METHODS

A Microelectrophoresis Chamber of Small Volume for Use with Biological Systems

By G. V. F. SEAMAN AND D. H. HEARD

THE B10° cylindrical chamber equipped with grey platinum electrodes described by Bangham et al.[1] requires about 10 ml. of fluid per mobility determination. The platinum electrodes of this chamber are of sufficient surface area to prevent appreciable polarization when used in suspending media of high ionic strength (0.1 to 0.2). The B7 cylindrical chamber[1] requiring about 3 ml. of fluid can however only be used for suspending media of ionic strength < 0.02, since the reduction in area of the platinum electrodes necessitated by the smaller volume of the end compartments leads to a high current density (amps $cm.^{-2}$) with consequent polarization at the electrodes in suspending media of ionic strength 0.1 to 0.2. For some biological systems which are available only in small quantity, even a chamber of volume 3 ml. is too large. With these difficulties in view, a cylindrical microelectrophoresis chamber of capacity < 1 ml., using reversible Ag/AgCl/KCl electrodes, has been devised. The apparatus is intended to be used for the examination of blood and tissue cells.

Reversible Ag/AgCl electrodes were chosen in preference to $Zn/ZnSO_4$[2] or $Cu/CuSO_4$[3] electrodes because of the risk of contamination of the biological fluids by copper or zinc ions. Loveday and James[4] describe a rectangular apparatus which uses Ag/AgCl electrodes, with the plaster of paris plug of the earlier apparatus[5] replaced by a sintered glass disc, but this apparatus could not normally be used for serum or tissue cell studies because of the relatively large volume of material required per mobility determination.

EXPERIMENTAL

The Microelectrophoresis Tube

The tube consists of a thin walled pyrex glass of refractive index 1.47 ± 0.01. The thickness of the wall of the tube should be about 0.6 mm.; the internal diameter (diameter of the bore) may have any value from 2.0 to 2.4 mm. provided that it is uniform to within ± 0.01 mm. throughout the length of the bore. A tube length of about 120 mm. is recommended for the elimination of significant end effects, such as electroendosmotic perturbation or leakage of potassium chloride through the sintered discs. A B7† pyrex socket is fused onto each end of the tube coaxial with this tube and B5‡ or B7 pyrex sockets fused onto each end at right angles to the tube for filling purposes (fig. 1). The viewing region is an optical flat about 2 cm. in length, formed on the

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°Equivalent to 10/18 American standard taper designation.
†Equivalent to 7/15 American Standard taper designation.
‡Equivalent to 5/12 American Standard taper designation.

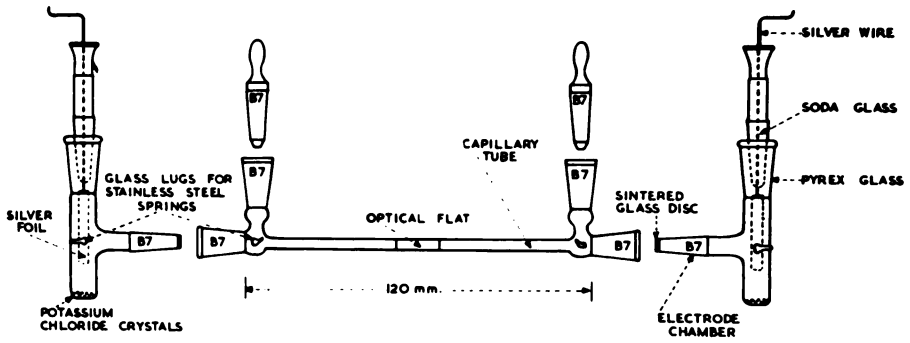

Fig. 1.—Diagram showing the essential features of an all glass microelectrophoresis chamber of small volume incorporating Ag/AgCl/KCl electrode systems.

capillary wall. In order to enable the use of x40 objectives of short working distance, the wall thickness in the viewing region is reduced to a value of about 0.05–0.10 mm. measured at the point of minimum thickness of the capillary wall.

Preparation of the Optical Flat

The optical flat is formed on the outer wall of the pyrex tube using a machined steel block (width 2 cm., depth 1.5 cm., length 15 cm., surface ground plane to within ± 0.001 mm.) as a grinding tool. Two aluminum rings are clamped 2 cm. apart on the glass tube, so as to act as guides during the grinding of the glass tube on the tool. Silicon carbide powders of 100, 320 and 600 mesh (British Standards Institution sieve sizes) are used in succession on the grinding tool to remove the portions of glass labeled A, B and C in figure 2. The resulting finely ground plane glass surface is polished by means of rouge on a lap of Swedish pitch formed on a machined steel block. Care should

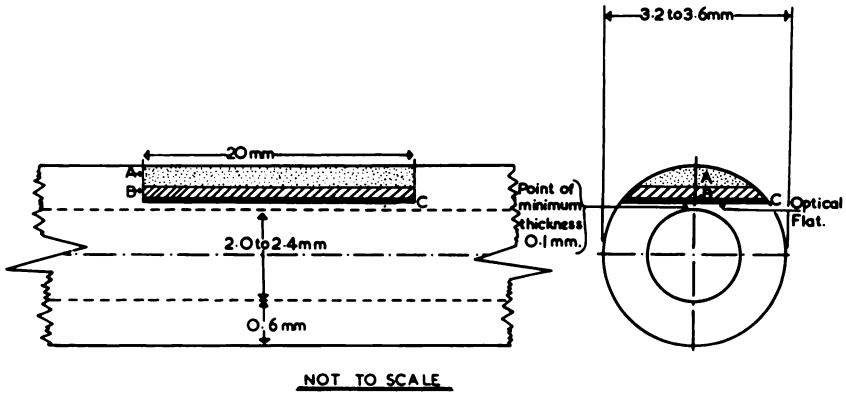

Fig. 2.—Preparation of an optical flat on a pyrex tube to be used as a component for a microelectrophoresis chamber. A. Depth of glass removed with 100 grade silicon carbide (about 0–30 mm.). B. Depth of glass removed with 320 grade silicon carbide (about 0–15 mm.). C. Depth of glass removed with 600 grade silicon carbide (about 0–05 mm.).

be taken to ensure a uniform pressure between glass tube and grinding or polishing tool; otherwise the resulting plane surface will not be parallel with the axis of the glass tube. A micrometer screw gauge should be used across the center and ends of the flat during preparation to ensure that it is parallel to the axis of the tube.

The Electrode Compartments

The electrode compartments comprise a B7 socket fused to a vertical pyrex glass chamber for holding the electrodes and a B7 male joint fused to the center of this chamber at right angles to the B7 socket. Circles of 5 mm. diameter were cut from a G3 or G4 Jena Glass sintered glass disc, cemented on the tips of the B7 male joints with Araldite epoxy resin and ground down to a thickness of about 0.5 mm. An average pore size of 10 to 20 μ for the sintered glass discs gives the optimum conditions for minimal resistance and minimal leakage of potassium chloride from the electrode chambers. Since the pore size ranges are 15 to 40 and 3 to 15 respectively for G3 and G4 sintered glass discs, a suitable disc must be obtained empirically. The electrodes comprise a cylinder of silver foil (3 x 2 x 0.013 cm.) welded to a 1 mm. diameter silver wire sealed onto a B7 tailed soda glass joint by drawing down the joint until the silver wire is a "push" fit and then pouring molten Apiezon Vacuum sealing wax W down to the wire-glass interface via the open end of the glass taper joint. The silver electrodes are anodized in 0.1M HCl using a current density of 2mA cm^{-2} for 15 minutes.[6] The differences in the coefficients of expansion of silver and pyrex glass are too great to achieve a satisfactory seal of silver in a pyrex B7 tailed joint and therefore soda glass has to be used. Saturated AnalaR potassium chloride solution is used as the liquid for the electrode compartments. A layer of crystals of potassium chloride is kept in the base of each electrode chamber to ensure that no appreciable concentration changes occur in the chamber during a mobility determination.

The effective electrical length (l_e) of the microelectrophoretic chamber between the sintered discs was obtained, using standard aqueous solutions of 0.1M, 0.01M and 0.001M potassium chloride at 25 C., from the relationship

$$l_e = \frac{K.V\ \pi\ a^2}{I}$$

where K, is the specific conductivity of the potassium chloride solution used, V, the applied potential, a, the radius of the capillary, and I, the current. The electrical length was found to be effectively constant over the concentration range (0.001 to 0.1M) and to correspond closely to the physical length. The correspondence of the electrical and physical lengths indicates that under these conditions the resistance of the sintered glass discs is small compared with the resistance of the capillary. Provided that an ammeter is incorporated in the circuit to check any spurious resistance effects, it is permissible to obtain the electrical field in volts $cm.^{-1}$ directly from the applied potential and effective electrical length. G4 sintered glass discs having a thickness > 0.5 mm. tend to give a chamber whose electrical length varies considerably with the

concentration of standard aqueous potassium chloride used for the length determination.

The electrode compartments were held in position by means of two pairs of 1" stainless steel springs. The B7 joints between capillary and electrode compartments were sealed with a thin film of Apiezon M grease to obviate risk of electrical leak between the interior of the apparatus and the contents of the water bath. Failure to grease these joints may result in an electrical circuit in parallel with the capillary.

The cylindrical microelectrophoresis chamber is suspended in a water bath as described previously.[1] The cell is filled via the two B5 or B7 sockets (fig. 1). The capacity of the chamber can range from about 0.8 to 1.4 ml. depending mainly on the bore and length of capillary tube selected. The cell holder and all other accessories associated with a mobility determination have been described by Bangham et al.[1]

Experimental Technic and Calibration

The chamber was flushed through with water distilled twice from pyrex ware and the leakage of potassium chloride through the sintered glass discs was measured by means of a Pye "Scalamp" shunted galvanometer, of maximum sensitivity 15.0 mm./μA on the direct reading switch position. The sensitivities on other positions which are shunted are $\times$ 0.05, $\times$ 0.01 and $\times$ 0.001 of the maximum sensitivity respectively. It was found that no significant leakage of potassium chloride occurred during the duration of the mobility determination.

Observations were made at the stationary level;[1] the optical correction of Henry[7] was found to be negligible for a thin walled chamber viewed in water. The source of current was a 120 volt dry battery fed through a variable potentiometer which was adjusted to give a potential field of about 2 volts $cm.^{-1}$ in the microelectrophoretic chamber. Ten observations in each direction were made, the polarity being reversed after each observation, the length of path of an individual particle being adjusted to give a time of traverse of about ten seconds. A stop watch reading to 0.1 second was used. The alignment of the apparatus was checked by the determination of the mobility of washed human erythrocytes in 0.0145M aqueous sodium chloride : 4.5 per cent sorbitol : 3 x 10^{-4}M sodium bicarbonate solution which has been found to be $-2.78 \pm 0.08\mu$ sec. $^{-1}v^{-1}cm.$ in the range pH 6.5 to 7.5.[8] The mobility of red cells was also checked in 0.145M aqueous sodium chloride and was found to agree with the published figure of $-1.08 \pm 0.03\mu$ $sec.^{-1}v^{-1}cm.$[1] These results also confirm that the electrical length of the capillary does not vary with the concentration of aqueous sodium chloride used as a suspending medium.

Serum Studies

In order to check the reliability of the apparatus for serum studies, a set of mobilities for unwashed human erythrocytes in serum was obtained.

Blood was drawn by venipuncture from ten fasting (12 hours) male subjects, of various blood groups in the 20- to 30-year old group, and defibrinated.

The blood was centrifuged and the supernatant serum removed. A small volume of the unwashed, packed erythrocytes was then resuspended in its own serum to give an approximately 0.05 per cent v/v suspension. Mobilities were determined in duplicate for each subject and the value obtained corrected for the relative viscosity of the serum (table 1). The viscosities of the sera, relative to water, were determined at 25 C. by the method of Hardwicke and Squire[9] using capillary microviscometers of internal bore 0.5 mm. and bulb volume 0.5 ml.

Although the effective electrical length of the capillary has been shown to be constant for simple strong electrolytes such as aqueous potassium and sodium chlorides, it is possible that resistances may arise in the sintered discs in the case of serum—as a result for instance of the salting out of protein within the pores of the sintered glass discs. Comparison of this Ag/AgCl system with an apparatus of large volume equipped with irreversible platinum electrodes[1] has shown that the mobilities of red cells suspended in their own serum are similar for the two apparatuses. It is uncertain however that the mobilities obtained can be given a quantitative significance. There are a number of difficulties involved in ascribing values to the ionic strength, dielectric constant, and viscosity of the serum and in addition there is the possibility of a spurious variation in the electrical length.

The agreement between the duplicate mobility values obtained for the red cells in their respective sera show that, under standard conditions, even in a system as complex as serum, reproducible electrophoretic mobilities can be obtained. There is, however, a somewhat greater variation in the electrophoretic mobility from one subject to another.

The apparatus which is now in continuous use is reliable. No difficulty is experienced with thermal convection or leaks from any of the joints. In addition, the small volume of fluid which is needed in this apparatus for a mobility determination makes its use in clinical studies feasible.

Table 1.—*Microelectrophoretic Mobilities of Human Erythrocytes in Their Own Serum Corrected to Unit Bulk Viscosity at 25 C.*

Subject	Viscosity relative to water at 25 C.	Mobility corrected to unit bulk viscosity μ sec $^{-1}$v $^{-1}$cm.			Deviation from the mean (1.41)
		Duplicate values		Mean	
1	1.64	1.37	1.41	1.39	−0.02
2	1.64	1.40	1.41	1.41	0.00
3	1.62	1.43	1.46	1.45	+0.04
4	1.64	1.40	1.42	1.41	0.00
5	1.62	1.34	1.36	1.35	−0.06
6	1.63	1.39	1.40	1.40	−0.01
7	1.69	1.51	1.51	1.51	+0.10
8	1.60	1.37	1.37	1.37	−0.04
9	1.67	1.42	1.41	1.42	+0.01
10	1.72	1.38	1.39	1.39	−0.02

Mean value for the ten cases: 1.41, apparatus error 3 per cent equivalent to ±0.04.

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Summary

A cylindrical microelectrophoresis chamber about 1 ml. in volume incorporating silver/silver chloride/potassium chloride electrode systems separated from the viewing compartment by sintered glass discs is described. The apparatus was designed for biological systems and the determination of mobility of red cells in human serum used as an example of its field of application. The apparatus gives reproducible results in good agreement with previous observations.

Summario in Interlingua

Es describite un cylindriforme camera de microelectrophorese de un volumine de circa 1 ml, con systemas electrodic de argento, chloruro de argento, e chloruro de kalium que es separate ab le compartimento de observation per discos de vitro sinterisate. Le apparato esseva ideate specificamente pro systemas biologic. Le determination del mobilitate de erythrocytos in sero human es usate como exemplo de application. Le apparato produce resultatos replicabile, con valores ben de accordo con previe observationes.

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