XXVI. NOTE ON THE PREPARATION OF BUFFER SOLUTIONS FOR THE DETERMINATION OF pH

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For instructional purposes it appears good practice to introduce into elementary courses of physiology and biochemistry at least some determinations of pH such as are readily carried out by means of the indicator-buffer method.

The one serious difficulty which probably prevents the more general introduction of such experiments into an elementary syllabus is the time involved in setting up the necessary buffer solutions to be used as standards, the use of the hydrogen electrode being precluded owing to its expense. Moreover, no buffer solutions so far devised keep indefinitely and, when made up according to the usual directions, their volumes are unnecessarily large and wasteful. If the standards are to cover an extended range, as for instance for use in plotting the titration curve of phosphoric acid, an added difficulty is the large number of solutions required when the standards are prepared according to Sørensen [1912] and Clark & Lubs [1916].

More recently some of these difficulties have been overcome by the introduction of the buffer mixtures of Prideaux & Ward [1924], and of Britton & Robinson [1931], both of which are obtainable commercially. Even when using these mixtures, however, according to the directions given by the authors, the methods are wasteful when only small volumes of each solution are required. The method described here is more economical both in time and material and has the added advantage that it reduces the number of measurements involved in the preparation of any particular standard pH solution. The method has been in actual use for teaching purposes in this laboratory over an extended period and is put forward in the belief that it will prove of value to others.

The mixtures of Prideaux & Ward, and of Britton & Robinson, when dissolved in water to a final volume of 1 litre, give practically straight line curves over the respective ranges pH 2.7–11.4 and 3.85–8.8 when treated with acid or alkali according to the formulae given.¹

The principle of the present method is to prepare stock solutions having pH at the limits of the range required. Solutions of intermediate pH values are then prepared from these by mixing them in varying proportions, the volume of the mixed solutions remaining constant throughout the series. For example, if solutions of pH 3.1 and 4.1 are available a series of solutions covering this range can be prepared by mixing them according to the data in Table I.

To prepare a series of solutions, the successive members of which differ from one another by 0.2 pH unit, the "acid" and "alkaline" stock solutions should differ from one another, preferably, by pH 1.0, 2.0, 2.5, 4.0 or 5.0. If then the

¹ Prideaux & Ward's formula is: $pH = 3.1 \pm 0.1185 V$, where V = number of ml. of 0.2 N NaOH (plus) or HCl (minus) added to 100 ml. of buffer solution, with subsequent dilution to 200 ml. with water.

Britton & Robinson's formula is: $pH = 3.91 + 0.0853 V$, where V = number of ml. of 0.2 N NaOH to be added to 100 ml. of the buffer solution.

Table I

pH required	3.1	3.3	3.5	3.7	3.9	4.1
ml. acid constituent (pH 3.1)	10	8	6	4	2	0
ml. alkaline constituent (pH 4.1)	0	2	4	6	8	10

volume of mixture prepared be 10, 20, 25, 40 or 50 ml., respectively, successive solutions, passing from those of lower to those of higher pH, will be prepared by decreasing the volume of the more acid solution and increasing that of the more alkaline solution by 2.0 ml. for each interval of 0.2 pH unit. The advantage of making the pH interval between the "acid" and "alkaline" stock solutions as stated is that even if only 10 ml. of any particular solution be required measurements of the stock solutions do not involve volumes smaller than 0.1 ml. This will be clear from the notes on Table II.

For the preparation of a series of solutions differing from one another by pH 0.1 the interval between the acid and alkaline stock solutions should be pH 1.0, or a multiple of this, and the volume of mixture prepared 10 times the interval selected. The volume of the "acid" constituent will then be decreased by 1.0 ml. and that of the alkaline constituent increased by 1.0 ml. for each increment of 0.1 pH unit.

It is sometimes convenient to make the stock solutions considerably more acid or more alkaline than are likely to be required, the pH interval between the solutions being determined by the volume of mixture required, the unit volume of mixture (that is the volume which must be prepared in order that a change of 0.2 pH unit shall be obtained by a change of ± 2.0 ml. in the volume of one solution and of ∓ 2.0 ml. in the volume of the other) being 10 times the pH interval between the stock solutions. Again, if standard pH solutions over an extended range, but each in small quantity only, be required, it may be preferable to prepare three stock solutions instead of two, e.g. if the whole useful range covered by the Prideaux and Ward mixture be required, it would be better to prepare stock solutions of pH 3.1, 7.1 and 11.1 and to obtain solutions at the acid end of the range by mixing the first two, and at the alkaline end by mixing the last two, than to cover the whole range by the suitable admixture of solutions of pH 3.1 and 11.1. In the instance cited above the solution of pH 7.1 would be the "alkaline" constituent when mixtures at the acid end of the range are being prepared, but the "acid" constituent when mixtures at the alkaline end of the range are being prepared.

For convenience in making up small quantities of solutions of definite pH rapidly the stock solutions can be stored in bottles each of which is provided with a cork carrying a 10 ml. graduated pipette.

The solutions given in Table II are those most convenient to use for the determination of the isoelectric point of caseinogen when carried out by students as a class experiment. The series required [Michaelis & Pechstein, 1912] ranges from pH 3.5 to 5.9; a solution of pH 3.1 is chosen as that of the acid constituent as this is the pH of Prideaux & Ward's mixture when dissolved in water to one litre, and for use as the acid stock solution it only requires dilution with an equal volume of water. The pH chosen as that of the "alkaline" constituent depends on the quantity of mixture required. For a class of 40 students, working in pairs, four sets of standard buffer solutions are sufficient, each set consisting of the following ranges of tubes: (1) pH 3.5-4.3 containing bromophenol blue, (2) pH 4.1-5.3 containing bromocresol green and (3) pH 5.1-5.9 containing chlorophenol red. Approximately 7 ml. of solution in each tube are sufficient for comparison purposes, so that for solutions which are put up with one indicator only 28 ml. are actually required for the four sets, and for those (pH 4.1, 4.3, 5.1, 5.3) which are set up with two indicators 56 ml. are required. To these quantities a little extra should be added in order that some of each solution

may be held in reserve. Suitable "acid" and "alkaline" stock solutions would thus be pH 3.1 and 7.1. The requisite volume of each of these may be measured from a burette into boiling tubes, 40 drops of the appropriate indicator being added and approximately 7 ml. of each mixture poured into four series of test tubes.

Table II

ml. of "acid" solution (pH 3.1) required when pH of
"alkaline" constituent is

pH required	ml. "alkaline" constituent	4.1 (1)	5.1 (2)	5.6 (3)	6.1 (4)	7.1 (5)	8.1 (6)
3.1	0	10	20	25	30	40	50
3.3	2	8	18	23	28	38	48
3.5	4	6	16	21	26	36	46
3.7	6	4	14	19	24	34	44
3.9	8	2	12	17	22	32	42
4.1	10	0	10	15	20	30	40
4.3	12	—	8	13	18	28	38
4.5	14	—	6	11	16	26	36
4.7	16	—	4	9	14	24	34
4.9	18	—	2	7	12	22	32
5.1	20	—	0	5	10	20	30
5.3	22	—	—	3	8	18	28
5.5	24	—	—	1	6	16	26
5.7	26	—	—	—	4	14	24
5.9	28	—	—	—	2	12	22
6.1	30	—	—	—	0	10	20
6.3	32	—	—	—	—	8	18
6.5	34	—	—	—	—	6	16
6.7	36	—	—	—	—	4	14
6.9	38	—	—	—	—	2	12
7.1	40	—	—	—	—	0	10
7.3	42	—	—	—	—	—	8
7.5	44	—	—	—	—	—	6
7.7	46	—	—	—	—	—	4
7.9	48	—	—	—	—	—	2
8.1	50	—	—	—	—	—	0
Total volume of "acid" constituent required to make up the whole range possible by admixture with "alkaline" constituent		30	110	169	240	420	650

Notes on Table II

1. It will be clear from what has been said that Table II can be extended to pH 11.1 by the use of Prideaux and Ward's mixture.
2. If only 10 ml. of each pH solution are required, the figures in columns 2, 3, 4, 5 and 6 will be divided by 2, 2.5, 3, 4 and 5 respectively, the volumes so obtained being made up to 10 ml. by the addition of the appropriate "alkaline" constituent.
3. From the Table it can be seen why an interval of 3 pH units between the "acid" and "alkaline" stock solutions is not suitable when only 10 ml. of mixture are required, for then the volumes of acid constituent become 10, 9.33, 8.66, 8.00, 7.33 ml. etc.
4. The total volume of the alkaline constituent required to make up the whole range possible from any two stock solutions will be the same as the volume of the acid constituent required (see figures at the foot of columns (1), (2), (3), (4), (5) and (6)).

SUMMARY

A method by which a series of standard buffer solutions may be prepared is described. The method has various advantages over those at present in use. These are:

1. The ease and rapidity with which solutions of any desired pH can be prepared from two stock solutions.

2. The method described here necessitates the storage of two or three solutions only, and of these only those strongly alkaline (e.g. pH 11.1) need be stored in a paraffined bottle.

3. The volume of mixture made up is known so that the correct amount of indicator can be added without further measurement.

4. The method is specially suitable for the preparation of standard buffer solutions for class experiments for which a particular range may be required only once or twice during the year, and then, perhaps, only in small quantities.

REFERENCES

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