pure ammonium salt gives a better color in the absence $Na_2CO_3$ and sulphite lessens the color markedly, facts which explain why Wu apparently did not recognize that his salt under proper conditions gave more color than Folin's original solution. Our method is simpler and yields more usable material than Wu's method would if used for the same purpose.

The actual method of blood analysis is somewhat modified. No sodium carbonate is used, the cyanide furnishing the requisite alkalinity. Benedict's standard must be used. The cyanide must be measured to an accuracy of 0.1 c.c.

## ABSTRACTS OF THE COMMUNICATIONS,

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### Colorimetric determination of hydrogen ion concentration by means of a double-wedge comparator.

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In a former paper[2] a method was outlined for determining hydrogen ion concentration colorimetrically without the use of buffer solutions, and data for making such determinations for $P_H$ values between 6.7 and 8.1 were given, using phenolsulphonephthalein as an indicator. The method consisted in the partition of a constant amount of indicator solution between pairs of test tubes of equal caliber, one tube of each pair containing 5 c.c. of weak acid, and the other tube 5 c.c. of weak base. When such pairs of tubes are viewed by transmitted light in the comparator of Hurwitz, Meyer and Ostenberg a series of colors is observed

[2] Barnett, G. D. and Chapman, H. S., 1918, "Colorimetric determination of reaction of bacteriologic mediums and other fluids," *Jour. Amer. Med. Assoc.*, lxx, 1062.

covering the range of the indicator, each color corresponding to a definite $P_H$ value. A committee of the American Association of Bacteriologists later investigated and reported on the method,[1] and published a similar series of hydrogen ion exponents for brom thymol blue. More recently Gillespie[2] has extended the method to all of the indicators described by Clark and Lubs,[3] determining the $P_H$ values of his tube pairs by comparison with buffer solutions whose hydrogen ion concentrations were checked by the gas chain method. The present work was in progress when Gillespie's paper appeared, and is largely a confirmation of his results.

Instead of dividing the indicator between two tubes, however, use has been made of a comparator consisting of a long narrow rectangular glass box containing a diagonal glass partition dividing it into two equal wedge-shaped compartments placed base to apex. One wedge is filled with acid indicator solution, and the other with alkaline indicator solution of the same concentration. Light transmitted horizontally through the box thus presents the complete range of color change of the indicator. For purposes of calibrating the color scale in terms of $P_H$, buffer solutions of known hydrogen ion concentration and containing the same indicator concentration were placed in a small glass box having the same fluid diameter as the large box. For any given buffer solution within the range of the indicator an exact color match is obtained. A scale along the lower edge of the comparator is divided into 100 parts and graduated from left to right. If the acid color of the indicator occupies the left end of the comparator, the readings of this scale will thus represent the percentage of alkaline indicator color present in the color blend observed at that point. The colors are best viewed against an oblique plate mirror reflecting the sky. The buffer solutions used were the phthalate, phosphate and borate mixtures of Clark and Lubs,[4] and their $P_H$ values were

[1] Conn, H. J., Harding, H. A., Kligler, I. J., Frost, W. D., Prucha, M. J., and Atkins, K. N., 1919, "Methods of pure culture study," *Jour. Bact.*, iv, No. 2, 107–132.

[2] Gillespie, Louis J., 1920, "Colorimetric determination of hydrogen ion concentration without buffer mixtures, with special reference to soils," *Soil Science*, ix, No. 2, 115–136.

[3] Clark, W. M. and Lubs, H. A., 1917, "The colorimetric determination of hydrogen ion concentration and its applications in bacteriology," *Jour. Bact.*, i.

[4] Clark, W. M. and Lubs, H. A., 1916, "Hydrogen electrode potentials of phthalate, phosphate and borate buffer mixtures," *Jour. Biol. Chem.*, 1916, xxv, 479–510.

checked with the hydrogen electrode, following the method of Clark,[1] and using the tables of Schmidt and Hoagland.

The comparator was made in the laboratory from the glass of discarded X-ray plates jointed with balsam. Since only extremely weak acid and base have been used there have been no leakage difficulties. The boxes are carefully dried with filter paper after each day's use.

Dimensions: Inside length 35 cm.
Fluid diameter 15 mm.
Height 2.5 cm.

Gillespie has shown that the indicator transformations follow the law of mass action within the limits of error of the method, and has calculated "apparent dissociation constants" for each of his observations from the modified mass-law equation

$$K = P_H + \log \frac{x}{100 - x}$$

where $x/(100 - x)$ represents the partition ratio of the indicator in per cent. Similar values are given in the tables below. This constant is the $P_H$ value of the mid-point of the indicator, *i.e.* the $P_H$ value at which the indicator is half transformed from acid to salt form.

It will be noted that the constants above calculated show somewhat less deviation than those of Gillespie, probably because the method permits an exact color match, no interpolation being necessary. That our values are slightly higher than those of Gillespie is doubtless due to the fact that our measurements were made in the close neighborhood of 20°, instead of at the higher temperatures he used. The greatest discrepancies are with brom phenol blue and brom cresol purple, with which we have had some difficulty in obtaining a perfect color match.

In order to determine the $P_H$ of unknown solutions we may construct a table or curve for each indicator, giving the theoretical value of the $P_H$ for scale readings at convenient intervals. It is, however, more convenient to graduate the scale directly in $P_H$

[1] Clark, William Mansfield, 1915, "A hydrogen electrode vessel," *Jour. Biol. Chem.*, 1915, xxiii, 475–486.

[2] Schmidt, Carl L. A. and Hoagland, D. R., 1919, "Table of $P_H$, H, and OH values," Univ. California Publications in Physiology, Vol. 5, No. 4, pp. 23–69.

TABLE OF DISSOCIATION CONSTANTS. (CALCULATED MID-POINT PH.)

| Indicator. | Solvent. | Percentage Indicator in Final Solution. | Scale[1] Reading. | $P_H$. | $K$. | Mean. |
|---|---|---|---|---|---|---|
| Brom phenol blue.... | Alcohol | 0.0004 | 27.1 | 3.52 | 3.95 | |
| | | | 41.4 | 3.72 | 3.85 | |
| | | | 52.8 | 3.94 | 3.89 | |
| | | | 67.2 | 4.16 | 3.85 | |
| | | | 78.7 | 4.40 | 3.83 | 3.87 |
| Methyl red..... | Water | 0.0004 | 30.0 | 4.65 | 5.02 | |
| | | | 38.5 | 4.82 | 5.02 | |
| | | | 50.0 | 5.03 | 5.03 | |
| | | | 58.6 | 5.19 | 5.04 | |
| | | | 61.4 | 5.22 | 5.02 | |
| | | | 64.3 | 5.26 | 5.00 | |
| | | | 72.9 | 5.41 | 4.98 | |
| | | | 75.6 | 5.50 | 4.99 | |
| | | | 81.5 | 5.65 | 5.00 | 5.01 |
| Brom cresol purple... | Alcohol | 0.0012 | 27.1 | 5.83 | 6.26 | |
| | | | 38.5 | 6.02 | 6.22 | |
| | | | 47.2 | 6.22 | 6.27 | |
| | | | 55.7 | 6.44 | 6.30 | |
| | | | 64.3 | 6.59 | 6.33 | 6.28 |
| Brom thymol blue ... | Water | 0.0004 | 24.2 | 6.59 | 7.09 | |
| | | | 35.7 | 6.83 | 7.09 | |
| | | | 47.2 | 7.03 | 7.08 | |
| | | | 55.7 | 7.19 | 7.09 | |
| | | | 67.1 | 7.37 | 7.06 | |
| | | | 75.6 | 7.56 | 7.06 | |
| | | | 81.5 | 7.75 | 7.09 | 7.08 |
| Phenol red..... | Water | 0.0004 | 18.5 | 7.12 | 7.77 | |
| | | | 30.0 | 7.37 | 7.74 | |
| | | | 38.5 | 7.56 | 7.76 | |
| | | | 49.0 | 7.74 | 7.77 | |
| | | | 58.6 | 7.93 | 7.78 | |
| | | | 70.0 | 8.16 | 7.79 | |
| | | | 81.5 | 8.43 | 7.78 | 7.77 |
| Cresol red..... | Water | 0.0004 | 30.0 | 7.74 | 8.11 | |
| | | | 35.7 | 7.91 | 8.17 | |
| | | | 47.2 | 8.15 | 8.10 | |
| | | | 58.6 | 8.28 | 8.13 | |
| | | | 64.3 | 8.39 | 8.13 | |
| | | | 71.5 | 8.52 | 8.12 | 8.13 |
| Thymol blue..... | Water | 0.0004 | 15.7 | 8.10 | 8.83 | |
| | | | 18.5 | 8.17 | 8.83 | |
| | | | 30.0 | 8.44 | 8.87 | |
| | | | 35.7 | 8.54 | 8.80 | |
| | | | 44.3 | 8.78 | 8.89 | |
| | | | 58.6 | 9.01 | 8.86 | |
| | | | 75.6 | 9.43 | 8.93 | 8.86 |

[1] The actual readings in this column were made on a centimeter scale, and are here converted to per cent.

intervals of 0.1 on each side of the mid-point. This may be done by giving to the quantity $\log x/(100 - x)$ successive values from $-0.9$ to $+0.9$ in intervals of 0.1. From these equations corresponding values of $x$ are readily obtained:

| $\log \frac{x}{100-x}$ ($P_H$ Difference). | $x$(Scale Reading). | $\log \frac{x}{100-x}$. | $x$ |
|---|---|---|---|
| −0.9 | 11.2 | +0.1 | 55.7 |
| −0.8 | 13.7 | +0.2 | 61.3 |
| −0.7 | 16.6 | +0.3 | 66.6 |
| −0.6 | 20.1 | +0.4 | 71.5 |
| −0.5 | 24.0 | +0.5 | 76.0 |
| −0.4 | 28.5 | +0.6 | 79.9 |
| −0.3 | 33.4 | +0.7 | 83.4 |
| −0.2 | 38.7 | +0.8 | 86.6 |
| −0.1 | 44.3 | +0.9 | 88.8 |
| 0.0 | 50.0 | | |

If these values are indicated on the scale by the figures representing $P_H$ differences, we may read the $P_H$ value of an unknown solution by adding to the mid-$P_H$ value (dissociation constant) of the indicator the differential quantity indicated by the scale reading obtained. Thus, if a scale-reading midway between +0.4 and +0.5 is obtained, using methyl red, the $P_H$ of the solution will be 5.01+0.45, or 5.46. With reasonable care the error of such a reading is certainly not greater than 0.02 $P_H$, especially in the region of the mid-point, where the indicators are most used. Compensation for colored or turbid solutions is made by placing a small glass compartment of the same fluid diameter behind that portion of the comparator in which the match is to be obtained.

## SUMMARY.

1. The method of determining hydrogen ion concentrations colorimetrically without the use of buffer solutions is extended to the group of indicators described by Clark and Lubs. Values of the dissociation constant at 20° of each of the indicators are given.

2. A double glass wedge comparator is described for making such determinations.