5. The use of alcohol containing perchloric acid and saturated with potassium perchlorate at the temperature of use for washing the precipitate after the sodium salt has been extracted with alcohol containing perchloric acid.

The saturated solution of potassium perchlorate is best prepared by dissolving the necessary amount of salt in hot conc. aqueous solution of perchloric acid and adding the solution to anhydrous alcohol.

By this procedure the error can undoubtedly be kept within a very few tenths of a milligram, and seems to us preferable to, although more troublesome than that of Gooch and Blake, who have advocated the procedure of keeping the volume of washing liquid, consisting of an alcoholic solution of perchloric acid, very small. The errors in their experiments varied from negative values of from 0.5 to 0.9 mg. in the absence of sodium salts, to positive values up to 2.6 mg. in the presence of sodium salts.

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[CONTRIBUTION FORM THE LABORATORIES OF SOIL FERTILITY, BUREAU OF PLANT INDUSTRY, U. S. DEPARTMENT OF AGRICULTURE.]

# COLORIMETRIC DETERMINATION OF TITRATION CURVES WITHOUT BUFFER MIXTURES.

BY LOUIS J. GILLESPIE.

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The writer is led to present this material by the experience of the late Dr. E. H. Walters (First Lieutenant, Sanitary Corps) and Dr. L. E. Wise, formerly in this laboratory, who found the titrimetric determination of molecular weight to be very useful in the identification of small quantities of unknown acids in cases where phenolphthalein could not be used and where no neutralization equivalents were given in the literature. The usefulness of the titration curves has been explained by Hildebrand,[1] who gave a comparatively simple electrometric procedure. The writer has aided both Dr. Wise and Mr. R. N. Harger in determining titration curves colorimetrically, using the assortment of indicators recommended by W. M. Clark and Lubs[2], and the 0.05 *M* buffer mixtures studied by them.[3] For titration curves a large number of buffer mixtures were required, and we in fact resorted only to the colorimetric method because the neutralization equivalent was required for an organic acid containing arsenic, which badly poisoned the hydrogen electrode. Since that time the writer has developed a system for the colorimetric determination of

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[1] THIS JOURNAL, **35**, 847 (1913).

[2] H. A. Lubs and W. Mansfield Clark, *J. Wash. Acad. Sci.*, **6**, 481 (1916); W. M. Clark and H. A. Lubs, *J. Bact.*, **2**, 1, 109, 191 (1917).

[3] W. M. Clark and H. A. Lubs, *J. Biol. Chem.*, **25**, 479 (1916); also described in *J. Bact.*, *loc. cit.*

hydrogen ion concentration which requires no buffer mixtures.[1] and the main inconvenience for the present purpose is thereby removed. The procedure requires now little beyond the indicators themselves, and may be taken up without special preparation.

The data upon which Table I was constructed were determined by means of Clarks and Lubs' buffer mixtures,[2] the hydrogen-ion exponents of which were determined at the working room temperature, and the data were smoothed by the use of the equation $P_H = k + \log$ (alk. form)/(acid form), where $P_H$ is the hydrogen ion exponent of Sörensen; $k$, the apparent or total dissociation constant, the logarithms are common, and "alk. form" and "acid form" are the concentrations of indicator practically completely transformed into the alkaline or acid form by excess of base or acid. The equation was verified for all the indicators of Table I within the limits of experimental error. The equation is the general equation of which Equation 7, page 1122, of Stieglitz[3] is the type, and comes from the application of the mass-action law to both the dissociation and any tautomeric equilibria when the indicators are assumed to be monobasic acids or monacid bases. The values obtained from Table I should be good to about 0.1 at room temperatures (25 to 30°). Reference may be made to the other forthcoming paper for further details and literature discussion.

Neutralization equivalents are seldom given in the literature for cases where phenolphthalein or methyl orange cannot be used, yet such values can still be obtained in many cases by titration to the proper end-point, expressed in terms of hydrogen ion exponent ($P_H$). A titration curve shows the point, if there is any, where the largest change of reaction ($P_H$) occurs per drop of alkali (or acid), and this is the end-point of the titration. This point is taken as the middle of the steep or comparatively steep portion of the curve. The method can be applied for learning whether the acidity of a given mixture, perhaps of weak and strong acids, or of weak acids and salts of weak bases, can be determined by direct titration. A. B. Clark and Lubs[4] recently gave a method for titrating 2 acids successively in the same solution in certain cases; other cases can be studied most directly by means of titration curves. The determination of the titration curve as outlined gives both the titration result required and at the same time information sufficient for the specification of conditions for simple titration and for the statement of the experimental error due to uncertainty of end-point.

[1] To be published in *Soil Science*.

[2] W. M. Clark and H. A. Lubs, *J. Biol. Chem.*, **25**, 479 (1916); also described in *J. Bact.*, *loc. cit.*

[3] THIS JOURNAL, **25**, 1112 (1903).

[4] *Ibid.*, **40**, 1443 (1918).

## Preparation of Indicator Solutions.

The indicators used are from the assortment recommended by Clark and Lubs,[1] after careful study in various biological fluids, in comparison with direct electrometric measurement. Some of them have also been tested in the same way in soil extracts,[2] of very low salt content. They are known to be a relatively high degree of reliability. With the exception of methyl red, they are sulfonphthaleins, a series which is the subject of an interesting series of papers by Dr. S. F. Acree and collaborators,[3] who have been studying especially the seat of color change, and the various tautomeric and dissociation constants involved.

TABLE I.—DATA FOR THE DETERMINATION OF HYDROGEN ION EXPONENT.

| | Hydrogen ion exponent for each pair of tubes. | | | | | | |
|---|---|---|---|---|---|---|---|
| Drop ratio. | Bromphenol blue. | Methyl red. | Bromcresol purple. | Bromthymol blue. | Phenol red. | Cresol red. | Thymol blue. |
| 1 : 9 | 3.1 | 4.05 | 5.3 | 6.15 | 6.75 | 7.15 | 7.85 |
| 1.5 : 8.5 | 3.3 | 4.25 | 5.5 | 6.35 | 6.95 | 7.35 | 8.05 |
| 2 : 8 | 3.5 | 4.4 | 5.7 | 6.5 | 7.1 | 7.5 | 8.2 |
| 3 : 7 | 3.7 | 4.6 | 5.9 | 6.7 | 7.3 | 7.7 | 8.4 |
| 4 : 6 | 3.9 | 4.8 | 6.1 | 6.9 | 7.5 | 7.9 | 8.6 |
| 5 : 5 | 4.1 | 5.0 | 6.3 | 7.1 | 7.7 | 8.1 | 8.8 |
| 6 : 4 | 4.3 | 5.2 | 6.5 | 7.3 | 7.9 | 8.3 | 9.0 |
| 7 : 3 | 4.5 | 5.4 | 6.7 | 7.5 | 8.1 | 8.5 | 9.2 |
| 8 : 2 | 4.7 | 5.6 | 6.9 | 7.7 | 8.3 | 8.7 | 9.4 |
| 8.5 : 1.5 | 4.8 | 5.75 | 7.0 | 7.85 | 8.45 | 8.85 | 9.55 |
| 9 : 1 | 5.0 | 5.95 | 7.2 | 8.05 | 8.65 | 9.05 | 9.75 |
| % in indicator solution | 0.008 | 0.008 | 0.012 | 0.008 | 0.004 | 0.008 | 0.008 |
| Cc. 0.1 $N$ NaOH per 0.1 g. portion | 1.64 | ..... | 2.78 | 1.77 | 3.10 | 2.88 | 2.38 |
| Produce acid color with | 0.05 $N$ HCl | 0.05 $N$ HCl | 0.05 $N$ HCl | 0.05 $N$ HCl | 0.05 $N$ HCl | 2% $H_2KPO_4$ | 2% $H_2KPO_4$ or $H_2O$ |
| Quantity acid used to produce acid color | 1 cc. | 1 drop | 1 drop | 1 drop | 1 drop | 1 drop | 1 drop |

The strengths given in Table I are suitable for test-tubes of about 15 mm. external diameter with moderately thin wall. The test-tubes should be of fairly uniform diameter and should have no flanges. Methyl red solution is made up in alcohol of about 60 vol. per cent. The free indicator acid is dissolved in acid-free alcohol after grinding and the volume made up with distilled water (6 volumes of alcoholic solution being made up to 10). The other indicators are prepared as monosodium salt in

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[1] H. A. Lubs and W. Mansfield Clark, *J. Wash. Acad. Sci.*, **6**, 481 (1916); W. M. Clark and H. A. Lubs, *J. Bact.*, **2**, 1, 109, 191 (1917).

[2] L. J. Gillespie, *J. Wash. Acad. Sci.*, **6**, 7 (1916); L. J. Gillespie and Lewis A. Hurst, *Soil Science*, **6**, 219 (1918).

[3] THIS JOURNAL, **41**, 1190 (1919), and previous articles.

water solution, 1.1 equivalents of alkali being used except in the case of dibromo-*o*-cresolsulfonphthalein, which requires more nearly 1.5 equivalents.[1] The strengths given are in terms of the free acid. The table gives the quantity of standard alkali furnishing the stated number of equivalents for 0.1 g. portions of the indicator acids. In dissolving the indicators, it is practically necessary to grind the indicator with the alkali, added in portions, before making up the volume. Heat may be used, but should be unnecessary. The indicator solutions are reasonably permanent, but the color standards prepared from them should not be trusted more than a day or so, especially the more alkaline indicators.

The indicators can now be purchased in this country or prepared.[2]

## Preparation of Color Standards and Use in Determination of Hydrogen Ion Exponent.[3]

A very similar technique has been proposed by Barnett and Chapman[4] for determination of hydrogen ion exponent with phenol-sulfonphthalein.

To take a concrete example, if 6 drops of methyl-red solution be put in a test-tube with the addition of a drop of dil. alkali and enough water to make the volume 5.5 cc., and 4 drops of methyl-red solution be treated in another test-tube with a drop of dil. acid and enough water to bring the liquid to the same height (the tubes should be nearly uniform), this pair of tubes constitutes a color standard, such that a solution of unknown hydrogen ion exponent containing 10 drops of indicator solution in the same volume will have the exponent 5.2, if its color, viewed through the side, matches the color seen when the light passes through both tubes of the standard pair held together (conveniently with rubber bands). The figure 5.2 is found from Table I, under the heading methyl red and opposite the drop ratio 6 : 4. The first figure of the drop ratio indicates the number of drops in the alkaline tube, and the second the number in the acid tube of the color standard pair. To secure a better optical effect a tube of clear water should be held behind the tube containing the unknown solution. The simple comparator shown in Fig. 1 (modified from the comparators used by Hurwitz, Meyer, and Ostenburg[5] and by Clark and Lubs[6] is of great convenience, and almost necessary if the unknown solution is colored itself. In this case some of the unknown without in-

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[1] Dr. H. A. Lubs, private communication.

[2] H. A. Lubs and W. M. Clark, *J. Wash. Acad. Sci.*, 5, 609 (1915); and 6, 431 (1916); E. C. White and S. F. Acree, THIS JOURNAL, 41, 1190 (1919), and previous articles.

[3] The hydrogen-ion exponent is the common logarithm of the reciprocal of the hydrogen ion concentration in gram equivalents per liter. As the solution becomes more alkaline, the exponent increases, passing through about 7.0 at physico-chemical neutrality.

[4] *J. Am. Med. Assoc.*, 70, 1062 (1918).

[5] *Johns Hopkins Hospital Bull.* 27, 16 (1916).

[6] W. M. Clark and H. A. Lubs, *J. Bact.*, 2, 1, 109, 191 (1917).

dicator is put in a test-tube back of the standard pair for compensation, and 2 tubes of clear water in back of the unknown plus indicator. This is the device of Walpole,[1] who also studied the titration curve of solutions which had themselves indicator properties. In this case standard alkali (or acid) is added to the unknown solution without indicator, just as it is added in the actual titration. The comparator illustrated is suitable for test-tubes of the above given dimensions.

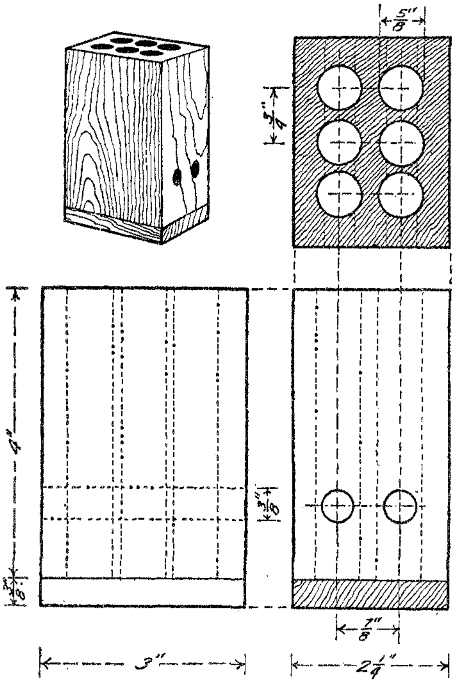

Fig. 1.—Comparator for hydrogen ion exponent measurements. The interior is painted black.

Table I gives the necessary data. The first figure of the drop ratio indicates the number of drops in the alkaline tube, and the second, the number in the acid tube of the color standard pair. The ratios 1.5 : 8.5 and reverse are included because of their occasional usefulness. Three and 17 drops are taken and the volume is made up two-fold, *i. e.*, to 11 cc. Extra ratios may be interpolated at will in this way wherever desired. The quantities of acid used to develop the full acid color in the standards need not be exact, even as to the number of drops. For producing the full alkaline color in the standards, one drop of 0.05 *N* NaOH is satisfactory, except for thymol blue, which requires 2 or 3 drops.

The indicators are entered in the table under their laboratory names: their chemical names being, in regular order: tetrabromophenol-sulfonphthalein, 4′-dimethylamido-azobenzene-2-carboxylic acid, dibrom-*o*-cresol-sulfonphthalein, dibromo-*o*-thymol-sulfonphthalein, phenol-sulfonphthalein, *o*-cresol-sulfonphthalein, and thymol-sulfonphthalein.

## Titration Procedure.

A definite quantity of substance is made up to definite volume with water,[2] and aliquot portions are used, one for each necessary indicator.

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[1] *Biochem. J.*, 5, 207 (1910).

[2] If the solution is not aqueous, but contains much alcohol or other solvent, the figures of Table I cannot be expected to apply. However, it still may be possible to

If the substance is rare, one portion may serve sometimes for 2 indicators, as detailed below. The indicators may be used in regular order for the complete curve, but generally the approximate turning point will be known well enough to avoid the necessity for using all, and the curve is constructed in sections, which may, of course, be determined in any order. A chosen indicator solution is added to the aliquot in the proportion of 2 drops per cc. of the aliquot. Standard alkali (or acid) is then added until the color begins to change. Two drops of indicator solution are then added for every cc. of standard alkali added, and, after mixing, a portion of the solution is poured into a test-tube and its hydrogen ion exponent determined by means of the color standards. The solution is then returned to the main portion, without rinsing the test-tube, more alkali is added until the color has changed significantly, and indicator solution is added as before and the exponent determined in the same test-tube. As the titration proceeds, the hydrogen ion exponents are plotted vertically on cross-section paper against the number of cc. of standard alkali (or acid) added. The titration is continued until the indicator has reached its limit. Another section of the curve is then secured by the use of another indicator. It is sometimes possible to economize valuable substance by applying 2 indicators in succession to the same solution; for this, the working ranges of the 2 must not overlap. In this way we have used phenol-sulfonphthalein after methyl red. Before the alkaline limit for methyl red was reached the curve had already become steep, so that a slight excess of alkali sufficed to bring the reaction into the phenol-sulfonphthalein region. It was desired to obtain more of the curve in order better to estimate the middle point. Methyl red solution was, therefore, diluted in water containing a drop of alkali, 2 drops of methyl red per cc., and this was placed in the comparator in back of the phenol-sulfonphthalein color standard. Phenol-sulfonphthalein was added to the aliquot used for methyl red, and the titration continued with the phenol-sulfonphthalein color standards.

The procedure for colored bodies, including such as may possess indicator properties, was outlined above under the preparation of the color standards.

The method has certain limitations. The data do not extend into the alkaline region beyond the useful range of phenolphthalein. If desired, phenolphthalein may be used in the same way as the others. It has the disadvantage of a relatively high salt error, as was shown by the work of

carry out the scheme with satisfaction, even if the various curve-portions found for the different indicators do not fit together, since the sudden change in the form of the titration curve may occur almost entirely within the range of one indicator. There are many cases where hydrogen ion determinations may be very serviceable, in solutions hardly aqueous, whether or not the absolute $P_H$ values are greatly in error.

Rosenstein.[1] He gave figures for various salt contents, for which reference must be made to his article. For the case where the salt content is 0.05 *N*, and the temperature 23–24°, the following hydrogen ion exponent values may be used. For the successive drop ratios, 1 : 9, 2 : 8, 3 : 7 4 : 6, 5 : 5, 6 : 4, 7 : 3, the values are 8.8, 9.1, 9.2, 9.3, 9.5, 9.7, 9.8, respectively. An 0.08% solution of phenolphthalein in alcohol is suitable. The values are calculated from the data of Rosenstein, but are modified by subtracting 0.1 in every case, in accordance with some measurements of the writer.

Another limitation relates to the possible inexactness of the method if applied to solutions containing substances which may have specific action on the dissociation or tautomerism of the indicators. The method may still be of sufficient value, however, as in solutions containing organic solvents, as discussed in a footnote under "Titration Procedure."

In case the removal of carbon dioxide is necessary, this should be done at room temperature, by passing air through soda lime and then through the solution.

## Summary.

A simple method has been described for securing titration curves colorimetrically, which requires the minimum of equipment. The hydrogen ion exponents are determined without the use of buffer mixtures. Instead of a buffer mixture containing indicator, a color standard is used, consisting of a pair of test-tubes containing together 10 drops of indicator solution of suitable strength, the drop ratio being varied from 1 : 9 to 9 : 1. One tube of the pair contains dil. alkali and the other contains dil. acid. Reference to a table gives the hydrogen-ion exponent corresponding to the drop ratio, or the exponents can be calculated from the relation $P_H = k + \log$ (drop ratio); where the logarithms are common, the drop ratio is the ratio of the number of drops (of the indicator solution) in the alkaline tube to the number in the acid, and the values of $k$ (good to about 0.1 at 25–30°) are the following: tetrabromo-phenol-sulfonphthalein, 4.1; methyl red, 5.0; dibromo-*o*-cresol-sulfonphthalein, 6.3; dibromo-thymol-sulfonphthalein, 7.1; phenol-sulfonphthalein, 7.7; *o*-cresol-sulfonphthalein, 8.1; thymol-sulfonphthalein, 8.8. Details of technique are given in full.

WASHINGTON, D. C.

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[1] THIS JOURNAL, 34, 1117 (1912). The writer has no definite comparison to offer for the relative values of thymol blue and phenolphthalein. No systematic study of the salt errors of thymol blue has yet appeared such as Rosenstein's study of phenolphthalein. Clark and Lubs studied thymol blue in solutions of various composition. The study in soil extracts has not been extended to cresol red or thymol blue. Furthermore, the titration curves determined hitherto in this laboratory have not included these 2 indicators. We suspect that calcium hydroxide may exert a specific action upon them, but have not settled the point. We used sodium hydroxide for our titrations.