CXCVIII.—*Universal Buffer Solutions and the Dissociation Constant of Veronal.*

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The large number of satisfactory standard buffer solutions available for the colorimetric determination of p_H values has been augmented in recent years by a class of solutions known as universal buffer solutions. These consist of mixtures of acids of diminishing strength, by the successive neutralisation of which the p_H values of the solutions are caused to vary continuously from about 2 to 12, the change in p_H being approximately proportional to the amount of alkali added. Two such buffer solutions containing phosphoric, acetic or phenylacetic, and boric acids have been described (Prideaux, *Proc. Roy. Soc.*, 1916, **92**,*A*, 463; Prideaux and Ward, J., 1924, **125**, 426), having a p_H range of 2—12, but the present authors have shown (this vol., p. 458) that the graph of the p_H of the buffer solution containing phosphoric, phenylacetic, and boric acids against the degree of neutralisation is not a straight line but is undulatory in character, and that the use of a linear interpolation formula may lead to errors of as much as 0·2 unit in p_H.

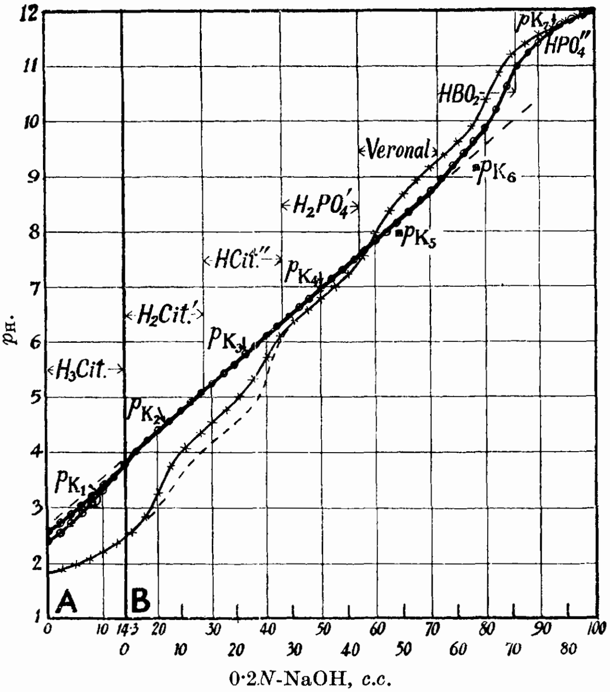
This communication contains a more detailed study of the original mixture of phosphoric, acetic, and boric acids and also a description of a new universal buffer solution which throughout a considerable portion of its neutralisation gives a rigidly rectilinear change in p_H. This has been made possible through the fact (see Kolthoff, "Gebrauch der Farbenindikatoren," 1923, p. 163;

Michaelis, *J. Biol. Chem.*, 1930, **87**, 33) that veronal [sodium diethylbarbiturate] on neutralisation with hydrochloric acid behaves as the sodium salt of a monobasic acid whose dissociation constant is of the order of 10^{-8}. This reaction has also been reinvestigated.

EXPERIMENTAL.

Titration of 100 *c.c. of a Solution of Phosphoric, Acetic, and Boric Acids, each* 0·04M, *with* 0·2N-*Sodium Hydroxide at* 18°.—This titration was performed with the hydrogen electrode against the normal

FIG. 1.



calomel electrode, a saturated solution of potassium chloride being used as the "salt-bridge." Measurements were made after the addition of each 2·5 c.c. of alkali. The p_H values so obtained are recorded in Table I and represented graphically by the thin line in Fig. 1. Only by following the neutralisation process at such small intervals can the fundamentally composite nature of the curve be brought out. As in the case of the other buffer mixture, in which acetic acid is replaced by phenylacetic acid (Britton and Robinson, *loc. cit.*), the neutralisation curve often diverges from Prideaux's straight line, which he obtained by plotting a much smaller number of points that happened to lie on or near it. Consequently, if this mixture is to be used in the colorimetric determination of p_H values, more satisfactory results will be obtained by referring to the complete curve.

The values obtained in the initial stages of the neutralisation are not comparable with those determined by Prideaux, since he diluted the mixture at different stages in the neutralisation to a constant volume. However, beyond 20% neutralisation the effect of dilution becomes very small and the p_H values here recorded are in good agreement with those obtained by Prideaux. The broken line in Fig. 1, corresponding to 20—40% neutralisation, represents the contribution made by phenylacetic acid if used in place of acetic acid.

TABLE I.

NaOH, c.c.	p_H.	NaOH, c.c.	p_H.	NaOH, c.c.	p_H.	NaOH, c.c.	p_H.
0	1·81	27·5	4·35	52·5	7·00	77·5	9·91
2·5	1·89	30·0	4·56	55·0	7·24	80·0	10·38
5·0	1·98	32·5	4·78	57·5	7·54	82·5	10·88
7·5	2·09	35·0	5·02	60·0	7·96	85·0	11·20
10·0	2·21	37·5	5·33	62·5	8·36	87·5	11·40
12·5	2·36	40·0	5·72	65·0	8·69	90·0	11·58
15·0	2·56	42·5	6·09	67·5	8·95	92·5	11·70
17·5	2·87	45·0	6·37	70·0	9·15	95·0	11·82
20·0	3·29	47·5	6·59	72·5	9·37	97·5	11·92
22·5	3·78	50·0	6·80	75·0	9·62	100·0	11·98
25·0	4·10						

These titration curves illustrate in a marked manner their composite nature. The existence of the inflexions is due to the ratio of the dissociation constants of the acids involved being somewhat too great, since it can be shown on theoretical grounds that the ideal mixture, giving an absolutely straight line, should consist of acids whose respective p_K values differ from one another by 1·2. Although this does not present any great difficulty in regard to acids that undergo neutralisation below p_H 7, it does in the case of the weaker acids that are neutralised between p_H 7 and 14. Veronal has recently been shown to set up buffer action between p_H 6·8 and 9·6, so much so that Michaelis has incorporated it in a series of buffer solutions that are especially useful in the determination of p_H values of biological fluids. By using citric acid, phosphoric acid, veronal, and boric acid, it is possible to obtain what is probably the nearest approach to the ideal conditions, at least between p_H 3·5 and 9·0, for the p_K values that are successively involved are p_{K_1} of citric acid, 3·0; p_{K_2} of citric acid, 4·6; p_{K_3} of citric acid, 5·8; p_{K_2} of phosphoric acid, 6·9; p_K of veronal, 7·96; p_K of boric acid, 9·2; and p_{K_3} of phosphoric acid, 11·6.

The Dissociation Constant of Veronal.—As the values of the dissociation constant of diethylbarbituric acid obtained by Kolthoff and Michaelis are not in satisfactory agreement, it has been redetermined from the p_H values obtained by means of the hydrogen electrode at 18° during the decomposition of 100 c.c. of 0·04M-veronal by 0·2N-hydrochloric acid. The salt was obtained from the British Drug Houses Ltd. The p_K values deduced from measurements at intervals between one-eighth and seven-eighths decomposition ranged from 7·92 to 7·98, and the mean of four such determinations was 7·96, *i.e.*, the dissociation constant of the acid is $1{\cdot}1 \times 10^{-8}$ at 18°, the p_K value being 0·1 unit higher than that found by Michaelis at 25°.

The following table gives the volumes of 0·2N-hydrochloric acid that must be added to 100 c.c. of 0·04M-veronal to set up p_H values at intervals of 0·2 unit.

p_H	6·8	7·0	7·2	7·4	7·6	7·8	8·0	8·2	8·4	8·6	8·8	9·0	9·2	9·4	9·6
0·2N-HCl, c.c.	18·4	17·8	16·7	15·3	13·4	11·47	9·39	7·21	5·21	3·82	2·52	1·65	1·13	0·70	0·35

The Citric Acid–Phosphoric Acid–Veronal–Boric Acid Universal Buffer Mixture.—This buffer mixture consists of four acids 0·2N with respect to the total replaceable hydrogen, giving rise to seven stages of dissociation which are successively neutralised with sodium hydroxide. Each stage of dissociation corresponds to an acid concentration of 0·02857N. The solution contains potassium dihydrogen phosphate, citric acid, veronal, boric acid, and hydrochloric acid dissolved in water and made up to a litre. The p_H values, determined by means of the hydrogen electrode, at stages in the titration of 100 c.c. of this mixture with 0·2N-sodium hydroxide are given in Table II under the heading p_H (T). Since in colorimetric work, a series of constant-volume mixtures is necessary, the p_H values of solutions of 100 c.c. of the buffer mixture with varying amounts of sodium hydroxide solution diluted to 200 c.c. have been determined. These values, given under the heading p_H (D), are slightly higher than those obtained by titration during the first 16% of the neutralisation; beyond this point, however, the effect of dilution is negligible.

Fig. 1 shows by means of the thick curve, the relation between p_H and percentage neutralisation; between 16% and 64% neutralisation the points lie very close to the straight line corresponding to the interpolation formula

$$p_H = 0{\cdot}0853x + 2{\cdot}686.$$

Between 0 and 16% neutralisation the points lie below this line by

TABLE II.

p_H Values of mixtures of 100 c.c. of 0·0286*M*-citric acid, 0·0286*M*-potassium dihydrogen phosphate, 0·0286*M*-boric acid, 0·0286*M*-veronal and 0·0286*N*-hydrochloric acid and *x* c.c. of 0·2*N*-sodium hydroxide at 18°.

x	0	2	4	6	8	10	12	
p_H (T).........	2·40	2·55	2·73	2·92	3·12	3·35	3·57	
p_H (D).........	2·58	2·72	2·86	3·03	3·21	3·43	3·66	
p_H (calc.) ...	2·86	3·03	3·20	3·37	3·54	3·71	3·88	
x	14	16	18	20	22	24	26	
p_H (T).........	3·80	4·02	4·21	4·40	4·57	4·75	4·91	
p_H (D).........	3·87	4·09	4·26	4·42				
p_H (calc.) ...	3·88	4·05	4·22	4·39	4·56	4·73	4·90	
x	28	30	32	34	36	38	40	42
p_H (T and D)	5·08	5·25	5·40	5·57	5·70	5·91	6·10	6·28
p_H (calc.) ...	5·07	5·25	5·42	5·59	5·76	5·93	6·10	6·27
x	44	46	48	50	52	54	56	58
p_H (T and D)	6·45	6·62	6·79	6·94	7·12	7·30	7·45	7·62
p_H (calc.) ...	6·44	6·61	6·78	6·95	7·12	7·29	7·46	7·63
x	60	62	64	66	68	70	72	74
p_H (T and D)	7·79	7·98	8·15	8·35	8·55	8·76	8·97	9·20
p_H (calc.) ...	7·81	7·98	8·15	8·32	8·49	8·66	8·83	9·00
x	76	78	80	82	84	86	88	
p_H (T and D)	9·41	9·65	9·88	10·21	10·63	11·00	11·23	
x	90	92	94	96	98	100		
p_H (T and D)	11·44	11·60	11·75	11·85	11·94	12·02		

0·3 p_H, although this deviation is lessened somewhat if each solution is diluted to a constant volume of 200 c.c. Beyond 64%, the experimental curve shows a very considerable inflexion, corresponding to the transition from the neutralisation of boric acid, $p_K = 9·2$, to that of the third stage of phosphoric acid, $p_K = 11·6$. Only by the inclusion of an acid of $p_K =$ *ca.* 10·4 could this inflexion have been avoided. If such an acid could be obtained it would be possible to make a buffer mixture whose p_H value would vary linearly over the whole course of the neutralisation.

If a buffer mixture of range p_H 3·85—12·0 should be required, the preparation may be simplified by omitting hydrochloric acid, the diethylbarbituric acid being liberated by interaction with the first hydrogen of citric acid. In these circumstances the titration begins at a point corresponding to 14·3 c.c. in the previous titration (B in Fig. 1), at which point $p_H = 3·85$. The titration curve is then identical with the curve given in Fig. 1. A solution of any p_H value given in Table II can therefore be prepared by adding to 100 c.c. of this modified solution y c.c. of 0·2*N*-sodium hydroxide, where $y = x - 14·3$, y being obtained by means of the interpolation formula, $p_H = 0·0853y + 3·91$. (This mixture is being supplied as a powder by the British Drug Houses, Ltd.).

Discussion.

As the titration curves of these two buffer mixtures are the resultant curves of a number of monobasic acids, at the middle point of any stage in the neutralisation, $p_{\mathrm{H}} = p_K$, where K refers to the acid neutralised in that stage, and at the end of one stage and the beginning of the next $p_{\mathrm{H}} = \frac{1}{2}(p_{K_1} + p_{K_2})$ where K_1 and K_2 refer to the two acids concerned. The agreement of the p_{H} values so calculated with those observed is good in the case of the first buffer solution, as the following table shows :

NaOH, c.c.	20	30	40	50	60	70	80
p_{H} (obs.)	3·29	4·56	5·72	6·80	7·96	9·15	10·38
p_{H} (calc.)	3·32	4·74	5·82	6·90	8·05	9·20	10·40

In the case of the second solution good agreement is obtained until the veronal comes into play :

NaOH, c.c.	7·15	14·3	21·45	28·6	35·75	42·9
p_{H} (obs.)	3·04	3·85	4·52	5·13	5·72	6·36
p_{H} (calc.)..................	3·0	3·8	4·6	5·2	5·8	6·35
NaOH, c.c.	50	57·15	64·3	71·45	78·6	85·75
p_{H} (obs.)	6·94	7·55	8·18	8·91	9·72	10·95
p_{H} (calc.)	6·90	7·43	7·96	8·58	9·20	10·40

At 64·3 c.c. of sodium hydroxide, the point of half neutralisation of the diethylbarbituric acid, the p_{H} value observed is about 0·2 unit higher than that observed in the direct titration of the acid, whilst at the half neutralisation of the boric acid the observed p_{H} is about 0·5 unit higher than the calculated.

Much difficulty has been experienced in elucidating this anomaly. On neutralising a mixture of diethylbarbituric acid and boric acid with sodium hydroxide, the p_{H} values at the half neutralisation of each acid gave p_K (veronal) = 7·85 and p_K (HBO_2) = 9·25, whilst the p_{H} at the mid-point of the complete titration was 8·51 instead of $p_{\mathrm{H}} = 8·58$, the mean of the two p_K values. Similarly, the titration curve of a mixture of potassium dihydrogen phosphate, diethylbarbituric acid, and boric acid gave p_K (H_2PO_4') = 6·8, p_K (veronal) = 8·0, and p_K (HBO_2) = 9·3, which again are in good agreement with the accepted p_K values, 6·9, 7·96, and 9·2 respectively.

When, however, a mixture of citric acid and boric acid was titrated with sodium hydroxide, the three p_K values calculated during the titration of citric acid were 3·00, 4·47, and 5·86 (accepted values 3·0, 4·6, and 5·8), showing that the neutralisation of the citric acid was proceeding without interference of the boric acid. The p_K value calculated at any point in the boric acid section of the curve was 0·3 of a p_K unit too high, apparently owing to the presence of the sodium citrate. This elevation in the p_K value of boric acid in the presence of sodium citrate will explain the lack of agreement between the calculated and the observed p_H values in the latter portion of the titration of the new buffer mixture.

The effect of an organic hydroxy-acid on the dissociation of boric acid seems to have an important bearing on the so-called " boric acid problem " (see Bancroft and Davis, *J. Physical Chem.*, 1930, **34**, 2479) to which we are giving further attention.

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