[CONTRIBUTION FROM THE BIOLOGICAL LABORATORY, LONG ISLAND BIOLOGICAL ASSOCIATION]

A Vertical Microelectrophoresis Cell with Non-Polarizable Electrodes

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The measurement of the electric mobility of microscopic particles in horizontal electrophoresis cells may be complicated by the settling out of the particles under observation. It would be of advantage to have the cell constructed so that the same particle could be studied for periods even as long as one hour. Furthermore, it is of importance that non-polarizable electrodes be employed. An apparatus of this type in which the electrophoresis cell itself is *vertical* is described in this communication. It should not be construed from this paper that the vertical cell is more suitable for all systems, but it may be of advantage in the investigation of particles similar to those described here.

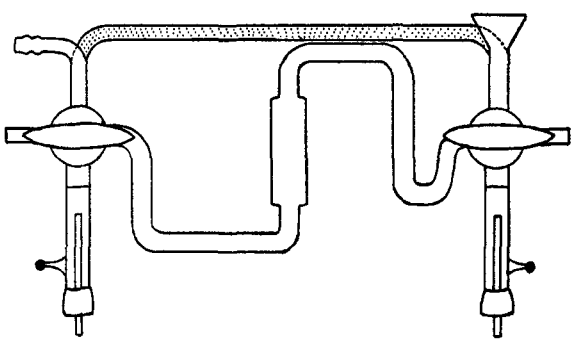

Fig. 1.

Description.—The instrument is constructed and calibrated similarly to that previously described by Abramson[2,3] (a modification of a type developed by Northrop and Kunitz) and discussed recently in some detail by Moyer.[4] As shown in Fig. 1, the apparatus is provided with the arrangement of electrodes developed for the horizontal cell. This arrangement combines the features of non-polarizability and use of plaster of Paris plugs designed to eliminate disturbances at the electrodes due to precipitation of sols. In addition, the instrument is constructed of one piece of glass with no rubber or other connections. This provides a device which may be fixed rigidly onto the stage of the microscope and hence eliminates completely the disturbing vibrations which are so commonly encountered in three-piece instruments. Cleaning is therefore facilitated and the cell may readily be removed for filling or for cleaning. For a detailed description of construction, electrical connections and calibration, see references 2, 3 and 4. The cell is used with the microscope tilted horizontally. The bar running across the top (Fig. 1) is fused onto the (L-shaped bore) stopcock sleeve. This bar (stippled) can then be suspended by hooks, made to fit from strap brass, in front of the objective and clamped to the stage by a spring brass clip. The clip can be adapted to the size and shape of the microscope stage and to glass microscope slides (not shown in the figure) which may be cemented to the horizontal bar and side arms to provide a flat resting surface for the cell against the stage of the microscope. The connections of the cell itself with the two electrodes may be altered to fit the needs of the investigator. Thus, the funnel may be made larger or replaced by other devices for filling; the side arms may be run directly to the cell from the stopcocks, etc. Both the horizontal and vertical instruments may be obtained from Mr. J. D. Graham of Haddonfield. New Jersey. Dimensions of the central chambers of the vertical and horizontal instruments used in these experiments are given in Table I.

TABLE I

DIMENSIONS OF CENTRAL CHAMBERS OF CELLS DISCUSSED

Dimension	Vertical cell, mm.	Horizontal cell, mm.
Length	42	37
Av. depth	0.615	0.795
Width	12.0	12.0
k	20	15

Use of the Cell.—Since the central chamber is of nearly rectangular cross section, the position of the stationary levels may be calculated. Calculation of the stationary levels from Komagata's equation[5] (which has been checked by Komagata as well as more recently by van Gils[6])

$$y_{(v_w = 0)} = \pm b[(1/3) + (128/\pi^5 k)]^{1/2} \qquad (1)$$

where y is the level measured from the central axis, k is the ratio of half the width to half the thickness, b (see Table I for values of k), yields levels which do not differ significantly from 0.2 and 0.8 of the total depth.

Any difficulties encountered in finding the true focus of the ceiling and floor may be obviated by filling the cell with powdered glass or quartz suspended in water and laying it flat for a sufficient time to permit a few particles to stick on both walls.

Since the electrical and gravitational forces operating on the particle are superimposed, the

(1) Research Fellow, Netherland-American Foundation.

(2) H. A. Abramson, *J. Gen. Physiol.*, **12**, 469 (1929); H. A. Abramson and E. B. Grossman, *ibid.*, **14**, 563 (1931).

(3) H. A. Abramson, "Electrokinetic Phenomena," Chemical Catalog Co., New York, 1934.

(4) L. S. Moyer, *J. Bact.*, **31**, 531 (1936).

(5) S. Komagata, *Researches Electrotech. Lab.* (*Tokyo*), No. 348 (1933).

(6) G. S. van Gils, "Electrophorese metingen." Thesis, Utrecht, 1936.

particle, although falling, tends to remain in the same level of the cell with respect to its thickness. If it is at the stationary level it will remain there and may be kept under observation for long periods of time. However a correction must be made for gravitational forces. This is done by timing the particle while moving it in one direction and then reversing the electrical field and timing for the same distance in the opposite direction. The effects of small convection currents, if not large enough to produce turbulence, are apparently also canceled out by this method, although such forces may be largely eliminated at the start by carefully equilibrating both the instrument and solutions to room temperature before filling the cell. Particles which move too rapidly under gravity may set up local turbulence which disturbs the normal mobility of smaller particles in their neighborhood. When such effects occur, letting the apparatus stand for a time without applying the field frequently eliminates the difficulty by allowing the large particles to settle out. Water-cooled lighting devices should be used, of course, for illuminating the sol.

In a given medium large particles are affected more by gravity than small particles of the same substance, even when both have the same electric mobility, so that the electric mobility *appears* to be dependent on size. However, when large and small particles are timed over the same distance, it is found that the differing effect of gravity cancels out and the resultant electric mobilities are not dependent upon size and shape. This is similar to the effects noted by Mooney.[7] Hence it is not sufficient to time a series of particles moving in one direction and then a series in the other and average, but each particle should be timed over the whole distance. For long observation where the eye is kept constantly at the microscope, a metronome may be used instead of a stop watch.

Interesting observations of these effects were made in a system consisting of gelatin-coated collodion particles (about 0.5 μ) and gelatin-coated, long asbestos needles (up to 50 μ). In the horizontal cell, both collodion particle and needle under these conditions migrate with the same mobility in either direction. On the other hand, in the vertical cell the long needles are operated on by a greater gravitational component which creases the observed mobility to such an extent, when particles are falling, that they migrate faster than the collodion particles. On reversing the current, however, so that the particles now migrate against gravity, the asbestos needles experience greater retardation and both particles are thus made to travel over the total distance in the same time.

Table II gives comparative measurements made on a mixed suspension of gelatin-coated collodion and asbestos particles in 0.01 M acetic acid. It will be noted that both types of particles move with the same mobility, irrespective of size, shape or orientation.

TABLE II

TIMES REQUIRED FOR GELATIN-COATED PARTICLES OF DIFFERENT SIZES TO TRAVEL A FIXED DISTANCE IN A CONSTANT ELECTRIC FIELD

	Collodion particles, sec.		Asbestos needles, sec.	
	12.5	11.6	11.8	12.6
	12.3	12.2	12.1	11.8
	11.8	11.2	12.0	12.6
	13.4	12.5	12.2	12.1
	12.3	12.5	12.9	12.5
Average	12.23		12.26	

Comparison with Other Cells.—As far as the writers are aware, the instruments under discussion here are the only ones which have been shown to yield values comparable with those obtained in U-tubes (although Bull[8] checked his cell against streaming potential) and therefore may well be used as standards in measurements of this kind.

The recent measurements of Howitt[9] of the electric mobility of mammalian red blood cells by a moving boundary method in a U-tube afford an additional direct comparison of the validity of the entire method. It had been shown[3] previously that the mobility of various mammalian red blood cells is constant for each species investigated. For instance, the human erythrocyte exhibits the same mobility in isotonic phosphate buffer at pH 7.4 independent of sex, blood group, age or race of the individual. Table III presents data obtained on the red cells of different mammals, suspended in 0.067 M phosphate buffer at pH 7.4. Note the agreement, well within the usual limits of error of the methods concerned.

To demonstrate the symmetry and flatness of the vertical cell, collodion particles were coated with 1% Coignet gelatin, which was also used to coat the walls of the cell. The gelatin was then

(7) M. Mooney, *Phys. Rev.*, **23**, 396 (1924).

(8) H. B. Bull, *J. Phys. Chem.*, **39**, 577 (1935).

(9) F. O. Howitt, *Biochem. J.*, **28**, 1165 (1934).

TABLE III

COMPARABLE MOBILITIES (μ/SEC.) OF RED BLOOD CELLS, FOUND BY DIFFERENT OBSERVERS WITH SEVERAL TYPES OF INSTRUMENTS

	Man	Rat	Rabbit	Mouse	Guinea pig
Moving boundary[9]	..	1.45	0.60	1.42	1.16
Horizontal[3]	1.31	1.45	.55	1.35	1.11
Horizontal[4]	1.30	..	..	..	..
Vertical	1.25	..	..	..	..

diluted to 0.1% by adding acetic acid so that its final concentration was 0.01 M. While keeping the field strength constant, mobilities were determined at various levels in the cell. It is clearly seen in Fig. 2 that no significant differences were

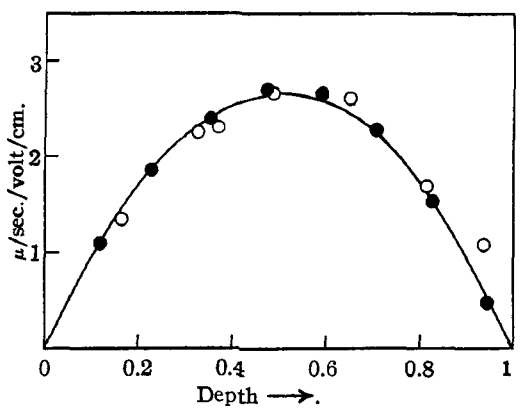

Fig. 2.

observed between measurements made in the two instruments (vertical, open circles, and horizontal, closed circles). The smooth curve has been drawn from the equation

$$V(x) = 10.65\,(x - x^2) \qquad (2)$$

where V is the mobility at any depth, x, after a plot of the data in the form of a straight line, as discussed in a previous paper,[10] showed that the intercept was not significantly different from 0 (*i. e.*, the ratio of the electroösmotic to the electrophoretic mobility was 1.0). It may be observed that the electrophoretic mobility (at the 0.2 and 0.8 levels, = 1.70 μ/sec.) agrees with that found for the asbestos needles in the previous experiment.

(10) L. S. Moyer and H. A. Abramson, *J. Gen. Physiol.*, **19**, 727 (1936).

Discussion

It is of some importance that the needle-shaped particles such as the asbestos ones investigated here assume a position with both ends of the needle at the same electroösmotic level. This is not found in the horizontal cell where needles are frequently observed with one end in one level and the other end at another level. The fact that the needles in the vertical cell thus assume a position with practically no velocity gradient acting upon them makes the vertical cell more suitable for investigations of this type of particle. It should be emphasized, however, that within the limits of time ordinarily adopted for a single experiment and within the limits of the field strengths employed, little or no orientation of these rod-shaped particles occurs. It is very remarkable indeed to see asbestos needles up to 50 microns in length migrate independently of their orientation under these new and more favorable conditions, thus confirming previous experiments.[3] In accord with our previous results[10] on the ratio of electroosmosis to electrophoresis, recently confirmed independently by van Gils,[6] a ratio of 1.0 was found for gelatin-coated surfaces in dilute solutions (here at an ionic strength of about 3×10^{-4} M). Under the seconditions, no motion was ever observed near the wall in either cell.

Summary

The horizontal microelectrophoresis cell has not been adapted to the measurement of particles which settle out of suspension. A vertical cell has been devised which embodies all of the useful characteristics of the one-piece glass cell described previously by Abramson and discussed more recently by Moyer. This vertical cell has been checked by comparing measurements on protein-coated collodion particles, asbestos needles, and blood cells with measurements obtained in a calibrated horizontal cell and a U-tube. Agreement within the usual limits of experimental error was obtained. The advantages and disadvantages of the vertical cell are discussed.

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