XCI. THE USE OF THE GLASS ELECTRODE IN BIOCHEMISTRY.

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THE purpose of this paper is to describe the glass electrode as a means of measuring hydrogen ion activity. By it a p_H determination can be made on 0·5 cc. or less of liquid, whether clear, coloured or turbid, easily reducible, or unstable in air, with an accuracy of 0·02 p_H, the experiment taking less than 5 minutes, and the sample being returnable chemically unchanged at the end.

Throughout this paper the term c_H is taken to designate the hydrogen ion activity, and p_H the negative logarithm of the hydrogen ion activity.

The possibility of the use of glass as an electrode for hydrogen ions was first discovered by Haber and Klemensiewicz [1909] and was confirmed by Freundlich and Rona [1920]; the glass electrode was compared with a hydrogen electrode by Hughes [1922], v. Steiger [1924] and Briske [1924] and correlated with the chemical composition of the glass by Horovitz [1923] and Schiller [1924] and described with some experimental detail by Brown [1924].

The chemical and physical theories as to the behaviour of the glass are not considered here, as the true explanation does not yet seem to have been found beyond dispute. Used empirically the method is simple, needing only extreme care in the technique. There has been some hesitancy in the adoption of the glass electrode—a hesitancy quite out of proportion to the unique advantages and facile manipulation it can present when once carefully set up.

General Principles.

Glass is used as a membrane to separate two solutions of different hydrogen ion activity. If the membrane be thin enough—about 25μ in its thinnest part—the electromotive force between the two solutions set up by the difference in activity may be measured. If the c_H of one solution be known, that of the other may be calculated from the electromotive force. Contact is made with the solutions by the arms of two saturated KCl-calomel electrodes arranged to oppose one another. The E.M.F. arising from this cell combination is balanced and measured potentiometrically.

The technical difficulties are caused by the high resistance of the glass membrane (50–100 megohms), the electric current naturally tending, if possible, to short-circuit the electrode by leaking through what would normally

be considered an insulating medium, and especially across dirty or moist surfaces of insulators. Dirt is always a difficulty, but the trouble with moisture varies, being more obvious in summer than in winter, as, in the summer, room air has a greater relative humidity.

Fig. 1 shows the general arrangement of connections.

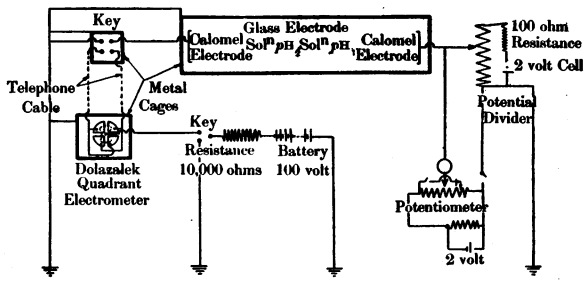

Fig. 1.

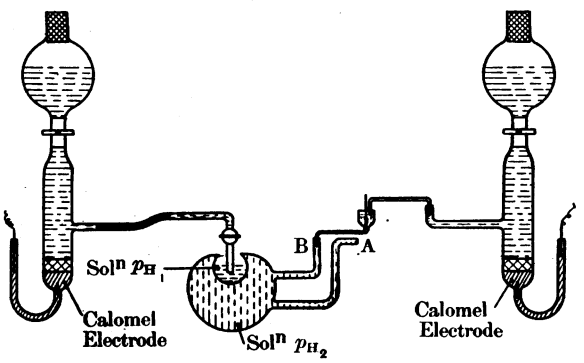

Fig. 2.

The Glass Electrode.

The electrode must be so designed as to present a very thin glass membrane, so held by stronger glass that it may separate two solutions. The simplest form used by all previous workers was that of a small bulb blown at the end of a piece of glass tubing. The one solution was put inside the bulb, while the other solution was held in a beaker in which the bulb was immersed. This shape has several disadvantages; for example, some 20–30 cc. at least of the liquid of unknown p_H are required in the beaker; it is not easy to fill and refill the bulb attached to the tubing as there is no exit for the air; and moreover the bulb is very easily broken. It is naturally under some strain, being of the limiting thickness capable of holding together as a bulb. In stirring the solution in the beaker, or in washing and drying the bulb, great danger of breakage is incurred.

A form of electrode found very convenient for biological purposes is illustrated in Fig. 2. The membrane in this case is in the shape of a deep spoon sucked inside a bulb of thicker glass. This is very easily glass-blown after a

little practice. The solution of known p_H may be filled into the outer bulb by turning the electrode on its side and putting the tube *A* under the jet of a pipette or burette, the air in the bulb escaping through *B*. The other solution, that of unknown p_H for example, is put into the spoon. The advantages of this form are self-evident. Filling, washing and emptying the inside solution are simple operations. The membrane blown in this way seems to be under less strain than when blown at the end of a tube, and does not break so easily, and the quantity of liquid required for the spoon is very small. The total capacity of the spoon is about 1 cc. and it need never be more than half-full. A smaller quantity than 0·5 cc. may be used if necessary. If the liquid under experiment is liable to air oxidation or to loss of CO_2 it may be covered with liquid paraffin without affecting the E.M.F.

Another form of electrode found convenient, especially when the samples of unknown p_H solution can be allowed to flow successively through the electrode or when it is desirable to work in a thermostat, is shown in Fig. 3. The solution of known p_H is placed in the outer vessel and the unknown solution in the narrow tube going through the ground stopper. A thin bulb is sucked into an enlarged part of this tube. The total capacity of the tube is about 1 cc.

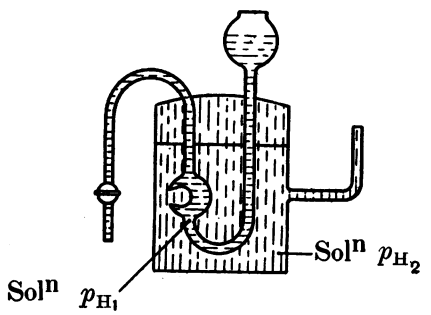

Fig. 3.

The glass used for the electrodes was an ordinary soft soda laboratory glass. Haber used originally a soft Thüringian glass. Horovitz [1923] and Brown [1924] found that certain borosilicate glasses act as electrodes for sodium as well as hydrogen in certain ranges of p_H, or as mixed electrodes. I have found that certain glasses which acted as mixed sodium and hydrogen electrodes, when a sodium phosphate buffer mixture was used as the known solution, acted accurately as H· electrodes when the Na phosphate solution was replaced by a K phosphate or a KCl solution. No results could be obtained with "Durosil" glass, or with pure silica electrodes, presumably because the resistance of these membranes was too large even when thin.

The Calomel Electrodes.

The calomel electrodes were of the type shown in the diagram and were filled with saturated KCl solution four times re-crystallised. The calomel was prepared electrolytically according to instructions given by Clark [1923].

The contacts with the glass electrode solution were made in the manner shown, special care being taken to avoid diffusion of the heavy saturated KCl into the other solution, which may alter the E.M.F. by 2 or 3 millivolts. A tap, ungreased in the middle race and turned off, allows sufficient KCl to pass for electrical contact, but allows no flow. Another useful device to avoid diffusion is to leave a small bubble in the end of the glass capillary tube dipping into the electrode, the sides of the glass being wet enough to conduct. Convenient latitude in manipulation is obtained by attaching the arm of the calomel electrode to a rack and pinion, so that it can be readily lowered to, or raised from, the solution in the spoon of the glass electrode. The contact to the other calomel electrode is by means of narrow tubes and a small cup with a ground stopper. As a further precaution these tubes are removed from the electrode between readings.

The Potentiometer.

Theoretically only one potentiometer should be necessary for the measurement of the E.M.F., but in practice it is convenient to use a Pye rotary potential divider, together with a 2-volt accumulator and a 100 ohm resistance, to tap off the current required to balance that from the electrodes, and a potentiometer to measure accurately the current thus tapped off. The latter is standardised by means of a standard cell, and is used in conjunction with a small pointer galvanometer; or a combination apparatus, such as that supplied by the Cambridge Scientific Instrument Co. for hydrogen ion concentration measurement, may be employed.

The Electrometer.

A quadrant electrometer of the Dolazalek type has been used as null point indicator in the electrode circuit. The difficulties which may be encountered in adjusting the instrument to give reliable and sensitive readings are described in the paper by Brown [1924]. A Lindemann electrometer, which is small and portable, is unfortunately not sensitive enough for the purpose.

The Switches and Connections.

Owing to the high resistance of the glass electrode, and the sensitivity of the electrometer, special precautions have to be taken with regard to insulation and to shielding. The high tension battery used for charging the electrometer needle was supported on paraffin wax blocks, and was on a wooden table separated by an air gap from the table carrying the electrodes. The latter were enclosed in an earthed cage of wire netting. The glass electrode was supported on a block of wax; and, as in the case of the calomel electrodes, was insulated by an amberite rod held in an earthed metal stand. All connections to earth were of bare thick copper wire which was soldered where required. In the first instance, other connections were of varnished copper wire sus-

pended by paraffined string, junctions being sunk in paraffin wax and the whole surrounded by a large cylinder of earthed wire netting. This arrangement was found unsatisfactory, as in time the wax became dirty, and in wet weather the surface was moist and the amount of leakage considerable. Such difficulties were entirely avoided by the use of lead shielded telephone cable. The Callendar Cable and Construction Co. kindly presented us with a length of their dry core cable, in which the insulating medium is virtually the air in the pores of paper dried in a vacuum at 250°. They also sent two wooden boxes soaked in wax, in which wax-embedded connections to lighter cable leading to the electrometer and to the switch could be made. This lighter cable for small length connections was made by putting varnished copper wire through narrow waxed glass tubing; this was surrounded with rubber tubing, and all the space inside filled up with wax. The rubber tubing was sheathed on the outside with copper tape, which could be earthed, as was the lead sheath of the heavier cable. Care was taken that the wax used had never been heated above its boiling point, when its insulating properties are impaired.

This arrangement eliminated all leakage troubles satisfactorily and allowed the work to be carried on even on wet days.

Porcelain switches embedded in wax were unsatisfactory for the reason given above, *i.e.* that surface leakage starts after a time on account of dirt and moisture. For charging the electrometer needle with 100 volts, and for discharging it, an ebonite switch with brass conductors and ivory handles was used. For connecting the glass electrode to one or other pair of quadrants of the electrometer, and for earthing these, a switch was made having amberite insulators. The terminals on the ebonite top of the switch were surrounded by amberite collars, and were covered with earthed metal shields.

Manipulation.

Newly-blown glass electrodes require careful cleaning with chromic acid, steaming for about 2 hours and soaking in distilled water for 24 hours before they can be used. Even after this treatment there may be a potential at the glass surface of 40 millivolts or more, which decreases slowly with time until after about 2 days it reaches a constant level of 4 or 5 millivolts which it will retain for weeks. (This contact potential may possibly be due to a state of strain in the glass.)

The system may be represented as follows:

$$\begin{array}{l|l|l|l|l|l|l|l|l} \text{Hg} & \begin{matrix}\text{Hg}_2\text{Cl}_2 \text{ in} \\ \text{sat}^{\text{d}}\ \text{KCl}\end{matrix} & \begin{matrix}\text{KCl} \\ \text{sat}^{\text{d}}\end{matrix} & \begin{matrix}\text{Sol}^{\text{n}} \\ p_{\text{H}_1}\end{matrix} & \Big\| & \begin{matrix}\text{Sol}^{\text{n}} \\ p_{\text{H}_2}\end{matrix} & \begin{matrix}\text{KCl} \\ \text{sat}^{\text{d}}\end{matrix} & \begin{matrix}\text{Hg}_2\text{Cl}_2 \text{ in} \\ \text{sat}^{\text{d}}\ \text{KCl}\end{matrix} & \text{Hg} \end{array}$$

Glass membrane

The E.M.F. of the combination is given by

$$E = \frac{RT}{F}(p_{\text{H}_1} - p_{\text{H}_2}) + Eg,$$

when E is the total E.M.F., Eg that occurring at the glass membrane. If E and Eg are measured in millivolts, $\frac{RT}{F}$ is equal to 57·7 at 18°.

One method of finding the value of Eg is to put the same solution on both sides of the glass so that $p_{H_1} = p_{H_2}$. Practically, however, the more accurate way of working is to have at hand a small quantity of a buffer solution of known p_H either freshly prepared from highly purified chemicals [Clark, 1923] or tested by the hydrogen electrode. The solution p_{H_2} may then be a stock buffer solution, say of potassium phosphates, whose p_H need never be accurately known. With this solution in the outer bulb and $\frac{1}{2}$ cc. of the standard solution in the spoon, a reading of the E.M.F. (E standard) is taken. The standard solution is then emptied from the spoon, the latter washed several times with distilled water and filled with the solution of unknown p_H (p_{Hx}). The E.M.F. is again measured (E_x). p_{Hx} is then readily calculated from the equation

$$p_{Hx} = p_{Hstd.} \pm \frac{E_{std.} - E_x}{\frac{RT}{F}}$$

$$= p_{Hstd.} \pm \frac{E_{std.} - E_x}{57{\cdot}7} \text{ at } 18°.$$

This method of standardisation eliminates any error due to slight inequalities between the calomel electrodes or to liquid-liquid contact potentials. One standardisation at the beginning of each day's series of experiments is sufficient, but it may conveniently be repeated at the end of the day for confirmation.

In measuring the E.M.F. it is often quicker to balance only approximately with the potentiometer and then to read the remaining small deflection of the electrometer; the sensitivity of this instrument may be determined by measuring the change in deflection for a known change in E.M.F.

Lastly, the need for great cleanliness cannot be too strongly emphasised.

Application.

The technique described has been employed for some 450 estimations of the p_H of various substances. A few typical examples are:

Material	p_H
Blood (human-oxalated)	7·42
Blood (dog-defibrinated)	7·75
[Blood by Dale-Evans' method	7·73]
Flour (turbid mixture with H_2O) ...	6·15
Bean seedlings (aqueous extract) ...	5·87
Phosphate solution	10·5
Phosphate solution	7·37
[Phosphate by hydrogen electrode ...	7·39]
Sycamore leaves (aqueous extract) ...	4·88
[Sycamore by hydrogen electrode ...	4·91]
Culture medium (broth, etc.)	5·95

I am indebted to Dr Chibnall for the measurements indicated on the hydrogen electrode.

A comparison also of the "buffering powers" of heart and skeletal muscle, by means of titration curves, has been possible by the use of the glass electrode. These results will be published later.

I am deeply grateful to Prof. A. V. Hill, F.R.S., for much help and advice during the course of this research, which was carried out with the aid of grants from the Scientific and Industrial Research Department and the Medical Research Council.

SUMMARY.

1. The use of the glass electrode as an instrument for biochemical investigation is discussed, and its technique described in detail.

2. Two modified forms of electrode requiring only 0·5 cc. of liquid for examination are described.

3. Examples are given of the use of the electrode for various biochemical substances.

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