A GLASS ELECTRODE APPARATUS FOR MEASURING THE pH VALUES OF VERY SMALL VOLUMES OF SOLUTION.

By D. A. MacINNES and MALCOLM DOLE.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

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In a recent article[1] the authors have described a new type of glass electrode, which is of convenient size, and at the same time of sufficiently low resistance for accurate work. Such an electrode is shown diagrammatically on the right-hand side of Fig. 1. The lower end of glass tube *A* supports the glass diaphragm *D* which in our experiments has been about 0.001 mm. thick. The tube *A* is partly filled with 0.1 N HCl into which is inserted the silver-silver chloride electrode *B*. More details concerning these electrodes are given in the article referred to above.[2]

Since glass electrodes of this type with glass diaphragms 4 mm. or less in diameter can be used, they seemed admirably adapted to measurements involving very small amounts of material. The need for such measurements arises frequently, especially in biological investigations. The apparatus shown diagrammatically in Fig. 1 was therefore designed to adapt the electrodes to this purpose. The vessel *C* holds a reference saturated calomel electrode. This electrode is connected, through a stopcock, with the reservoir *R* which contains

[1] MacInnes, D. A., and Dole, M., *J. Ind. Eng. Chem.* (Analytical Edition), 1929, i, 57. This paper contains references to the previous work with glass electrodes, with the exception of a recent article by Mirsky and Anson (Mirsky, A. E., and Anson, M. L., *J. Biol. Chem.*, 1929, lxxxi, 581.

[2] Through the kindness of Dr. Alexis Carrel we have tested the utility of these electrodes in connection with tissue culture work. The usual vessel for that work was provided with three outlet arms. One arm contained a tube through which the medium could be drawn to make contact with a saturated calomel electrode, and the other two arms held glass electrodes. One glass electrode was kept in the medium and the other in the immediate vicinity of the tissue studied. In this way it was possible to follow accurately the change of pH with time of the tissue and the medium separately.

a supply of saturated KCl solution. Another branch of the tubing connects with the tip T. An additional branch tube F is closed by a piece of rubber tubing (a so-called "policeman") on which a screw pinchcock is placed. The whole apparatus is mounted on adjustable screw clamps, as shown in Fig. 2, so that the glass electrode and the calomel electrode with its attachments can be independently raised and lowered. The clamp holding the glass electrode is insulated from the rest of the support with a piece of Bakelite.

To make a pH determination the following steps are necessary. The rubber tube on F is compressed by the pinchcock. The stopcock below the reservoir

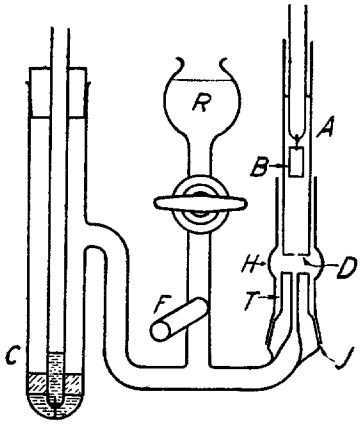

FIG. 1. Diagram of apparatus

R is then opened slightly and saturated KCl is run out on the tip T. With a piece of filter paper this solution is removed from the tip so that the solution lies in the capillary and flush with the surface. A drop of the solution whose pH is desired is then placed on the tip. By loosening the pinchcock on F slightly this drop is drawn into the capillary tube so that the liquid junction is lowered a few millimeters below the surface of the tip. The remainder of the drop is then removed with filter paper. This procedure has the effect of removing any KCl-bearing solution from the tip. Another drop of the solution under observation is then added, and the protecting tube H is put in place as shown. The glass electrode is next lowered, by means of the screw adjustment, until the glass diaphragm D comes in contact with the drop. An E.M.F. measurement may then be made with an electrometer and potentiometer.

It is necessary to put a thin coating of paraffin on the tubing up to the edge of

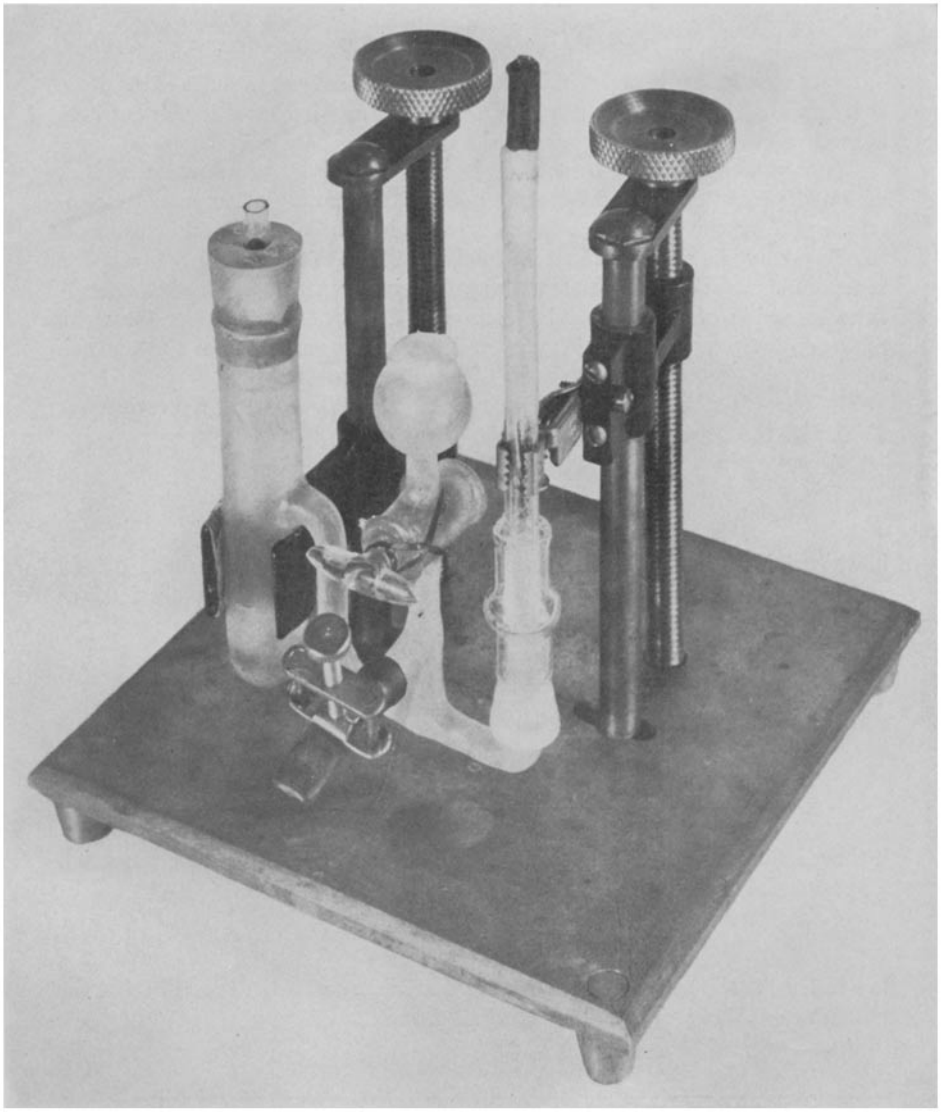

FIG. 2. Glass electrode apparatus for measuring the pH of very small volumes of solution

the tip T to prevent the drop of solution from spreading. A similar coating around the lower edge of the glass electrode is also desirable for the same reason. The protecting tube H was added to the apparatus after finding that the potentials observed were unsteady and drifting due to evaporation from the edge of the drop. It is important to have a close fitting ground joint at J so that rising air currents are prevented.

Our measurements have been made in a constant temperature room, at 25°, with a "Type K" Leeds and Northrup potentiometer, using a Compton electrometer, made by the Cambridge Scientific Instrument Co., as a null instrument. Readings could be made to about 0.2 millivolt. Due to the relatively low resistances of the glass electrodes (about 10 megohms in our most recent measurements) little screening of the electrical system was found necessary. For the same reason ordinary care in insulation was ample. The connections from the potentiometer to the electrometer were made with lead-screened wire.

The formula to be used in computing pH values from the measurements at 25°C. with the apparatus as described is

$$\text{pH} = \frac{E + G + 0.1066}{0.05915}$$

in which E is the measured potential, and G the potential (to be discussed below) which may be present in the glass. The constant 0.1066 can be obtained as follows. The potential of the system

$$\text{Hg} \mid \text{HgCl, sat'd KCl} \parallel 0.1\ \text{N HCl} \mid \text{glass} \mid 0.1\ \text{N HCl, AgCl} \mid \text{Ag} \qquad \text{(A)}$$

will be the same as the combination

$$\underset{\longleftarrow\!\!\!\!\!\!\!\!\!\!\!\!\!\!\text{B}\!\!\!\!\!\!\!\!\!\!\!\!\!\!\longrightarrow}{\text{Hg} \mid \text{HgCl, sat'd KCl} \parallel 0.1\ \text{N HCl} \mid \text{H}_2} \; - \; \underset{\longleftarrow\!\!\!\!\!\!\!\!\text{C}\!\!\!\!\!\!\!\!\longrightarrow}{\text{H}_2 \mid 0.1\ \text{N HCl, AgCl} \mid \text{Ag}}$$

if the glass acts as a hydrogen electrode and is the source of no other potential. The potential of the cell B is,[3] at 25°,

$$E = 0.2458 - 0.05915 \log a_H$$

in which a_H is the activity of the hydrogen ion in 0.1 N HCl. The cell C has the potential −0.3524.[4] The total potential is therefore

$$E = 0.2458 - 0.3524 - 0.05915 \log a_H \qquad \text{(D)}$$

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[3] Clark, W. M., The determination of hydrogen ions, Baltimore, 3rd edition, 1928, p. 672.

[4] From the work of Scatchard, G., *J. Am. Chem. Soc.*, 1925, xlvii, 641, involving a slight interpolation.

Now if we raise the hydrogen ion activity of the acid solution nearest the calomel electrode in the cell A to unity a potential will arise at the glass surface equal to

$$E = +0.05915 \log a_H \qquad (E)$$

so that the new potential will be equal to the sum of D and E or

$$E_o = 0.2458 - 0.3524 = -0.1066$$

which is the constant in the equation

$$E = E_o - 0.05915 \log a_H = E_o + 0.05915 \text{ pH}$$

or the potential of the cell A when the hydrogen ion activity between the glass and the saturated KCl solution is unity.

The potential G in the glass may be determined by placing the glass electrode (with the solution and silver-silver chloride electrode) in a beaker containing 0.1 N HCl and another silver-silver chloride electrode. In our more recent work we have used glass of a composition which gives membranes in which this potential is nearly zero. This and other information we have obtained concerning the relation of the composition of glass to its behavior when made into electrodes will be published elsewhere.

A number of tests were made to see whether the apparatus as described would yield correct pH values. For this purpose four glass electrodes were used with a buffer solution of pH 7.76, as determined by the hydrogen electrode. The results are shown in Table I.

TABLE I.

Electrode	Date	pH
1	Apr. 24	7.84
2	" 24	7.74
3	" 24	7.77
3	" 24	7.75
3	" 25	7.76
4	" 25	7.76
1	" 25	7.76
1	May 2	7.74

Except for Electrode 1 (which initially gave an error of 0.08 pH unit) the measurements are all within 0.02 unit (about 1.2 millivolts).

Extensive tests (not made with this apparatus) have shown that

electrodes, made with the glass we have found most suitable, begin to deviate 0.02 pH unit from the correct values at pH 9.5 and show rapidly increasing deviations at higher pH values, if the solution measured is 0.1 N in sodium ion. The deviations begin at lower pH values if the sodium ion concentration is greater. In alkaline solutions the potentials may be dependent on the nature of the positive ions present and may vary with time. A more complete description of these tests will appear in another article.

An opportunity for testing the usefulness of this apparatus arose in connection with the work of Dr. Marian Irwin of this Institute. An important question to be investigated was whether the penetration of a basic dye changes the pH value of the vacuolar sap of living cells of *Nitella*. A few drops only of this sap can be conveniently obtained at one time. The question could not be settled by means of the hydrogen electrode since it is "poisoned" by the sap, and by the dye, brilliant cresyl blue, which was used. Such poisonings, which are, in many cases at least, due to irreversible oxidation-reduction potentials, do not appear to have any effect on the glass electrode.[5] Furthermore, the use of hydrogen gas would affect the concentration of CO_2 on which the pH value partly depends. The use of indicators with these dye solutions is obviously impossible. By employing the apparatus described above on samples of the sap prepared by Dr. Irwin it was shown that the entrance of the dye raises its pH value considerably. The results of these experiments will be fully described elsewhere.

[5] So far as we have been able to test the glass electrodes they seem, up at least to pH 9, in dilute salt solutions, to react only to changes in hydrogen ion activity. For instance, the paper referred to ([1]) gives data on the electrometric titration of sulfuric acid in the presence of potassium permanganate. The potentials followed the course to be expected from the change of hydrogen ion activity and were uninfluenced by the strong oxidation potential of the permanganate. On the other hand, the glass electrodes showed no change in potential when ferrous sulfate was titrated with potassium dichromate in the presence of an excess of sulfuric acid. In this case the hydrogen ion concentration remained substantially constant during the titration although there was a change in an oxidation-reduction potential of roughly 0.3 volt. We have other evidence, bearing on this matter, which cannot be conveniently summarized and will be published later.

SUMMARY.

A glass electrode apparatus is described with which pH measurements can be made with as small volumes as 2 drops (about 0.14 cc.) of solution.

Using this apparatus the change of pH of the vacuolar sap of *Nitella*, due to the penetration of brilliant cresyl blue, has been readily followed. The sap and the dye have been found to poison the usual type of hydrogen electrode.