions are different in the test solution and in the check solution. Since the anions due to the salt bridge are in such excess, this objection is not likely to be serious at the present level of accuracy

This type of correction is found to be rather large (Table 1) and would destroy the constancy of the apparent  $pK_a$  for alanine. The values given in Table 3 are readings of e.m.f. given on a scale with fixed points determined with aqueous buffer solutions (solutions of constant hydrogen-ion activity and e.m.f., and thus of constant available energy). The constancy of  $pK_a$  in Table 1 thus implies a constancy in pH and thus in e.m.f. and available energy.

When the pH scale is calibrated in terms of buffer solutions the meter readings are related as follows for two solutions X and Y:

$$\begin{aligned} M_X - M_S &= \log a_S - \log a_X + (E_X^1 - E_S^1)/\phi \\ M_Y - M_S &= \log a_S - \log a_Y + (E_Y^1 - E_S^1)/\phi \end{aligned}$$

When  $M_X = M_Y$ , as with water and 20% acetone solutions of alanine,

$$\text{then } \log a_Y - \log a_X = (E_Y^1 - E_X^1)/\phi$$

Thus for a solution of the acid in two different solvent mixtures the change in the liquid-junction potential is balanced by a change in the activity of the hydrogen ion.

The fact that the observed e.m.f.s of cells containing alanine at half-neutralisation in various acetone-water and ethanol-water mixtures do not vary suggests that a kind of buffering action may occur since with the strong acid, hydrochloric, there is a definite difference in the e.m.f.s observed in the two solvents. This buffering effect is not observed with the weak acids since their dissociation constants vary too greatly with the solvent. It is impossible to compare directly the effect of the components of the mixed solvents on the e.m.f. of a strong acid in the region of pH greater than 7; comparison with solutions of strong alkali are not direct since the electrode is sensitive to hydrogen ions only and above pH 7 operates by virtue of the ionic product, which is not constant as between different solvent mixtures.

\*  $\phi M_A = E_A - E^1$ ;  $\phi M_A^s = E_A^s - E^1$ ;  $E_A^s = -\phi \log a_A^s + E^{1s}$   
 $\phi M_B = E_B - E^1$ ;  $\phi M_B^s = E_B^s - E^1$ ;  $E_B^s = -\phi \log a_B^s + E^{1s}$   
 $M_A - M_A^s = \log (a_A^s/a_A) + (E^1 - E^{1s})/\phi$ ;  $\phi = (E_{s,1} - E_{s,2})/(M_{s,1} - M_{s,2})$

$E^1$  and  $E^{1s}$  include the standard cell potentials and the respective liquid junction potentials.

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The dissociation of amino-acids has been compared with that of the ammonium ion.<sup>13</sup> It seemed interesting to see how far the results obtained for amino-acids are paralleled by the dissociation of ammonium chloride. These results are given in Table 4; those for

TABLE 4. *Apparent pK<sub>a</sub> of the ammonium ion at 20° in mixed solvents.*

Solvent	pK <sub>a</sub>	pK <sub>s</sub>	Solvent	pK <sub>a</sub>	pK <sub>s</sub>
Water .....	9.41	14.04	44.2% Acetone .....	9.17	15.76
44.1% Ethanol .....	9.17	14.90	50.9% Dioxan .....	9.17	14.86
14.6% Formaldehyde .....	3.68	13.88			

*Solutions.*—25 ml. of 0.0509N-ammonium chloride solution diluted with 25 ml. of water, acetone, dioxan, or 25.3% formaldehyde solution.

formaldehyde solution cannot be exact because formaldehyde reacts slowly with ammonium chloride giving substituted ammonias.<sup>14</sup> It is noteworthy that there is a definite decrease in the apparent pK<sub>a</sub> on the introduction of 50% of organic component into the solvent. Thus, the liquid-junction potential being neglected, the activity of the hydrogen ion is apparently greater in the mixed solvents than in water. This agrees with the results obtained by Gutbezahl and Grunwald<sup>15</sup> for anilinium and ammonium salts in ethanol-water mixtures but contradicts the findings for aliphatic and hydroxy-acids.<sup>16</sup> Braude and Stern<sup>17</sup> have discussed this type of effect in terms of the quasi-crystalline structure of aqueous solutions, suggesting that in a solution containing some organic diluent the crystalline structure will be less evident and the freedom of the ions and thus their activities greater. In this case an alternative explanation may lie in the liquid-junction potential.

#### EXPERIMENTAL

A Cambridge portable pH meter calibrated to 0.02 pH unit and read to 0.01 unit was used. It was standardised with electrometrically checked buffer solutions (British Drug Houses Ltd.) at pH 3.96 and 9.21. The cell was a Cambridge wide-range glass electrode in conjunction with the standard calomel electrode.

*Procedure.*—In each titration 50 ml. of the acid (0.025N) in a deep 100 ml. beaker were titrated with 0.5N-sodium hydroxide solution, a micro-burette fitted with a fine hypodermic needle as the jet being used. The beaker was immersed in a copper thermostat tank (at 20° ± 0.1°) which acted as an electrical screen. A steady stream of nitrogen from a cylinder was passed through all solutions. For alkaline solutions the mouth of the beaker was packed with cotton wool (under these conditions a solution of sodium hydroxide maintained a constant pH of 9.48 for ½ hr., but without the cotton wool the pH fell to 7.93 in ¾ hr.

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<sup>13</sup> Ref. 4, p. 418.

<sup>14</sup> Werner, *J.*, 1917, **111**, 844.

<sup>15</sup> Gutbezahl and Grunwald, *J. Amer. Chem. Soc.*, 1953, **75**, 559.

<sup>16</sup> Grunwald and Berkowitz, *ibid.*, 1951, **73**, 4939.

<sup>17</sup> Braude and Stern, *J.*, 1948, 1976.