

The upright straps also serve to support a standard  $\frac{1}{4}$  in. brass pipe *D* into both sides of which, at intervals of  $1\frac{3}{4}$  in. center to center, a  $\frac{3}{4}$ -in. length of  $\frac{1}{4}$ -in. stainless-steel tubing *E* is silver soldered.

An electrically controlled valve *F* (2) is connected to the water-supply line and mounted at the center of the standard  $\frac{1}{4}$ -in. brass pipe. It is recommended that a vacuum breaker be installed in the water-supply line to obviate any possibility of back syphonage.

The valve is connected to a "time clock" *G* (listed in most scientific catalogs) which is set to turn the water on at the end of the desired periods of fixation and to turn the water off when washing is completed.

For fixation, properly identified tissues are placed into Moss Embedding Bags (3) that are inserted into vials. The water outlet for each vial is fitted with rubber tubing, and a glass spout extends from the tubing into the vial.

The design of the wash rack may readily be modified to meet the needs of individual laboratories.

#### Notes

1. We wish to express appreciation to Edwin Herskind, Institute of Tuberculosis Research, who constructed the apparatus to our specifications.
2. Skinner Valve No. 5-6260, 115 v, 60 cycles.
3. A. S. Aloe Co., No. 64690.

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## Versatile Anaerobic Spectrophotometer Cell

Arnold Lazarow\* and S. J. Cooperstein

Department of Anatomy, Western Reserve University,  
School of Medicine, Cleveland, Ohio

This paper describes an anaerobic cell that is similar to the Thunberg tube but permits the measurement of reaction rates in the Beckman Model DU Spectrophotometer. Strict anaerobiosis is obtained by bubbling oxygen-free  $N_2$  or helium through the cell in a manner similar to that described by Singer and Kearney (1). The visible absorption spectrum can be measured under anaerobic conditions during or at the end of the reaction. Provision is also made whereby one component can be reduced by catalytic hydrogenation and then added to the body of the cell under anaerobic conditions.

The apparatus (2) is illustrated in Fig. 1. The total height of the body should be at least 11 cm in order to prevent protein solutions from foaming into the side arm during deoxygenation. The bubbling tube is fabricated in two parts to decrease its fragility, these parts are joined by a section of volttron plastic tubing. The lower part of the bubbling tube is bent to fit in one corner of the body, out of the way of the optical path (see Fig. 1, right); it almost reaches the bottom of the body.

*Anaerobic addition from the side arm.* The main body, side arm, and bubbling tube (Fig. 1, left) are used. One component necessary for the initiation of the reaction is placed in the side arm either by injecting it through stopcock *A* by means of a hypodermic

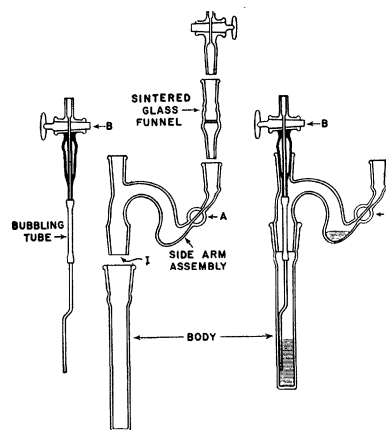


Fig. 1. Component parts of anaerobic Beckman cell.

needle or by inverting the side-arm assembly and carefully pipetting it through opening *I*. The other components are placed in the body. The joints are lubricated and the apparatus is assembled as shown in Fig. 1, right. Oxygen-free nitrogen or helium, prepared by passing the gas through heated copper screens, is bubbled through the cell. After 10 min, stopcock *A*, and then stopcock *B*, are closed, and the cell is transferred to the spectrophotometer.

Owing to the height of the assembly, it is necessary to build a light-tight box that fits over the spectrophotometer cell compartment mounting block, carriage, and phototube compartment. After a zero reading is taken, the contents of the side arm are tipped into the body and the reaction is started. Readings are taken in the usual way. It is possible to run two anaerobic cells simultaneously by placing them in positions 1

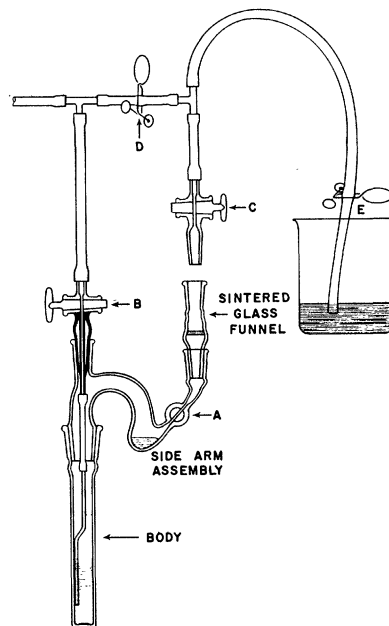


Fig. 2. Anaerobic Beckman cell assembled.

and 3 of the cuvette holder. Standard cuvettes containing appropriate blanks can be placed in positions 2 and 4.

**Catalytic hydrogenation.** The sintered glass funnel is used in the side-arm assembly, and the manifold is connected to the anaerobic cell as illustrated in Fig. 2. With all pinchclamps and stopcocks open, the manifold system is flushed for a few minutes. Stopcock *A* is then closed while the nitrogen continues to flow through stopcock *C* and tube *E*. The catalyst (platinum black, palladium black, and so forth) and the solution to be reduced (0.2 ml maximum) are then placed on the sintered disk, and stopcock *C* is inserted into the upper joint of the sintered glass funnel. Pinchclamp *D* is closed and stopcock *A* is immediately opened, thus shunting the flow of nitrogen through the cell, up through the sintered glass funnel, and out tube *E*. When reduction is complete, stopcock *B* is closed, pinchclamp *D* is opened, and the pinchclamp on tube *E* is closed, in that order. The pressure builds up and forces the solution through the sintered glass plate, through stopcock *A*, and into the side arm (3). When all the solution has been filtered, pinchclamp *E* is opened, stopcock *A* is closed, and the sintered glass funnel is removed. The subsequent steps are carried out as previously described.

This apparatus has been used successfully to study the enzymatic reduction of cytochrome oxidase. Since this enzyme is readily reoxidized by small amounts of oxygen (4), and since no difficulties were encountered in maintaining this enzyme in its reduced state, adequate anaerobic conditions are provided by this assembly.

#### References and Notes

- \* Present address, Department of Anatomy, University of Minnesota Medical School, Minneapolis.
- 1. T. P. Singer and E. Kearney, *J. Biol. Chem.* **183**, 409 (1950).
- 2. Obtainable from A. F. Bittel, 368 E. 123 St., Cleveland 8, Ohio.
- 3. On forcing the solution through the sintered plate, a positive pressure of 8 mm-Hg is built up in the anaerobic cell.
- 4. E. G. Ball, C. F. Strittmatter, and O. Cooper, *J. Biol. Chem.* **193**, 635 (1951).

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## New Electrophoresis Cell for Dual Analysis

Harold Corey and Irwin Oreskes

*Department of Physical Chemistry,  
Division of Laboratories,  
Jewish Chronic Disease Hospital, Brooklyn, New York*

Most of the cells in use today for moving-boundary electrophoresis are based on the design and principles of Tiselius (1). These cells are of two types. One, following Tiselius' original design, consists of a sliding sectional U-tube and detachable electrode vessels. The other type, which has come into use in recent years, is a one-piece cell. The essential feature of both cells is that the U-tube has vertical limbs of rectangular cross section with optically flat faces and electrode ves-

sels attached to both of its sides. In the one-piece cell there are no sliding sections, and the boundaries are formed by careful layering rather than by shearing.

One disadvantage of moving-boundary electrophoresis is that, with the cells currently available, only one experiment at a time can be performed. Since from 1 to 3 hr is required for an experiment, the usefulness of the method is limited by the amount of time required per run. To overcome this difficulty, many investigators have resorted to paper electrophoresis, which has sufficient capacity for multiple simultaneous runs. However, this method is not always a satisfactory substitute, because its resolution of components is often inferior to that obtained with the moving-boundary technique, and the determination of mobilities is subject to various correction factors (2).

Electrophoretic cells capable of multiple analysis have been developed by Polson (3). These employ the center section of a sliding sectional cell and a semi-permeable membrane across the bottom of the channels. Apparently only descending patterns can be obtained with these cells.

In the course of a project performed at this laboratory it was found necessary to do pairs of electrophoretic analyses under as nearly identical conditions as possible. To satisfy these conditions, we have developed the dual analysis cell described here. Because of the relative ease of filling and handling, the one-piece

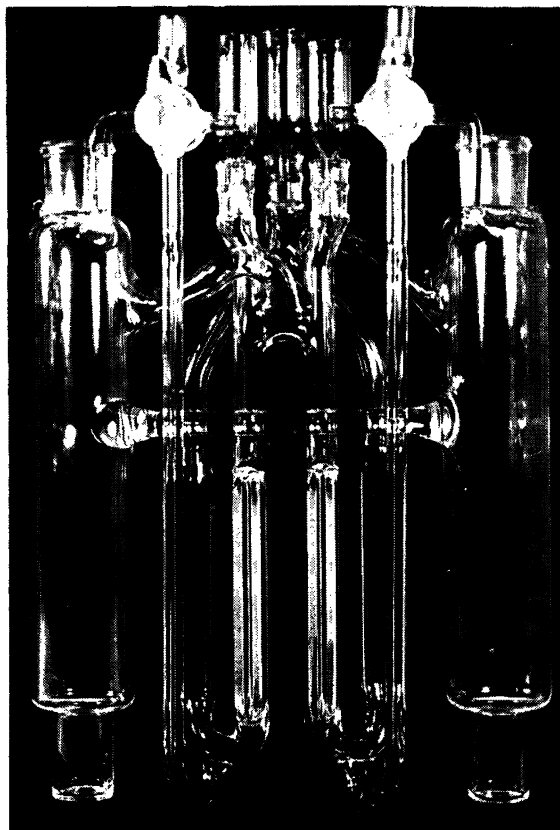


Fig. 1. The dual electrophoresis cell.