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Bicolor Determination of pH Using Standard Duboscq Colorimeter with Light Filter.

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With the Assistance of P. James English.

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The successful application of light filters in the Folin-Malmros[1] method for sugar suggested the possibility of compensating, in a similar way, the acid color of the bicolor indicators. Employing light filters consisting of the appropriate indicators in an acid solution, a procedure was worked out for determining pH with phenol red, brom cresol purple and brom cresol green.

The acid filter solutions for phenol red and brom cresol purple consists of 0.01% solutions of each indicator made up in acetate buffers having pH values of 5 and 3 respectively. In the case of brom cresol green ethyl alcohol must be added to the acid solution to prevent precipitation of the indicator. A 0.02% solution of the indicator is made up in hydrochloric acid and alcohol so that the final solution is equivalent to 0.1 N hydrochloric acid and contains 35% alcohol.

Battery jars 10 x 10 x 10 cm. or museum jars 16 x 10 x 8 cm. of clear, white glass make satisfactory containers for the filter solutions. The sides of the jars should be painted black, except for a 5 x 8 cm. window to allow the light to pass through. The prepared filter is placed in position between the colorimeter and light, and the instrument "set" in the usual way with the standard in both cups. Readings, which should agree within 0.1 mm. are taken with the left cup set at 1, 5 and 8 mm.

The standard solutions are made by adding 1 cc. of 0.02% phenol red and brom cresol purple, and 0.03% brom cresol green to 20 cc. of the alkaline buffer solutions. The buffers used should have pH values which will completely transform the indicator to the alkaline form. Hastings[2] employs a very dilute solution of sodium hydroxide for this purpose but we prefer buffer solutions. Clark[3]

* George Alexander Research Fellow in Biochemistry, 1930-32, sponsored by The Leopold Schepp Foundation, New York.

1 Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, **83**, 115.

2 Peters, J. J., and Van Slyke, D. D., Quantitative Clinical Chemistry, Methods, Baltimore, 1932, 801.

3 Clark, W. M., The determination of hydrogen ions, Baltimore, 1928, 94.

gives the pH values required by the different indicators for transformation to the alkaline form.

Add 0.5 cc. of the dilute indicator solution to 10 cc. of the unknown, place in left cup and set at 10 mm. The right cup containing the standard is adjusted until the fields match. The reading of the standard multiplied by 10 gives the per cent alkaline form of the indicator in the unknown. pH values are readily obtained from a dissociation curve of the indicator. The standard solutions should be made up fresh every few determinations since the strong colorimeter light seems to affect the intensity of color.

The method is sensitive, under favorable conditions, to changes of ±0.02 pH. However the absolute accuracy of the procedure depends upon a correct evaluation of pK′ in the solution being investigated. Also it is essential to check the pK′ for each new lot of indicator used. The value obtained is constant over the main pH range of the indicator, which is approximately 1.1 pH units for the dyes studied. A wider range can be utilized if the changing values for pK′ are employed in plotting the extremities of the dissociation curve.

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Note on the Susceptibility of Certain Strains of Hemolytic Streptococcus to a Streptococcus Bacteriophage.

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A bacteriophage active against certain mucoid strains of hemolytic streptococci, originally isolated by Clark and Clark[1], was used in tests with a series of 119 strains of hemolytic streptococci which had been obtained from a variety of sources, some pathological and some normal. Of these, 37 strains were isolated from human sources, including 5 throat cultures from normal persons; 42 strains were recovered from mastitis in cows and from milk and cheese; 18 from lymphadenitis in guinea pigs; 7 from spontaneous rabbit infections with hemolytic streptococcus; 7 from pleuro-pneumonia, chronic endometritis and distemper in horses; 2 from pneumonia in

[1] Clark, P. F., and Clark, A. S., *J. Bact.*, 1926, **11**, 89; PROC. SOC. EXP. BIOL. AND MED., 1927, **24**, 635.