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NASA TECHNICAL MEMORANDUM

NASA TM-78269

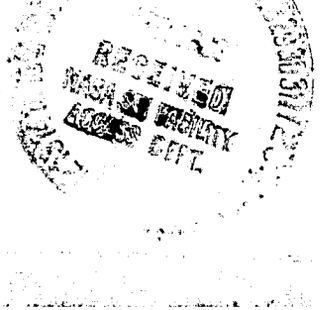
IMPROVED SAMPLE MANAGEMENT IN THE CYLINDRICAL-TUBE MICROELECTROPHORESIS METHOD

By Adam J. K. Smolka

February 1980

NASA

George C. Marshall Space Flight Center
Marshall Space Flight Center, Alabama



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16. ABSTRACT

A modification to an analytical microelectrophoresis system is described that improves the manipulation of the sample particles and fluid. The apparatus modification and improved operational procedure should yield more accurate measurements of particle mobilities and permit less skilled operators to use the apparatus.

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INTRODUCTION

Analytical microelectrophoresis is commonly used to investigate the nature of the ionized groups responsible for the surface charge of biological cells and other particles in aqueous suspension. The method provides a measurement of the electrophoretic mobility of test particles and is based on microscopic observation and timing of particle motion in an applied electric field. The theory and applications of microelectrophoresis have been recently reviewed by Seaman [1].

Briefly, the microelectrophoresis apparatus consists of a glass electrophoresis chamber, either cylindrical or rectangular in cross section, equipped with platinum or silver chloride electrodes; a microscope fitted with a calibrated reticule in the ocular, so that particle migration rate ($\mu m.sec^{-1}$) can be measured; and dial indicators accurate to ± 2 μm for precise positioning of the microscope. The use of a cylindrical chamber is generally preferable for cell electrophoresis since exact solutions are available for the hydrodynamic equations defining the location of the 'stationary level', the region in the electrophoresis chamber where observed mobilities are unaffected by electro-osmosis [2].

Cylindrical microelectrophoresis equipment has been described by Bangham et al. [3], Shaw [4], and Mehrishi [5] and is available commercially from Rank Bros., Bottisham, Cambridge, England. This apparatus, as presently supplied, uses an electrophoresis chamber based on the design of Mehrishi (Fig. 1). The complete Rank microelectrophoresis apparatus, including thermostatted water bath and power supply, is shown in Figure 2.

Some problems have been encountered with the operation of this chamber in the laboratory. Since cell populations show a Gaussian distribution of electrophoretic mobilities, it is necessary to observe and time as large a number of individual cells as possible to derive statistically significant mobility values. At the field strengths used in microelectrophoresis (~ 4 Vcm^{-1}), a single cell timing is approximately 6 sec in each direction. Another 10 sec may elapse before a fresh cell is located at the stationary level (i.e., in sharp focus). Due to gravity-induced sedimentation, no cells are left in the field of view after approximately 10 min, and it is then necessary to resuspend the cells or to introduce a fresh sample into the chamber. The latter processes are time-consuming even with skilled operators. Both sample plugs must be removed and the cell suspension aspirated

through a fine catheter. Next, the chamber should be rinsed with fresh buffer and then resuspended or fresh cells reintroduced, again with a catheter. Finally, the sample plugs must be replaced, ensuring that no bubbles are trapped in the chamber and that a leak-proof seal has been established.

Tightening of the sample plugs imposes hydrodynamic pressures on the enclosed electrophoresis buffer, which results in cell motion even in the absence of the applied field. Careful adjustment of the plugs is necessary to stabilize the cells, leaving less time for the mobility measurements to be taken before the cells sediment out of view. In addition, the continual manipulation of the plugs may disturb the precise positioning of the electrophoresis chamber with respect to the microscope, and further adjustments of the dial indicators may become necessary to ensure accurate results. In routine use under these conditions, the acquisition of mobility data is exceedingly tedious. Each filling of the chamber takes approximately 15 min, so only 80 to 100 data points can be accumulated per hour.

Short of automating cell recognition and timing at the stationary level, which is an elegant though expensive possibility [6], the cell observation phase of microelectrophoresis cannot be speeded up. However, the intermediate phase, i.e., emptying, rinsing, and refilling of the chamber, is a more tractable problem, which we have approached as follows.

ELECTROPHORESIS CHAMBER MODIFICATION

Each of the plexiglas sample plugs was modified by drilling a 2 mm channel down its long axis (Fig. 1). A 12-cm length of Tygon capillary tubing (2 mm OD, 1.5 mm ID) was inserted into this channel from the top of each plug and cemented in place with RTV sealant. The other end of each capillary was connected to a 13-gauge hypodermic needle, which was in turn cemented by its waist into an 8 × 2 × 0.75 cm plexiglas block (Fig. 3). The block was then fixed by a retaining screw to the upper surface of the cast-iron yoke which supports the electrophoresis chamber in the water-bath (Fig. 4). Three-way stainless-steel stopcocks (Becton-Dickinson, MS 11) were fitted into each of the hypodermic needles. Finally, both sample plugs were replaced in the plexiglas end-blocks of the electrophoresis chamber, adjusted to give a proper seal, and left permanently in that position.

A reservoir containing rinsing buffer (0.15 M NaCl, adjusted to pH = 7.2 with 0.5 M $NaHCO_3$) was connected by a length of tubing to the side inlet of one stopcock, and a low-pressure vacuum line and waste reservoir were connected to the same inlet on the other stopcock. The complete assembly is shown in Figure 3.

The Becton-Dickinson stopcocks have three valve positions. In the first position (valve handle vertical), syringes attached to the stopcocks communicate directly with the electrophoresis chamber. In the second position (valve handle 45° from the vertical), the syringes and rinse circulation system are sealed off from the chamber. In the third position (valve handle horizontal), the syringes are still sealed off, but the rinse circulation system communicates with the electrophoresis chamber.

With both valves in position 1, cell suspensions are applied to the chamber by fitting a sample-containing syringe to the Luer-Lok connection on one stopcock (Fig. 4). An empty syringe barrel fitted to the other stopcock receives excess sample and any air bubbles displaced from the chamber. The cells are then observed briefly to ensure there is no drift, and the stopcock valves are pushed to position 2, thereby establishing a closed fluid system within the electrophoresis chamber. Cell mobility measurements are then carried out, at the end of which the stopcock valves are returned to position 1 and the cell suspension withdrawn. Alternatively, the stopcock valves may be pushed to position 3, in which case the cells are flushed out of the chamber into the waste reservoir, and the chamber is rinsed out by the flow of fresh electrophoresis buffer. Five seconds of rinsing are sufficient to remove all cells from the chamber. If cells sediment out of view in the course of mobility measurements, the dc power is switched off, the valves are returned to position 1, and cells in the chamber are resuspended by gently raising and lowering the plunger of the sample syringe. At no time during this cycle of operations are the sample plugs disturbed.

Using the modification previously described, chamber emptying, rinsing, and refilling can be carried out in 30 sec, realizing a considerable saving of time and energy in the collection of mobility data. Since the electrophoresis chamber is not disturbed in any way, and no air can be admitted to the system, there are no problems with cell drifting through maladjustment of the plugs or through occlusion of the chamber by air bubbles. Routine use of the modification in our laboratory has shown that mobility distributions for given cell populations are highly reproducible and can be collected in approximately half the time required when using the unmodified chamber.

The modification is particularly suited for use in conjunction with the automated analytical electrophoresis apparatus designed by Bartels [6], in which 50 cells per minute are identified and measured by a vidicon-computer system, making the intermediate phase of chamber filling and rinsing the major rate-limiting step in the accumulation of data.

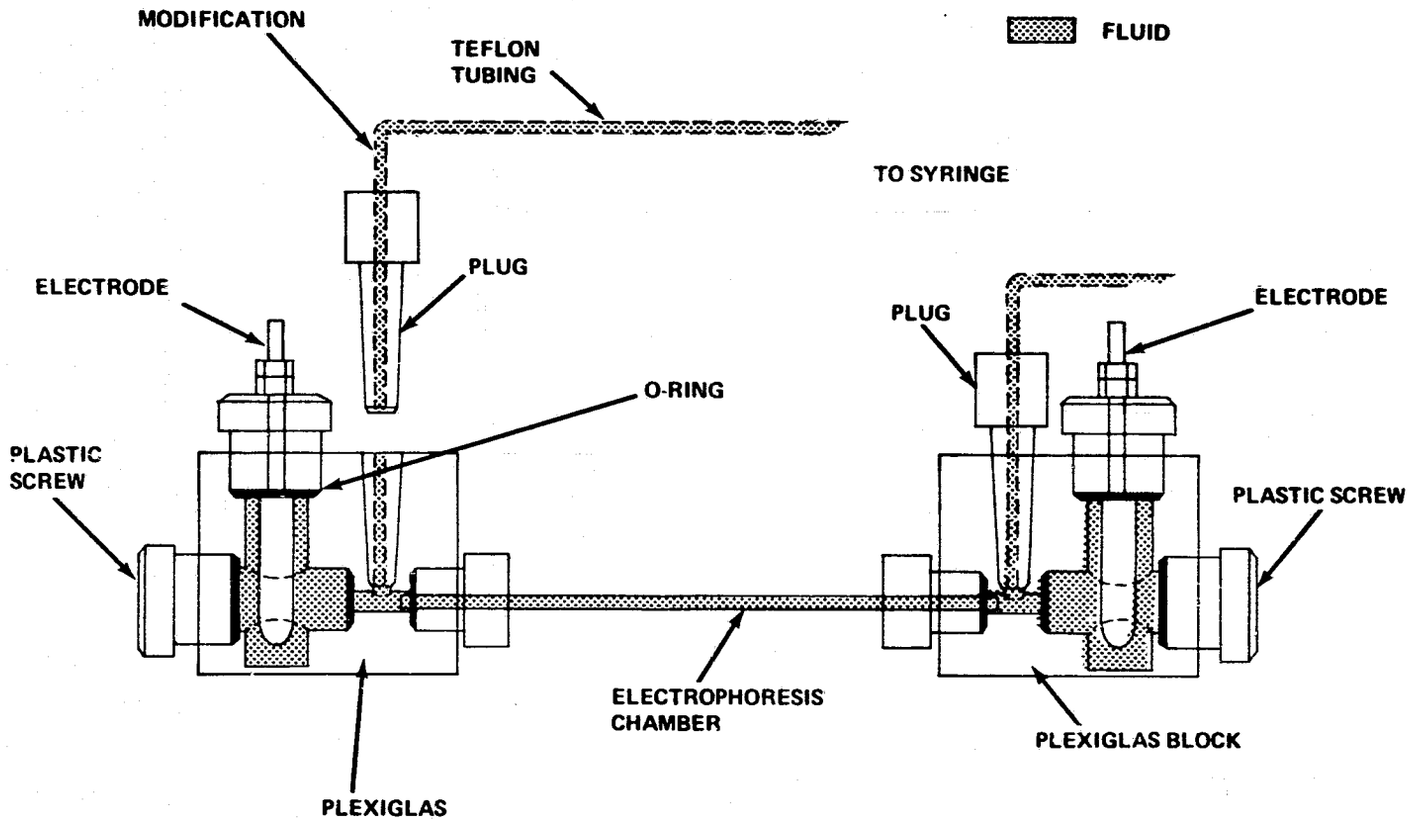

Figure 1. Rank Brothers Microelectrophoresis chamber assembly.

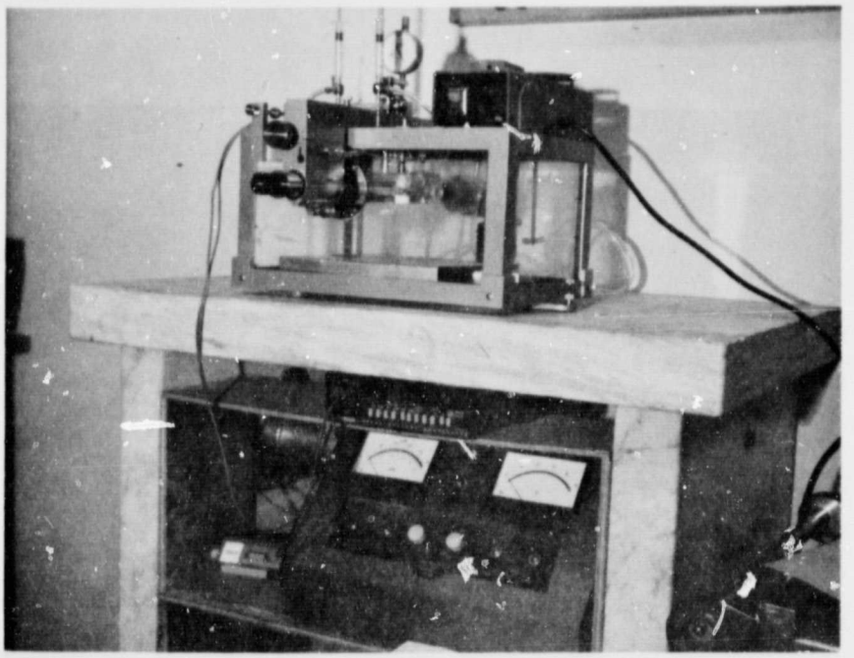

Figure 2. Rank Brothers microelectrophoresis apparatus showing water-bath, microscope assembly, heater unit, and power supply.

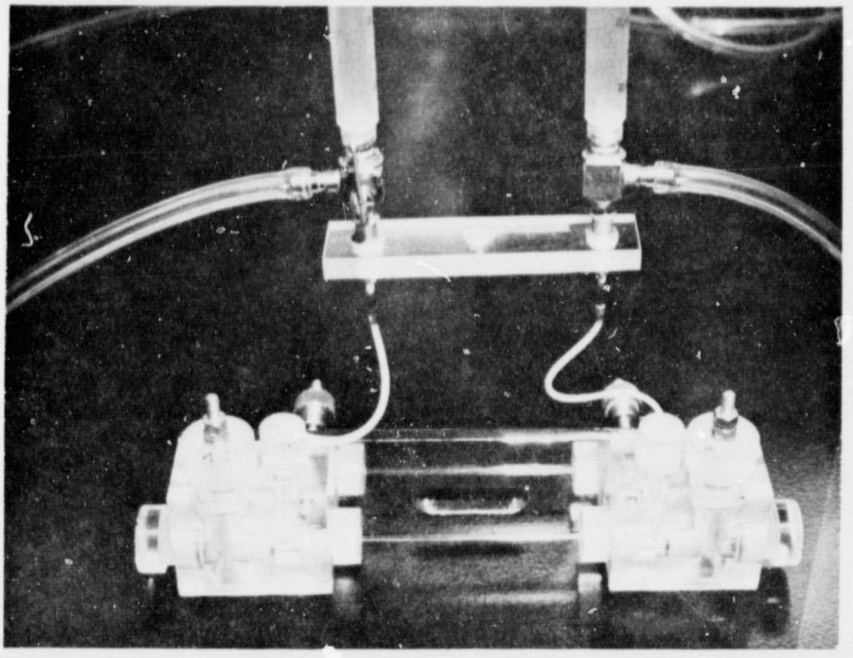

Figure 3. Electrophoresis chamber showing attached modification consisting of plexiglas block with Tygon connecting tubing, stopcocks, and sample application syringes.

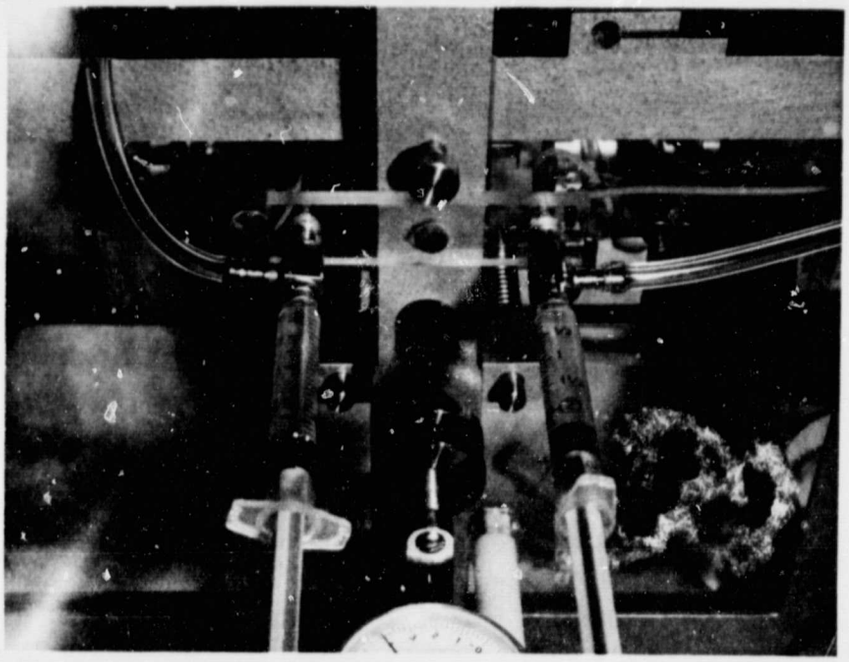

Figure 4. Modification to the microelectrophoresis apparatus mounted in place on the yoke which supports the electrophoresis chamber.

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APPROVAL

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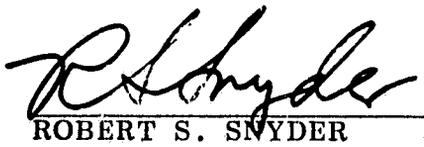
By Adam J. K. Smolka

The information in this report has been reviewed for technical content. Review of any information concerning Department of Defense or nuclear energy activities or programs has been made by the MSFC Security Classification Officer. This report, in its entirety, has been determined to be unclassified.



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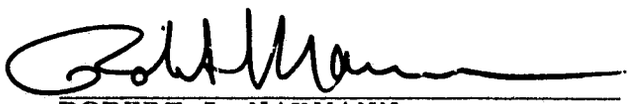

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