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A Sealed-In Micro Glass Electrode.

GRACE E. PICKFORD. (Introduced by Ross G. Harrison.)
From the Osborn Zoölogical Laboratory, Yale University.

A sealed-in glass electrode essentially similar to that described by Michaelis[1] but of much smaller capacity can readily be adapted for pH measurements of biological fluids when only rather small volumes are available and under conditions which exclude contact of the sample with air.

The design is shown in the accompanying diagram (Fig. 1); the inner tube of Corning O15 glass may be of capillary dimensions but care must be taken to minimize the capacity of slight but unavoidable dilations at the two ends where this capillary is sealed, through heavy walled graded glass, to the outer jacket. When this unit, the capillary electrode within its jacket, has been tested and found free of electrical leaks or other defects it is attached permanently to a stopcock of the type described by Stadie, O'Brien and Laug.[2] For testing it is convenient to connect it temporarily with rubber tubing to a spare stopcock of the same type. Redescription of this stopcock is not necessary but two points should be mentioned: (1) in order that the electrode may be permanently attached, as at X in the diagram, the stopcock must be made of soft glass similar to that of the electrode jacket. (2) In order to reduce the volume of the test sample to a minimum the diagonal bore for filling must be as fine as possible and without unnecessary widening at the ends. This bore has a diameter of 1 mm. in the electrode assemblies in use by the author; the bore of the filling and connecting tube is about 0.5 mm.

The method of filling the electrode depends, of course, on the technique and requirements of sampling. In an investigation of the digestive juice and blood of tarantulae it has been convenient to withdraw the sample directly from the animal into a 1 ml. Luer syringe (the short "Insulin" type is preferable). The needle is then removed and a short piece of haemocytometer tubing (3 mm. internal, 5 mm. external diameter) is fitted over the nozzle. The filling inlet on the stopcock is provided with a cup which exactly fits the nozzle when such a piece of rubber tubing has been slipped over it (see diagram). In order to avoid contact of the sample

[1] Michaelis, L., *Science*, 1936, **83**, 213.

[2] Stadie, W. C., O'Brien, H., and Laug, E. P., *J. Biol. Chem.*, 1931, **91**, 243.

with air the syringe may be first filled with freshly boiled distilled water which is then ejected so as to leave water rather than air in the bore of the needle and nozzle. The total volume of such remaining water is about 0.05 ml. This about equals the capacity of the smallest electrode at present in use so that very small samples are diluted with about an equal volume of water. When larger volumes are available it is of course better, if possible, to fill the syringe with a preliminary rinse sample so that there shall be no subsequent dilution of the test sample.

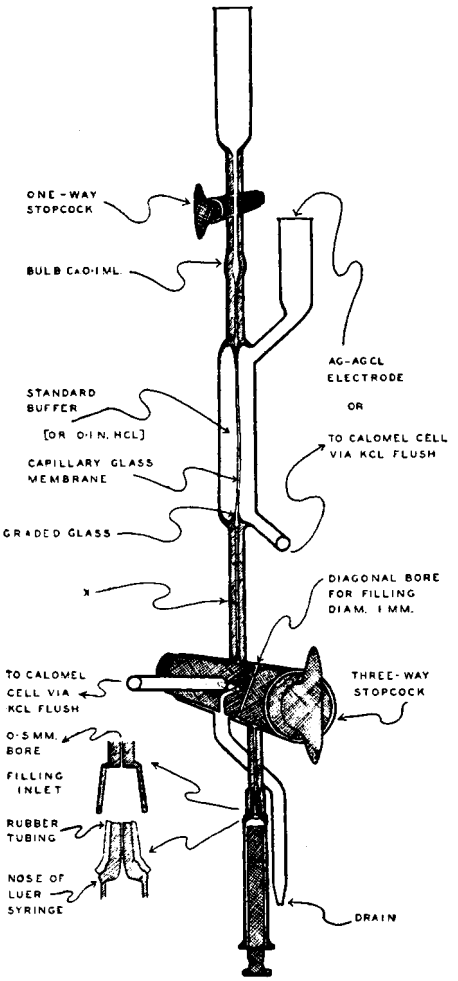

FIG. 1.

The electrode is calibrated with a standard buffer, washed and then dried with alcohol and ether; I can confirm the findings of

Stadie, *et al.*, that this treatment has a negligible effect on the asymmetry potential. The sample is then introduced slowly from the syringe into the electrode. Preferably, when more than the minimum volume is available, the first part of the sample is passed over into a small dilation above the electrode, thus eliminating that part which came into contact with the atmosphere. Both upper and lower stopcocks are then closed, after which the sample may be allowed to remain in the electrode for a considerable time without deterioration. Before making a measurement the lower stopcock is rotated clockwise so as first to flush saturated KCl through the groove and right angle bore—the position shown in the diagram—and then to make liquid junction between the sample and the saturated calomel half cell on the left. If the sample is required for other purposes it is not necessary to put it in full contact with the KCl provided the inner race of the stopcock is wetted with this solution. Under these conditions the sample is scarcely contaminated and may be withdrawn into the syringe after measurement and used, for example, in digestion experiments.

After use the electrode is washed with dilute salt solution and kept filled with distilled water. The outer jacket may be filled with N/10 HCl and provided with a permanent silver-silver chloride electrode, as recommended by Michaelis. In one assembly in use by the author the jacket is kept filled with distilled water; just before use this is replaced with M/20 K H Phthalate or some other standard buffer solution and connected by a lower outlet with a second KCl reservoir and calomel half cell on the right. The 2 calomel half cells agree within 0.1 mv. This arrangement permits immediate determination of changes in asymmetry potential, uncomplicated by other factors; on the other hand it is somewhat more troublesome and quite unnecessary for routine measurements of pH.

Two of these micro electrodes, one with a total capacity of 0.25 ml. and the other of 0.06 ml., have been in use in this laboratory for periods of nearly a year and 6 months respectively. A third sealed-in electrode of larger capacity, similar to that described by Michaelis except that it is filled by suction from above, is provided with an upper stopcock and is connected to the lower stopcock by an interchangeable grinding, is also in constant use and has proved most satisfactory. The asymmetry potentials were at first high but have since dropped almost to zero. The resistance is high, ca. 219 and 292 megohms for the 2 micro electrodes, ca. 146 megohms for the larger electrode, but this is of little importance since the assembly is used in conjunction with a Pliotron tube amplifier of the type de-

scribed by Du Bridge and Brown.[3] The circuit includes a Leeds & Northrop type K potentiometer and type R galvanometer; 1 mv. gives a deflection of about 4 mm. on the scale at one metre. No erratic behavior has been encountered on the part of these electrodes; there may be slight hysteresis after changing from one buffer solution to another of widely different pH but the effect wears off within a few minutes after careful washing. With all ordinarily well buffered solutions, including blood samples from spiders, we regularly obtain steady readings within ± 0.2 mv. or better. The value of the constant RT/nF became practically normal after the first few weeks of use.

Although the initial outlay may be greater than for many designs hitherto published, the assembly here described is so convenient that it seems probable that it might find a use in other laboratories. An assistant can be trained to make routine measurements without difficulty. The amplifier is simple to operate provided proper shielding requirements are observed, in particular the grid lead should be thoroughly shielded and insulated. As pointed out by Hill[4] the high input resistance of the Pliotron eliminates the need for making fragile glass membranes of very low resistance.

The electrodes described above were made by the Macalister Bicknell Company of Cambridge and New Haven. The amplifier was built by Dr. A. Petrunkevitch of this department, who has designed a very convenient non-capacity switch (unpublished). We are indebted to Mr. D. G. Brubaker and the Sloane Physics Laboratory of Yale University for measuring the constants of the Pliotron used and also for assistance in determining the resistance of the electrodes.

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Preparation and Properties of a Specific Polysaccharide from a Strain of *Vibrio Cholerae*.*

D. L. SHRIVASTAVA AND S. C. SEAL. (Introduced by R. W. Linton.)

From the Cholera Inquiry, Indian Research Fund Association, All-India Institute of Hygiene and Public Health, Calcutta.

After a number of trials the method described below gave satisfactory results in the preparation of a specific polysaccharide of the

[3] Du Bridge, L. A., and Brown, H., *Rev. Sci. Instr.*, 1933, **4**, 532.

[4] Hill, S. E., *Science*, 1931, **73**, 529.

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