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Imidazole Buffer: Its Use in Blood Clotting Studies.*

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In making studies on blood clotting we found it desirable to control pH in the range 6-8. Diethylbarbituric acid buffers have been recommended,[1] but the effective range is rather high (pH 7-9) and, furthermore, their solubility is not great. Buffers utilizing other weak acids, or salts, such as acid phosphate, tend to precipitate calcium or to form undissociated compounds. To avoid these difficulties we directed our attention to a study of the buffering action of weak bases. It was soon found that satisfactory results could be obtained with imidazole (glyoxaline). Shortly after our tests were carried out, Kirby and Neuberger[2] published a physicochemical study of imidazole derivatives. Working with a very dilute buffer solution (0.01 M), they found the pK_o value of imidazole to be 6.95. From this value one can calculate with reasonable accuracy the pH of dilute (0.01 M) buffer mixtures of imidazole and HCl. However, for the higher concentrations which are in more general use, the calculated values become approximations. To supply data, convenient for use, we wish to describe the preparation of imidazole buffers. We have chosen the concentration of 0.05 M, as being one which is more nearly suitable for many problems in biological research.

Imidazole was synthesized from tartaric acid by the method of Fargher and Pyman.[3] The white crystalline needles were dried over P_2O_5 and were found to have a melting point of 89° (corr.) and a nitrogen content which was 99.6% of the theoretical value. A portion of the imidazole used subsequently was obtained elsewhere.‡ Hydrogen ion determinations on buffer mixtures (Table

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1 Ransmeier, J. C., and McLean, F. C., *Am. J. Physiol.*, 1938, **121**, 488.

2 Kirby, A. H. M., and Neuberger, A., *Biochem. J.*, 1938, **32**, 1146.

3 Fargher, R. G., and Pyman, F. L., *J. Chem. Soc.*, 1919, **115**, 217.

‡ Part of our supply of imidazole was obtained through the courtesy of Dr. C. S. Marvel, University of Illinois. The rest was obtained from Eastman Kodak Company, Rochester, N. Y. The Eastman product, which is now generally available, should be dried before use.

TABLE I.
Imidazole Buffer.*

pH	0.1 N HCl cc	pH	0.1 N HCl cc
6.20	42.9	7.20	18.6
6.40	39.8	7.40	13.6
6.60	35.5	7.60	9.3
6.80	30.4	7.80	6.0
7.00	24.3		

*To the amounts of 0.1 N HCl listed in the table are added 25 cc portions of 0.2 M imidazole, and the mixtures are diluted with water to 100 cc.

In our original experiments, we used slightly different quantities of HCl (round number quantities), with corresponding differences in values of pH. The data actually obtained were plotted on a large-scale curve, and the values given in the table were obtained by interpolation.

I) were made at 25° ± 0.05°, using a glass electrode and a saturated KCl-calomel electrode (Beckman pH meter). Standardization was effected with standard acetate buffer having a pH of 4.62 at 25°. The pH values of newly prepared mixtures check the values in Table I within less than 0.02 pH. Buffer mixtures which were allowed to stand for 2 months at room temperature showed no growth of microörganisms and no detectable change in pH. It is of interest to note that imidazole buffer covers a pH range which is almost identical to that covered by mixtures of primary and secondary phosphates.[4]

In blood clotting studies we use the various reagents in concentrations which are isosmotic with 0.9% NaCl. Imidazole buffer, pH 7.25, which is isosmotic with 0.9% NaCl, as determined by the freezing point method, is prepared by dissolving 1.72 g of imidazole in 90 cc of 0.1 N HCl, and diluting with water to 100 cc. For the past year, we have included this imidazole solution among the reagents used in the 2-stage technique for the titration of prothrombin.[5] Tests show that the buffer has no effect upon either the conversion of prothrombin into thrombin, or upon the reaction of thrombin with fibrinogen, even when the buffer solution comprises more than one-half of the total volume.

Summary. Mixtures of imidazole and hydrochloric acid covering the pH range 6.2 to 7.8 are described. These mixtures are recommended as buffers for reactions which require the presence of calcium ion.

[4] Clark, W. M., and Lubs, H. A., *J. Biol. Chem.*, 1916, **25**, 479.

[5] Warner, E. D., Brinkhous, K. M., and Smith, H. P., *Am. J. Physiol.*, 1936, **114**, 667; *J. Exp. Med.*, 1937, **66**, 801.