COLOR STANDARDS FOR THE COLORIMETRIC MEASUREMENT OF H-ION CONCENTRATION

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In an article of the above title recently published in this journal, Medalia (1920) presents a system of color standards somewhat similar to one published by me a little before (Gillespie, 1920). The work is evidently independent of mine, but the proposed tables are in serious disagreement with the results of my work.

The cause of the disagreement apparently does not lie in a conflict of observations, but in the plan followed by Medalia in preparing the tables.

It is stated that a test of this plan with the indicator, brom-thymol blue, "succeeded perfectly, i.e., the green color was found at (pair no. 4) pH 7; or slightly yellowish green at (pair no. 3) pH 6.8 according to this range. (The change of color of this indicator was found by the writer to start with pH 6.2 instead of pH 6 as given by Clark and Lubs.)"

Unfortunately, this test is not sufficient to afford evidence in favor of the plan as against the method used by me to "smooth out" experimental errors for the preparation of tables, because the mass action equation used for this smoothing requires that such a limited test of the plan shall succeed perfectly, the error involved being only 0.02 pH, well within the experimental error.

In fact, the mass action equation requires that, if one is able to determine both limits equally distant from the half-transform-

1 Although there are practical limits to the useful range, there is of course no real point of pH where the indicator "starts in" to change color, but only a subjective point "over the threshold" where it may appear to do so.
mation point (at pair 4), then pairs 3, 4, and 5 will be substantially correct as calculated by the plan in question, but pairs 2 and 6 will be in error by nearly 0.10 pH, and pairs 1 and 7 by about 0.25. The mass action equation is, however, in accord over the useful interval of pH with the measurements of Tizard (1910) for methyl red, of Barnett and Chapman (1918) for phenol red, and of the present writer for all indicators studied by Medalia, except the acid range of thymol blue, which was not studied.

We do not need to assume the applicability of the mass action equation in order to show that the proposed tables are in disagreement with these measurements. It is only necessary to plot the results to be compared on one diagram in any uniform manner, and the discordance will be apparent. The proposed tables must therefore be considered incorrect, since the plan on which they are mainly based lacks a solid foundation, and is not supported by enough data to put into question the conflicting measurements.

In the article, mention is made of measurement of acid production of bacteria by means of pH determinations. A word of warning seems justified by the fact that the idea is apparent in the writings of others. The definition of acid production in terms of a difference between initial and final pH values is decidedly not superior to definition in terms of titration, but rather false, or at least of slender and involved significance. To measure how much acid is produced we must titrate. If the composition of the culture medium makes impossible a true titration on the direct culture, then we may distil the volatile acids and titrate

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6 This has been shown by me (Gillespie, 1920).

8 For instance, the percentage of indicator placed in the alkaline solution may be plotted against the pH pertaining to it, or better, the logarithm of the ratio between the quantities of indicator as distributed between the alkaline and the acid tubes of the color standards may be plotted against pH. By the second procedure a straight line is required by the mass action equation. Mathematically, the plan of Medalia consists of a pure guess as to the form of the curve obtained by such plotting of the data.

* Measurement of change of pH may, in some cases, give us the acid production, if we have already incorporated the results of titration in a titration curve.
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them, or possibly change the composition of the culture medium ("standard methods" notwithstanding), or resort to even graver expedients, but the last expedient indeed should be the measurement of pH for the given purpose. Measurement of pH and titration furnish two distinct methods of attack, each with its own object and interpretation. The principles involved have been carefully discussed by Clark and Lubs (1917).

As to a statement to the effect that the electrometric method is more accurate than the colorimetric, but that the apparatus which it requires is beyond the possibilities of the average bacteriological laboratory; the writer can subscribe to neither part in the unqualified form, but would refer again to the article of Clark and Lubs (1917) for a discussion of the first part, and to the recent book of Clark (1920) for the second. The writings of Clark and Lubs also contain full discussions of other principal topics, such as titration of culture media, effect of bacterial growth and of sterilization upon the indicators, etc.

It is pleasing to note that Medalia was able to preserve his color standards. The standards prepared by me were not permanent, and the main difference seems to be in the means taken by Medalia to avoid microbial decomposition, this point having been neglected by me.

It seems well to describe in this article, otherwise not very constructive, an instrument for further study of the indicator constants and behavior, which was devised too late to be of service in the work published (Gillespie, 1920). The necessary improvements in method, for work substantially better than that already published, must include temperature control of the buffer solutions in which the indicator is placed, and more precise measurements of the percentage transformation. The apparatus shown schematically in figure 1 can easily be made to satisfy both requirements. The writer has not seen it described. It is a colorimeter for two-colored indicators, and by an obvious modification it can be used to determine, if desirable, both the percentages of the two colors present and the total concentration. A simple apparatus was improvised5 with which the percentages

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5 In the Laboratories of Soil Fertility, Bureau of Plant Industry, Washington, D. C.
could be determined with far greater ease and precision than is possible with a one-colored indicator in the usual colorimeter, since the quality changes very rapidly with the adjustment. Plane polished surfaces are desirable in the optical system, but were not used.

The glass vessels $A$ and $C$ are fixed in position, and $B$ can be moved up or down, the motion being measured by a pointer (not shown) fixed to $B$ and moving upon a scale divided into 100 parts. The instrument is so made that the pointer moves from 0 to 100 when $B$ moves from contact with $C$ to contact with $A$. The acidified indicator solution of suitable strength may be placed in $B$ and an alkaline indicator solution of the same strength placed in $C$. $A$ is left empty. Then, if the scale reads 70, the path of light along the left-hand dotted line passes through the alkaline form during 70 per cent of its path in the indicator, and through the acid form during 30 per cent. The light along the right-hand dotted line traverses an indicator solution in tube $E$, again of the same strength, and over a path equal in length to the

For use in the determination of pH, a tube containing unknown solution without indicator can be slipped into tube $A$ in order to compensate for color or turbidity without lengthening the apparatus unduly. In this case, water would be introduced into $D$ to equal height in order to equalize absorption and the meniscus effects.
total path on the left. The merit of the instrument consists in the fact that the length of this total path is not affected by the motion of tube B, though the percentages of the path lengths in the two solutions are varied directly thereby. The indicator solution in tube E consists of a buffer mixture (or solution, the pH of which is to be determined) to which the proper amount of indicator has been added. If conditions are such that 70 per cent of the molecules encountered along the path on the right are in the alkaline modification, and 30, in the acid, then the eye will perceive identical impressions upon looking through the two systems from above. This will be the case, even if each modification is not pure, but admixed with the other, or if each modification absorbs to some extent like the other, or if the indicator exhibits dichromatism. Consequently the apparatus may be used to determine the apparent percentage transformation of the indicator at different hydrogen-ion exponents; the relation being studied at different temperatures and subsequently being used to determine unknown hydrogen-ion exponents.

To control the temperature of the buffer solutions or of the unknown solution, water can be circulated in a jacket (not shown in the figure) about the tube E. The temperature should be controlled to about one degree, or possibly better.

It is evident that titrations can be carried out in the tube E, a proper quantity of strong indicator solution being added for every cubic centimeter, or smaller unit, of added reagent.

7 It need not be the case if the indicator is grossly contaminated with another indicator of different apparent dissociation constant, or if the indicator behaves like a dibasic or polybasic acid. Wegscheider (1915) has made statements equivalent to those in the text above.

8 The instrument can of course be used at once and dependence put for the time being on the apparent dissociation constants and tables published (Gillespie, 1920). If the indicator used, the temperature, and what information as may be available as to the salt content of the solution, be recorded, the corrections can be applied at any time when better values for the indicators and other data are obtained. Although the writer can not admit that the method previously published or the use of a double colorimeter is to be classed as approximate because of doubtful optical assumptions, it is of course only approximate until precise calibration of the standards is made. At present the instrument is capable of giving more precision than could be obtained in the calibration made without it, and it may possibly disclose some small deviations from the simple dissociation curve.
It is well known that the simple law used in ordinary colorimetry, namely—the thickness of the solution times the concentration equals a constant when the thickness and concentration are varied in such a way as to match a standard color—does not hold for solutions of potassium dichromate. Indeed, with a color standard of different composition from the solution itself, the colors shown by a solution of potassium dichromate, as it is progressively diluted, can not be matched either by dilution of the color standard or by changing the depth of the layers. On the other hand, the changing colors can be matched in the double colorimeter. For standards (in tubes B and C), may be used a highly acid solution of potassium dichromate, and a solution of potassium (yellow) chromate. As the solution in question is diluted, it becomes necessary to change the ratio of the path lengths through the red and the yellow "forms", as well as to increase the path length through the solution (in tube E). It is generally assumed that a change of ionization occurs when potassium dichromate solution is diluted; and there seems to be no reason to doubt that the usual law of absorption holds for the constituents of the solution. There would appear to be no ground for a suspicion that the "dichromatism" of the sulfonephthalein indicators may interfere with their use in the double colorimeter.

In fact, to derive the law upon which ordinary colorimetry is based, we assume that light passing through a solution is affected independently by each particle of colored material, these particles usually being alike in kind. In order to apply the law to double colorimetry, we need only the further assumption that the same is true when the particles are not alike in kind, and it appears difficult to doubt this in the given case. Consideration of the expression for the intensity of the emergent light: \( Ia^e \), where \( I \) is the intensity of the entering light, \( a \) is the fraction absorbed by each particle, \( e \) is the thickness, and \( e \) is the concentration of particles, leads to the following conclusions.\(^9\)

\(^9\) The expression is applied to the different wave-lengths entering, the constant \( a \) being assumed different for each wave-length.
The variation of the constant \( a \) with wave-length, which leads to the dichromatism of the two-colored indicators, does not lead to any difficulty in the case of the double colorimeter. Dichromatism leads to the detection of errors made when turbidity of the solution to be measured is balanced optically in the usual manner. With a one-colored indicator the error made is no less because of the absence of dichromatism, but the error is not disclosed. In routine work, white light is advisable as a source for comparisons, when it can be used, so that such error may be made evident by dichromatism. When the subjective difficulties become too great for the use of white light, a screened light (Clark and Lubs, 1917) may be a valuable means of obtaining an approximate result.

SUMMARY

The recently published tables of Medalia are in disagreement with other published data and are not correct.

A colorimeter for two-colored indicators is described for use in accurate study of the indicators and for the measurement of hydrogen-ion exponent. The optical assumptions underlying its use are practically the same as those upon which ordinary colorimetry is based.

REFERENCES


Clark, W. Mansfield 1920 The Determination of Hydrogen Ions, 318 pp., Baltimore.


