A COLORIMETRIC METHOD FOR DETERMINING THE HYDROGEN ION CONCENTRATION OF SMALL AMOUNTS OF FLUID.

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The importance of the maintenance of the state of neutrality in practically all vital phenomena has become well recognized through the efforts of Sørensen (1), Michaelis and his collaborators (2), Henderson (3), Clark (4), and numerous others. The methods devised by them require the use of a considerable amount of fluid. While this fact is not a disadvantage in most types of investigation in which the pH value is of importance, studies where only single drops of fluid are available render them inadequate. Having in mind an investigation in which only exceedingly small amounts of liquid could be obtained—namely the influence of the H ion concentration on tissue cultures in vitro—and also realizing a definite need for other problems, the author has attempted to modify the technique of the colorimetric method that it might be applicable to small quantities of fluid, the gas-chain electrometric method being clearly out of the question. Haas (5) advised the use of indicator papers to meet these requirements. Although this method was not put to a severe test, the inability to secure reliable results and the complexities which arise in the use of such indicators led us to abandon this procedure.

Our first plan was to use tubes of small dimension and employ the dilution method as advised by Clark and Lubs (6) as a practical means by which to titrate bacteriological media. Accordingly, tubes of 2 to 3 mm. were chosen to each of which were added 1 drop of the fluid to be tested, 4 drops of distilled water, and 1 drop of indicator solution. In some of the fluids used there was a very low salt content and buffer action, so that the dilution
with the distilled water changed the pH markedly. Thus it became necessary to perform the determinations with undiluted fluid.

A piece of opal glass had been employed as a background in comparing the colors in the small tubes. This suggested the use of the plate instead of the tubes upon which to make the determinations. The first trial proved its worth, the color tint being just as easily judged as in large tubes and much more readily than in the tubes of 3 mm. diameter. In checking the method with buffer mixtures ranging from pH 2 to 9.6 it was found that an accuracy of at least 0.1 pH could be obtained.

The equipment necessary for performing the test consists of standard buffer solutions, indicators, opal glass plate, and small stirring rods. The buffer solutions used were Clark series kindly furnished by Dr. Clark and the La Motte Chemical Products Company. Although a number of indicators were tried out, the series advised by Clark and Lubs was clearly the most satisfactory. Yet, these indicators have a disadvantage or limitation in that the respective range of a single indicator is relatively short—not short compared to other indicators but in comparison with the possible range of H ion concentration met with in our work. An ideal indicator for our purpose is one that would cover the range from pH 5 to 9. Although it may be impossible to find such an indicator, it occurred to us that a combination could be made of two indicators having opposite color changes; that is, one the color of which became more intense in solutions of increasing H ion concentration and one in which the color intensified as the H ion concentration decreased. Methyl red and brom-thymol blue were tried and found to have a good working range from pH 4.6 to 7.6, the variation in color from pH 5.6 to 6.2 being somewhat indefinite. The combination of methyl red and brom-cresol purple makes a very useful indicator with a range of pH from 4.6 to 7. As an indicator to make only rough estimations of the H ion concentration of any fluid, that is whether it lies between pH 4.6 and 9, a mixture of methyl red and thymol blue is very satisfactory. In like manner a combination of thymol blue and brom-phenol blue makes an exceptionally good double indicator in the range between pH 1.2 and 4.6. The distinct advantage of these combined indicators lies in the fact that they make good "feelers"
for test fluids and, within certain ranges, the exact pH can be
determined with a single drop of fluid. These mixtures have
been made, and remained unchanged for a period of 3 months.

The concentration of the indicator is important because of the
small amount of fluid to be tested. As long as the drop of liquid
contains acids of low dissociation and their salts, the strength of
the indicator gives a proportionate color intensity of appropriate
pH value; if the electrolytic concentration is low and the H ions
and the hydroxyl ions are about the same in number, the indicator
does not change in color according to the pH of the liquid but is
simply diluted with only a partial change. According to Ostwald's
theory of indicators, in such cases the H ions are not in sufficient
numbers to react with the indicator molecule, the resultant color
being a mixture of the unchanged indicator and the part that has
reacted with the H ions present in the fluid.

The exact concentration of the indicator varies with the char-
acteristic of the fluid to be tested; highly colored or turbid fluids
demand the use of a strong indicator solution, while clear, almost
colorless, liquids are best tested with a weaker dilution of the
indicator. However, for general work we recommend the con-
centrations as follows:

Methyl red, brom-cresol purple, phenol red, and cresol red in
0.01 per cent solution in 25 per cent alcohol, while thymol blue,
brom-phenyl blue, brom-thymol blue, and thymol blue are made
0.02 per cent. The combined indicators are made by mixing
equal parts of a double strength indicator solution; that is, double
the concentration employed when the indicators are used singly.

Inasmuch as we are dealing with a final concentration of indi-
cators in the test fluid, three to five times as great as is used in the
ordinary test-tube colorimetric method, it should be emphasized
that the indicator salts should be very pure.

There is a question as to the stock container for the buffer
solutions and indicators for this method. Glass-stoppered bottles
with the end of the pipette drawn out to a fine point are perhaps
the most convenient. Fig. 1 represents the types of devices tried,
of which we think Type C is the most suitable. This particular
type was used over a period of 2 months, the buffer solutions
remaining unchanged.1

1 The dropper outfit with buffer solution of any pH and indicators can
be obtained from the La Motte Chemical Products Company, Baltimore.
Although an opal glass plate was used upon which to perform the test, it is self-evident that any smooth white surface will answer the purpose; the one requisite being the absolute neutrality of the plate. Ordinary white porcelain pans, evaporating dishes, and China dinner plates were all found to be quite satisfactory. Occasionally a plate which liberates OH ions can be rendered
neutral by soaking in bichromate cleaner for from 24 to 96 hours. It should be rinsed thoroughly with tap water and then with distilled, and finally dried with a clean towel or allowed to dry in an almost vertical position. This procedure for cleaning was used routinely, the bichromate cleanser being necessary only occasionally.

Procedure of the Test.

The method consists simply in mixing a drop of the fluid to be tested and a drop of an appropriate indicator, noting the color, and then placing in close proximity drops of several buffer solutions that are judged to give the same color. The exact match of color can readily be made and the pH determined according to which buffer mixture gave the same color tint. Because of the large relative surface area of a drop, allowing a rapid exchange of gases and evaporation, the reading is made within about 1 minute. Although the alcoholic solution of the indicator facilitates the mixing of the 2 drops, especially if the indicator is allowed to drop on the fluid from a height of several inches, in all fluids of rather high viscosity, it becomes necessary to make mixing complete by means of a small stirring rod.

Theoretically, the drops of indicator and fluid should be of the same size. However, we were able to obtain accurate and consistent results by using only approximate sizes, care being taken to let the drops fall from the pipette held in a vertical position. In learning the color changes of the indicators, it was found helpful to try all the indicator series with the different buffer solutions. That is, there were placed on a large opal glass plate, in rows, as many drops of the different buffer solutions as indicators to be used. To each drop of the buffer mixtures a drop of one of the indicators was added. This was repeated with each indicator solution. There were, then, on a single plate the various ranges of all the indicators, rendering possible a comparison of their different ranges. For example, a buffer solution of pH 6 is yellow with methyl red, brom-thymol blue, phenol red, etc., but a definite greenish blue tint with brom-cresol purple. This fact has a definite application in making pH determination by the drop method, due to the ease with which one can check results. For instance, if one has 5 drops of a fluid the pH of which is desired, a drop of
each of five indicators may be added, thus getting a series of colors depending on the pH of the fluid. If the colors given by methyl red and thymol blue are yellow, brom-cresol purple is very purple; brom-thymol blue, very blue; phenol red, quite red; cresol red, slightly pink; and one may safely state that the pH is somewhere around 7.6. This checking system cannot be over-emphasized in minimizing gross errors.

The method was not standardized by the gas-chain determination except from the fact that the buffer salts furnished by Dr. Clark were checked by him and shown to have maximum errors of not over 0.02 pH. However, all types of bacteriological media, serum, spinal fluid, fruit juices, milk, tissue extracts, etc., were tested both by the test-tube method and drop method and there was practically no difference discernible.

CONCLUSIONS.

A colorimetric method by which H ion concentration can be determined with reasonable accuracy on single drops of fluid has been described. Clark and Lubs series of indicators were used with certain combinations to make double indicators. These combinations make indicators of fairly wide range; thymol blue and brom-phenol blue between pH 1.2 and 4.6; methyl red and brom-thymol blue from pH 4.6 to 7.6; methyl red and brom-cresol purple from pH 4.6 to 7; and for only a very rough "feeler" methyl red and thymol blue from pH 4.6 to 9.

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