A Sodium Carbonate-bicarbonate Buffer for Alkaline Phosphatases

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For the study of alkaline phosphatase systems a non-phosphate-containing buffer is required. The buffers in common use for the alkaline pH range are ammonia, borate, glycine and veronal. Ammonia and borate have a retarding effect on the rate of enzymic hydrolysis of phosphoric esters. Glycine activates phosphatase when present in very low concentrations (0·1–1 mM), but in the concentrations used in the buffer system (0·01–0·1 M) it has an undoubted inhibitory effect. Veronal is much better in this respect, but its useful pH range (6·8–9·6) is not sufficiently high to cover the pH optima of the enzyme acting on aromatic phosphoric esters (Delory & King, 1943). To satisfy the criteria of non-inhibition of enzymic action, useful alkaline pH range, simplicity and reasonable stability, a mixture of sodium carbonate with bicarbonate has been investigated. The range covered, 9·2–10·8, is suitable for enzymic study with phenolic esters of phosphoric acid. A sodium carbonate buffer was described by Kolthoff (1925), in which a partial transformation into sodium bicarbonate was effected by the addition of hydrochloric acid. We have found it difficult to prepare buffer mixtures in this manner because of the ease with which carbon dioxide can be lost from the solution when the hydrochloric acid is added. The mixtures of sodium carbonate and bicarbonate, on the other hand, are easy to prepare, and have proved to be surprisingly stable. We have kept the mixtures described below for as long as 6 months in well-stoppered waxed bottles without any demonstrable change in their pH.

SOLUTIONS

Freshly distilled water boiled immediately before use to free it from CO_2 should be used. Only sodium carbonate and sodium bicarbonate of A.R. quality are suitable. The solutions should be kept in well-waxed tightly stoppered bottles in a cool place.

0·1 M-*Sodium carbonate.* 10·599 g. anhydrous Na_2CO_3 are dissolved in water and diluted to 1 l.

0·1 M-*Sodium bicarbonate.* 8·4 g. $NaHCO_3$ are dissolved in water and diluted to 1 l.

Determination of pH. The apparatus used embodied a hydrogen electrode unit employing the Moloney (1921) platinum electrode, and a saturated calomel electrode. The potentiometer was accurate to ½ mV., so that the pH values could be measured to nearly 0·01 pH. The apparatus was checked with standard buffer mixtures prepared according to Britton (1932). Through the courtesy of Prof. E. C. Dodds and Mr M. E. H. Fitzgerald of the Courtauld Institute of Biochemistry, London, we were able to check the determinations on their glass electrode apparatus (Beckmann). The two methods checked with a maximum difference of 1 mV. equal to about 0·02 unit of pH.

pH OF BUFFER MIXTURES

In Table 1 are listed the pH values at 20 and 37° for nine mixtures of sodium carbonate with sodium bicarbonate. These figures have been checked many times with different solutions and have agreed well both with repeated determinations of the same and with fresh solutions, and with the values recorded by Kolthoff (1925) for his Na_2CO_3-HCl mixtures, whose composition in terms of Na_2CO_3 and $NaHCO_3$ was calculated. The mixtures have been in continuous use for phosphatase studies and for routine work for several years, and have been satisfactory for their purpose and the range of pH specified. It is not recommended that this buffer system be used for pH lower than 9·2 (8·8 at 37°). For this purpose a carbonate-veronal buffer (King & Delory, 1940) is suggested.

Table 1. pH *values of sodium carbonate-bicarbonate buffer mixtures*

0·1 M-Na_2CO_3 (ml.)	0·1 M-$NaHCO_3$ (ml.)	pH (20°)	pH (37°)
1	9	9·16	8·77
2	8	9·40	9·12
3	7	9·51	9·40
4	6	9·78	9·50
5	5	9·90	9·72
6	4	10·14	9·90
7	3	10·28	10·08
8	2	10·53	10·28
9	1	10·83	10·57

SUMMARY

A sodium carbonate-bicarbonate buffer, covering the range pH 8·9–10·8, is described. The mixtures are easily prepared and are reasonably stable if kept in stoppered waxed glass bottles.

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