

## A BUFFER SOLUTION FOR COLORIMETRIC COMPARISON.

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In the use of standard buffer solutions for colorimetric comparison where more than a restricted range of reaction is required, it has been necessary in the past to make use of several solutions. The author has developed a system requiring but two stock solutions and covering a range of from pH 2.2 to pH 8.0 which approximately includes the limits of reaction for arable soils and physiological media. The materials used are as follows: 0.2 M disodium phosphate<sup>1</sup> and 0.1 M citric acid, combined in such volumes as to make 20 cc. of the mixture.

The disodium phosphate employed was recrystallized three times. A 0.2 M solution was prepared by titration against HCl, using methyl orange as indicator. Although the titration end-point was not distinct, the error in the resulting mixtures was usually not appreciable, seldom exceeding 0.01 pH. On account of the variable water of crystallization content of the phosphate salt<sup>2</sup> the stock solution was standardized by titration in order to have a reproducible method. Upon exposure to the air for a period of 2 weeks the water of crystallization of disodium phosphate is reduced to 2 molecules. If, after exposure, a quantity of the salt is kept on hand in a closed container and the correct weight of the salt required to produce the proper concentration of stock solution determined by titration, it is possible at any time to make up the stock solution by simply weighing out the salt. The citric acid was recrystallized at least twice before using.

<sup>1</sup> Molds may develop in phosphate solutions under suitable conditions, but this source of trouble has been obviated by Martin<sup>2</sup> by shaking the solutions with a little calomel for a few minutes and then filtering.

<sup>2</sup> Martin, C. J., *Biochem. J.*, 1920, xiv, 98.

The 0.1 M stock solution was standardized by titration against NaOH solution which had been prepared with boiled water and protected from CO<sub>2</sub>. Barium hydroxide was also used as recom-

TABLE I.

pH required.	0.2 M Na <sub>2</sub> HPO <sub>4</sub> .	0.1 M citric acid.
	cc.	cc.
2.2	0.40	19.60
2.4	1.24	18.76
2.6	2.18	17.82
2.8	3.17	16.83
3.0	4.11	15.89
3.2	4.94	15.06
3.4	5.70	14.30
3.6	6.44	13.56
3.8	7.10	12.90
4.0	7.71	12.29
4.2	8.28	11.72
4.4	8.82	11.18
4.6	9.35	10.65
4.8	9.86	10.14
5.0	10.30	9.70
5.2	10.72	9.28
5.4	11.15	8.85
5.6	11.60	8.40
5.8	12.09	7.91
6.0	12.63	7.37
6.2	13.22	6.78
6.4	13.85	6.15
6.6	14.55	5.45
6.8	15.45	4.55
7.0	16.47	3.53
7.2	17.39	2.61
7.4	18.17	1.83
7.6	18.73	1.27
7.8	19.15	0.85
8.0	19.45	0.55

mended by Sorensen and given by Clark,<sup>3</sup> but sodium hydroxide was found to be as accurate and more convenient. The correct weight of citric acid required to make the stock solution can also be determined by titration.

<sup>3</sup> Clark, W. M., The determination of hydrogen ions, Baltimore, 1920.

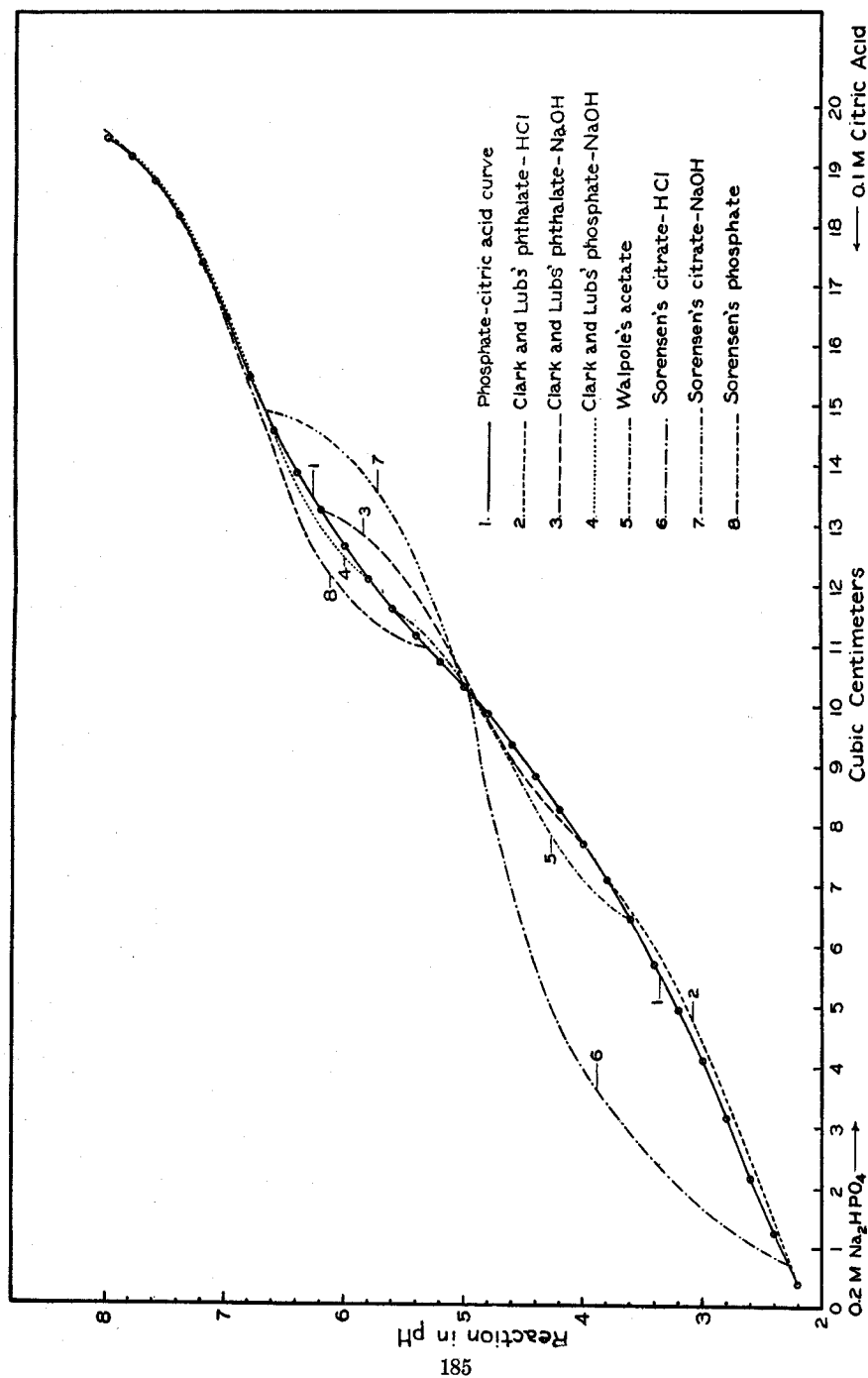


Fig. 1.

The pH values of the mixtures were determined electrometrically by use of the chain;  $\text{Hg}|\text{HgCl}|\text{N KCL}|\text{saturated solution of KCl}|\text{H}_2|\text{Pt}$ . No allowance was made for liquid potential. No attempt was made to maintain a constant temperature. However, the temperature of both calomel electrode and buffer solution were taken into account in computing the pH values. Clark's<sup>3</sup> extension of Sorensen's values for the normal calomel electrode was used with the necessary interpolations. Three extra calomel electrodes were employed for checking the accuracy of the one in general use. A Leeds and Northrup type K potentiometer and type R sensitive galvanometer were used for making the electrometric measurements. The electrode was of the platinum wire variety. The hydrogen was generated electrolytically and passed first through an acid permanganate solution, next through a hot tube, and finally through a wash bottle containing distilled water.

A graph was constructed in which the pH values (determined electrometrically) of various mixtures of phosphate and citric acid solution, so arranged that the total volume was in all cases 20 cc., were plotted against the volumes of the two solutions. By interpolation, using the curve so obtained, it was possible to arrive at the proper volumes of the two solutions which when mixed would give 20 cc. of a solution having any desired reaction. The values given in Table I were obtained in this way and checked by actually preparing the solutions and measuring the pH values by the electrometric method. In all cases the variation of the observed from the calculated pH was 0.01 or less.

Fig. 1 gives the titration curve for the foregoing system of buffers. For comparison, there is also given the titration curves of well known standard buffer solutions.<sup>3</sup>

The shape of the phosphate-citric acid curve of Fig. 1 indicates that the mixtures are well suited for colorimetric determinations of pH.

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