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A CONVENIENT MICROELECTROPHORESIS ASSEMBLY

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DISCLAIMER

Electrophoresis performed by means of the microscope method provides a sensitive technique for determining the surface charge of living cells and other biological colloids (BRINTON and LAUFFER, 1959; AMBROSE, 1965; WEISS, 1969; TENFORDE, 1970). Microelectrophoresis has also proved valuable in clinical studies on cellular surfaces in normal and pathological states (RUEFF et al., 1963; VASSAR, 1963; AMBROSE, 1967). Following the original development of a microelectrophoretic apparatus by NORTHROP (1921-22) and by KUNITZ (1923-24), a number of designs have been utilized in different laboratories (BRINTON and LAUFFER, 1959; GITTEN and JAMES, 1960; NADELL and CREGER, 1962; RUEFF et al., 1963; SEAMAN, 1965; SHER and SCHWAN, 1965). In this paper, a microelectrophoresis system is described that has several advantageous features:

(1) rapid assembly and cleaning;

(2) convenient sample input;

(3) freedom from bubbles and air leaks that promote particle drift within the microelectrophoresis cell;

(4) $Zn/ZnSO_4$ electrodes that are operationally stable at high currents;

(5) buffer chambers to prevent Zn^{++} and SO_4^{--} ion migration into the microelectrophoresis cell.

The basic design is shown in Fig. 1. The dimensions presented here are suitable for use with the commercially available Zeiss Cytopherometer (Carl Zeiss Inc., New York), but can easily be modified to accomodate other forms of microelectrophoresis equipment. The system shown in Fig. 1 can be assembled within a few minutes prior to an experiment, and disassembled and cleaned with running tap water immediately after use. The components of this design are described in the following paragraphs.

ELECTRODE CHAMBERS

The electrodes consist of zinc posts (A in Fig. 1; Arthur H. Thomas Co., Philadelphia, Pa.) immersed in saturated $ZnSO_4$ solutions. The commercial zinc electrodes are milled down to a diameter of 3/16 inch, and a tapered ring of silicon rubber (B in Fig. 1) is then permanently molded onto each electrode post. This sleeve forms a friction seal when the electrode is inserted into the end of a $\frac{1}{2}$ inch i.d. pyrex glass tube that serves as the electrode chamber (C in Fig. 1). The zinc posts are sanded before each run to remove oxidation products from their surfaces. The $ZnSO_4$ solution is introduced by means of a short pyrex tube (D in Fig. 1) annealed to the top of the electrode chamber. After filling, this tube is closed off with a rubber plug (E in Fig. 1). The electrode posts are fitted with L-shaped BNC connectors (F in Fig. 1), and the leads attached to a polarity reversing switch placed in series with a constant current power supply (for example, Model C631, Electronic Measurements, Eatontown, N. J.). Inclusion of a polarity reversing switch allows particle velocities to be measured with the applied electric field in alternate directions, thereby averaging out any component of the velocity associated with mechanical fluid drift in the microelectrophoresis cell.

BUFFER CHAMBERS

In order to prevent migration of Zn^{++} and SO_4^{--} ions into the sample chamber, a side arm tube containing the appropriate electrophoresis buffer solution (G in Fig. 1) is connected to the electrode chamber. This buffer chamber and the $Zn/ZnSO_4$ electrode chamber are constructed from the same $\frac{1}{2}$ inch i.d. pyrex filter tube. Separation of the solutions is maintained with a "fine" porosity sintered glass disk (H in Fig. 1). Although Fig. 1

indicates horizontal electrode chambers, which are specially adapted to the Zeiss Cytopherometer rotating stage, inverted vertical electrode chambers have also been constructed. The latter configuration prevents convection of the more dense $ZnSO_4$ solution into the buffer chambers. For purposes of filling, each side arm buffer chamber is connected to a thistle tube (I in Fig. 1) by means of a two-way teflon stopcock (J in Fig. 1). The effectiveness of the buffer chambers in preventing contamination of biological samples with Zn^{++} has been studied by means of atomic absorption spectrophotometry. Under normal operating conditions, the maximum level of Zn^{++} in the sample chamber is less than 0.05 ppm.

SAMPLE CHAMBER

The sample chamber containing the microelectrophoresis cell is connected to the side arm buffer chambers through three-way teflon stopcocks with 2 mm bores (K in Fig. 1). In order to reduce convective mixing at the stopcocks, the side arm buffer solutions are made approximately 10% wt/vol with dextrose. The permanently mounted microelectrophoresis cell (L in Fig. 1) is attached to the right and left hand electrode assemblies by means of 10/30 ground glass joints (M in Fig. 1). The female part of each joint is connected to the microelectrophoresis cell with 1/8 inch i.d. rubber tubing (N in Fig. 1). When setting up for operation, the filled electrode assemblies are mounted and the ground glass joints sealed with silicone vacuum grease. Stable support for the two electrode assemblies is provided by mounting plates constructed from $\frac{1}{4}$ inch lucite (O in Fig. 1). With the Zeiss Cytopherometer, these plates can be permanently connected to the rotatable stage by means of wing nuts. Attachment of the electrode assemblies to the lucite plates can be made either with plastic straps, or by snapping each electrode assembly into several specially machined lucite clasps (P in Fig. 1).

SAMPLE INPUT

The microelectrophoresis cell is filled by way of a conical centrifuge tube (Q in Fig. 1) permanently mounted above the three-way stopcock of the right hand electrode assembly. An outflow tube (R in Fig. 1) is attached to the three-way stopcock of the left hand electrode assembly. In order to flush out bubbles, a rubber squeeze bulb is placed on the centrifuge tube and pressure exerted to force solution through the sample chamber at a rapid flow rate. The electrophoresis cell can initially be filled in this manner using a buffer solution, and the biological sample then introduced. The total sample volume required is approximately 3 ml. After filling, the three-way stopcocks are turned to connect the sample chamber with the side arm buffer solutions. Between measurements, the microelectrophoresis cell can be cleaned by running through several volumes of liquid bleach, followed by distilled water and buffer. More thorough cleaning can be accomplished with chromic acid.

OPERATIONAL STABILITY

For maintenance of operational stability at high currents, the $Zn/ZnSO_4$ electrode system has proved in our experience to be superior to platinum and Ag/AgCl electrodes. The electrodes described here can be operated at an applied current of 10 mA without the onset of instability in the form of electrode gassing. Platinum electrodes with a comparable surface area become unstable at currents in excess of 2 mA, and Ag/AgCl electrodes at currents greater than 5 mA.

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FIGURE CAPTION

Fig. 1. Photograph of complete microelectrophoresis system. Letters refer to components described in the text. Inset at upper left shows the Zeiss Cytopherometer with electrode assemblies attached by means of lucite plates. Rectangular microelectrophoresis cells can also be obtained from the Arthur H. Thomas Co. (Philadelphia, Pa.) under the trade name "Cataphoresis Cell A."

FIGURE 1