50. AN IMPROVED CELL FOR MEASUREMENTS OF OXIDATION-REDUCTION POTENTIAL

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(*Received 19 January 1940*)

For the work described in the following paper [Dixon & Zerfas, 1940] we required an apparatus for conveniently making measurements of oxidation-reduction potential during certain enzyme reactions. This apparatus had to fulfil the following requirements: (*a*) It must contain some arrangement for adding several reagents successively to the enzyme solution without opening the apparatus, introducing any traces of O_2 or interrupting the readings. (*b*) It must contain provision for removing all traces of O_2. (*c*) There must be no possibility of contamination of the solution with traces of the reagents used in previous experiments. The latter point was of great importance, because the possibility that some coenzyme might have been carried over from a previous experiment would have destroyed the evidential value of the results.

A number of cells have been designed by previous workers, among which we may mention those of Lehmann [1930] (two types), Wurmser & Geloso [1931], Dixon & Kodama[1] [Tsukano, 1932] and Borsook & Schott [1931]. None of the existing cells fulfilled the conditions given above and we therefore had to develop a special cell for our purpose. This cell proved very satisfactory and we give here a detailed description of it in the belief that it may be useful for general work.

In general freedom from O_2 may be secured either by passing a stream of O_2-free N_2 or by evacuation. The first method is not very convenient, as it involves setting up apparatus for the supply in fairly considerable quantity of N_2 from which every trace of O_2 has been removed. Unless this is done very efficiently, appreciable quantities of O_2 may be passed into the solution in the course of an hour or two, along with the N_2. The vacuum method is much more convenient, and all the cells mentioned above depend on this principle. It must be pointed out, however, that it is not sufficient merely to exhaust the apparatus on a water-pump and to close it off immediately. Under these conditions the O_2 dissolved in the liquid is not removed and remains in the apparatus throughout the experiment. It is best to wash out the cell once or twice with N_2 before the final evacuation, and even then some provision should be made (e.g. a side-bulb containing alkaline pyrogallol) for the removal of any remaining O_2 traces. Only one of the above-mentioned cells (that of Dixon & Kodama) contains such provision.

The usual way of adding a reagent to an electrode vessel is from a burette leading through the stopper of the vessel. This involves the protection of the upper end of the burette from the air and the careful removal of dissolved O_2 from the solution in the burette. When several different solutions have to be added this method becomes very troublesome, and it is much better to have some arrangement inside the cell itself for adding the different solutions. Only

[1] The original form differed from Tsukano's illustration by the inclusion of a built-in agar salt bridge and an extra side-bulb for alkaline pyrogallol.

one of the above-mentioned cells (the "large" electrode vessel of Lehmann [1930, p. 243]) contains such provision. The upper part of this cell contains four cups mounted on pivots in such a way that they can be inverted by a movable arm, spilling their contents into the solution in the lower part of the electrode vessel. We have adopted this arrangement in principle, but using an electrical method which is very simple in construction and avoids an extra joint with possibility of leakage.

It is necessary to have some method of making contact between the solution in the electrode vessel and the standard half-cell which forms the second electrode of the system. The usual way of effecting this is by means of an agar-KCl bridge, and all the cells except one (that of Wurmser & Geloso) have such a salt bridge built in as a permanent part of the apparatus. This is undesirable for the following reason. During an experiment substances diffuse from the solution into the end of the salt bridge through the agar. During the next experiment these substances contaminate the solution by diffusing out from the salt bridge. Filling the vessel with KCl solution and allowing it to stand between experiments reduces, but does not eliminate, the effect, as some substances may diffuse into the bridge for some distance. The amounts involved are usually small, but we have found that they are important in some cases.

The apparatus of Wurmser & Geloso does not involve a salt bridge, but makes use of the conductivity of the film of water on the inner surface of the glass. This conductivity is of course extremely small compared with the salt bridge and it is necessary to use some form of thermionic potentiometer.

In order to overcome the difficulty we have arranged the standard half-cell so that a separate salt bridge is used; the bridges are easily prepared in quantities of fifty or more, and each bridge is used once only and then discarded. By this means all risk of contamination is avoided.

The apparatus is shown in Fig. 1. The main vessel carries on one side the side-bulb *S* for the pyrogallol and on the other the part *H* for the standard half-cell. The lower part, in which the solution to be studied is placed, is blown out as shown to form a bulb about 1 in. in diameter, in order to facilitate mixing of the solution. A gentle oscillation of the apparatus is sufficient to keep the liquid round the electrode thoroughly mixed. The vessel is closed at the top by a well-fitting rubber bung,[1] through which pass (*a*) the glass tube carrying the main electrode *E*, (*b*) a tube with a well-ground tap *T* for evacuating the vessel, (*c*) a wide tube, closed at the lower end, through which are sealed five platinum wires. The part *H* is closed with another rubber stopper, which carries the second electrode for the half-cell. The upper rims of the vessel are expanded so as to form water-seals round the stoppers, and thick liquid paraffin is run round all the tubes where they pass through the stoppers in order to prevent all possibility of leaks. The tube carrying the main electrode is bent as shown so that the electrode is brought near the central axis. The electrodes are both of bright platinum and contact is made through mercury in the tubes with the leads to the potentiometer (not shown) which are dipped into the open ends of the tubes. The salt bridges *B* are made of glass tubing, 2·5 mm. internal diameter, and are bent to fit a template. Their shape should be carefully adjusted so as to allow them to be readily inserted with forceps through the passage from the main vessel into the part *H*, and at the same time to allow their ends to dip well into the solutions in both parts of the vessel. Their preparation is described below.

[1] No doubt the apparatus could be improved by an all-glass construction with ground joints, but we have found the rubber stoppers quite satisfactory and the cell as described is easy to construct.

The central tube with its wires is for adding the reagents and the arrangement operates as shown in the detailed view (Fig. 2). The parts of the wires passing

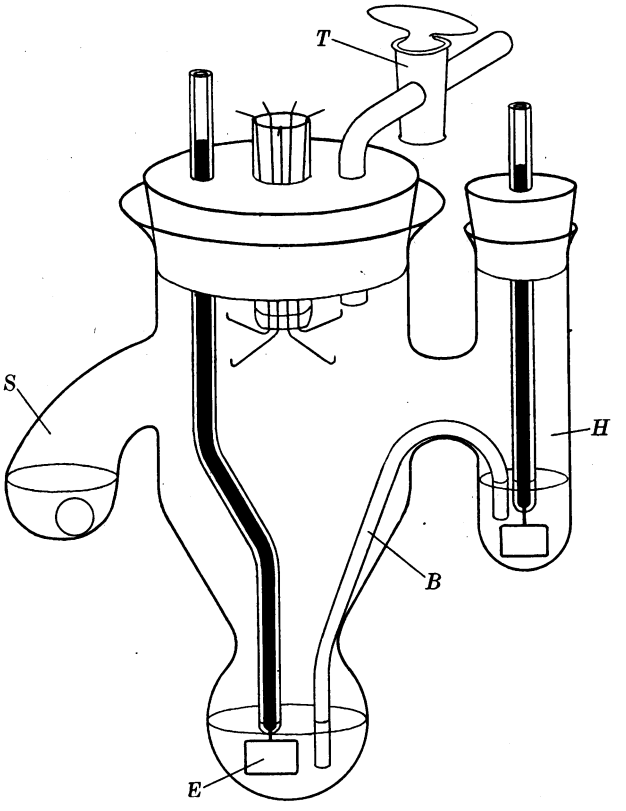

Fig. 1.

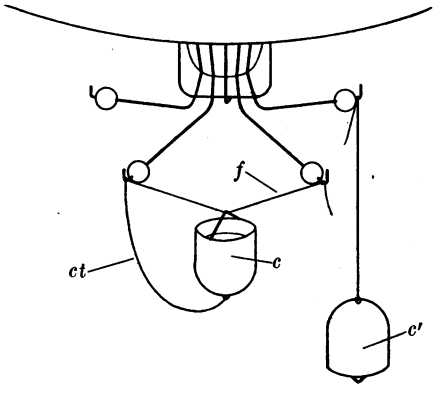

Fig. 2.

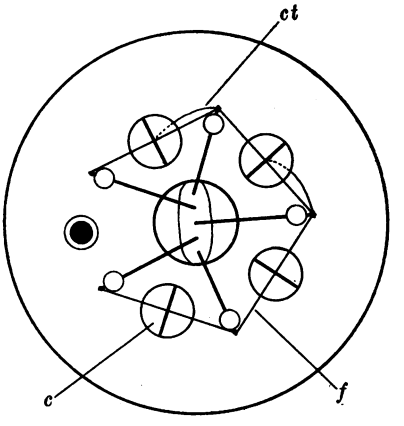

Fig. 3.

up through the wide tube are insulated from one another by lengths of narrow glass tubing slipped over them. A differently coloured bead is placed on the

upper end of each wire for identification, and a few mm. of bare wire should project so that connexion to an accumulator can be made through leads carrying "crocodile clips". The lower ends are bent radially outwards horizontally like the spokes of a wheel. The end of each wire carries a bead corresponding in colour with that on its other end, and is bent upwards to form a small hook. Each reagent to be added is placed in a small glass cup (*c*) holding about 1 ml., which has a handle formed of a piece of platinum wire fused on across its open end as shown. By means of this it is hung from a length of fuse wire (*f*) fastened across the ends of two of the platinum wires. Thus on connecting the upper ends of these two wires to a battery a current passes through the fuse wire, which melts and parts in the centre so that the cup falls. It does not however fall into the solution in the electrode vessel. A small loop of platinum wire is fused on to the bottom of each cup and to this is fastened a short length of cotton thread (*ct*) terminating in another small platinum loop. The latter is hung over the end of one of the supporting wires, so that when the cup falls it hangs in an inverted position as shown at *c'*, delivering its contents into the solution below. The cups are coated inside with a thin layer of high-melting paraffin wax and this ensures that the delivery of the contents is complete.

As there are five supporting wires four cups can be used, as shown in plan in Fig. 3, and any cup can be dropped at any time independently of the others by connecting the correct pair of wires to the battery. The wires must of course be stout enough to take the weight of the cups without bending. They are bent out so that the gap in the ring comes where the electrode stem passes up, as shown in the plan.

The arrangement is assembled as follows. The rubber bung with its attachments is held in a convenient stand. A piece of fuse wire (fine tinned copper wire rated at 3 amp.) of sufficient length is attached to the end of one of the supporting wires by winding it round several times with forceps. It is then threaded through the handle of one of the cups and attached to the next supporting wire in the same way. Another cup is then threaded on and the fuse wire attached to the next supporting wire and so on until all the cups are hanging. The colours of the corresponding beads are noted down at the same time. It is important that the wires should be free from grease as otherwise a bad contact may possibly give trouble. The loops on the ends of the threads are then dropped over the hooks on the adjacent supporting wires and the cups are examined to make sure that they hang freely in the centre of their length of fuse wire. This part of the apparatus is then ready. The lengths of thread should be long enough for the cups to hang upright, but short enough to keep them from touching the walls of the vessel when they fall. The rims of the cups should not be covered with a thick layer of wax, as if this touches the fuse wire the heat may soften it and cause it to stick. The current for melting the fuse wire is obtained from an 8 V. accumulator, which is connected either directly or if preferred through an ammeter. A fairly large current flows for about $\frac{1}{2}$ sec., when the wire parts and the cup empties. The accumulator should be of the ordinary plate type, not the block type. Provided due attention is paid to the points mentioned above, the arrangement has proved thoroughly reliable.

The salt bridge, the stem of the electrode and the sloping sides of the vessel may if desired be coated with paraffin wax, in order to make sure that the whole of the contents of the cups reaches the main solution and does not adhere to the walls in the form of drops. This is not always necessary, as it is found that as long as the glass is clean all drops flow easily down into the solution. In any case a high-melting wax should be used. We found that a sample melting at 68°

was satisfactory, as water ran freely off the coating without the slightest tendency to adhere, but with all the samples we examined which melted at lower temperatures there was a tendency to leave droplets of water on the surface.

The standard half-cell chosen was the quinhydrone electrode at *p*H 2·0. This is exceedingly constant and reproducible and is very quickly made up. It was of course freshly made for each experiment. A few ml. of a HCl : KCl buffer mixture *p*H 2·0, prepared according to the directions of Clark [1928], are placed in *H* and stirred up with excess of solid quinhydrone.

The whole experimental procedure is as follows. The cups containing the reagents to be added are prepared and suspended as described above, the arrangement being mounted in a stand. The solutions are added to the half-cell as just described, a salt bridge is placed in position and the half-cell closed with its stopper. A glass bead is introduced into the side-bulb *S*, followed by a solution of pyrogallol in water, for which a special curved pipette is used. The enzyme solution to be tested is then placed in the main vessel. Using a curved pipette, strong alkali is then run under the pyrogallol solution in the side-bulb. The main stopper is then inserted and the tube *T* connected with a water pump through a T-tap connected also with a N_2 reservoir. The apparatus is then exhausted, water being run round the water seals at the same time. By turning the T-tap the vessel is twice filled with pure N_2 and exhausted, and after the final exhaustion the tap *T* is closed and detached from the evacuating apparatus. By gently rocking the vessel the bead in *S* is caused to mix the pyrogallol with the alkali. The vessel is then immersed in a water bath at 20° (or room temperature). The method is not suitable for work at 37°, owing to the boiling of the liquids at that temperature in the vacuum. For this reason it is advisable to cool all the solutions under the tap before filling the apparatus. The vessel is held in a retort clamp. If a piece of rubber tube is slipped over the stem of the clamp where it is held in the boss the mounting will be sufficiently elastic to allow it to be shaken gently so as to mix the solution while still holding it securely. The vessel is immersed so deeply in the bath that the water covers the stoppers and only the tubes project; thus there is no possibility of air leaking in. The leads to the potentiometer are then connected with the electrodes and the leads for the accumulator are connected to the correct wires in preparation for the addition of the first reagent. Meanwhile the pyrogallol is absorbing any traces of O_2 which may remain in the vessel, and a short period is allowed for this process to become complete, after which the accumulator can be connected, so as to add the first reagent, and readings begun. The other reagents can then be added at suitable time intervals as desired.

The salt bridges were prepared in lots of fifty at a time as follows. The glass tubes were chemically cleaned, dried and put in an air oven to heat. 100 ml. of saturated KCl solution were heated to boiling and chopped agar added gradually while boiling until no more would dissolve. The solution was then filtered while hot through a hot water funnel, and placed in the hot air oven. The bridges were filled with the hot agar by suction. For this purpose a short length of rubber tube was attached to one end of the bridge and dipped into the agar. A longer rubber tube was attached to the other end for aspiration. The agar was drawn to about $\frac{1}{2}$ in. beyond the end of the glass tube, and it was then allowed to cool in the air. After a few moments it became solidified and the aspirating tube was then cut off about $\frac{1}{2}$ in. beyond the end of the bridge. After all the bridges had been filled in this way they were allowed to cool and then, held by the rubber end pieces, they were dipped momentarily into the hot paraffin wax. They were then allowed to cool for several hours, after which the rubber tips were carefully

split longitudinally with a razor blade and removed, and the projecting agar cut off so as to leave about 1 mm. beyond the ends of the tubes. The purpose of this was to prevent bubbles from forming in the ends of the tube under the vacuum during the experiment, so breaking the contact. The bridges are stored with their ends upwards in a vessel filled with saturated KCl solution. Before use the bridge selected should be rinsed outside with distilled water to remove excess KCl.

The arrangement adopted in this cell avoids any pressure difference tending to drive the agar through the bridge. The agar does not have to withstand the pull of the vacuum, as in certain other types of cells in which one end of the bridge is exposed to atmospheric pressure while the other end is exposed to the vacuum. A source of leakage is thus removed; and at the same time it is possible to use a larger bore for the tubes, with consequently a lower resistance. With bridges filled as described above there is no tendency for the agar to part in the middle of the bridge owing to the formation of gas bubbles under the vacuum.

After use the bridges are emptied by warming slightly and blowing out the agar. They are then cleaned and refilled with fresh agar as already described.

SUMMARY

An anaerobic electrode vessel for oxidation-reduction potential measurements is described. It contains provision for the addition of several reagents successively without opening the apparatus and for removing any traces of O_2 which may be present. It is free from the risk of contamination of the solution with substances from the preceding experiments, which is usually associated with built-in salt bridges.

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