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Performance Characteristics of a New Continuous-Flow Electrophoresis Instrument

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Summary

An instrument is described for the continuous separation of particles by means of electrophoresis. The effect of field strength, electrolyte flow rate, and sample flow rate on migration distance and particle band width were investigated. The interrelationships between the various operational parameters and particle band resolution are discussed.

The ζ potential exhibited by inorganic and biological particles has long been recognized as an important physical characteristic. The proceedings of a recent symposium (*1*) amply illustrate the value of this physical property for studying biological cells. ζ-Potential measurements generally have been derived from electrophoretic mobility measurements performed by the classical microelectrophoretic method (*2*). Although this technique has aided in the characterization of cell surfaces, it lacks the ability to utilize differences in the electrophoretic properties to physically separate particles in a liquid medium.

Various continuous-flow electrophoresis techniques have been developed to permit preparative scale separations. These techniques utilize a continuous flow of sample and electrolyte through an electrical field to separate the different types of particles into individual streams or "bands." This development has been impeded by the difficulties in obtaining adequate migration in a practical instrument without impairing resolution. The primary problem has been to minimize thermal convective disturbances

which tend to arise when excessively high field strengths are used. The electrophoresis cell developed by Barrolier et al. (3) and later improved by Hannig (4) utilized low-velocity electrolyte flows coupled with low field strengths. Although good separations are possible with this technique, the low velocity necessarily limits the sample capacity and increases the amount of diffusion. Kolin and Cox (5) successfully suppressed thermal convective disturbances in a serpentine-shaped cell. Kolin (6) also developed a cylindrical cell in which a magnetic field was used to impart a circular motion to the liquid film. Both of these techniques provided good separations but require elaborate machining and careful control of operating procedures. Strickler et al. (7) utilized a rapid electrolyte flow to suppress thermal convective disturbances. The theoretical aspects of this operational mode have been previously published (8). Successful utilization of the rapid-flow technique requires a cell which provides smooth laminar flow characteristics. The electrophoresis cell described herein is believed to provide the required hydrodynamic flow characteristics while retaining a high degree of operational simplicity.

DESCRIPTION

The basic electrophoresis cell design is shown in Fig. 1. Two Lucite plates separated by a 1.5-mm Teflon spacer and sealed by an O-ring are used to define the active electrophoresis area or "curtain." The width of this curtain is 44 mm. After the curtain buffer enters the top of the cell, it flows between the two Lucite plates in a laminar fashion and leaves the cell through a full width 0.8-mm slot near the bottom. An air vent tube is situated in the uppermost portion of the cell to facilitate filling.

The sample is introduced into the cell by gravity feed or by a motor-driven syringe. The stainless steel inlet tube is positioned above the electrical field to prevent the formation of electrolysis products. This position also provides sufficient equilibration time so that high-conductivity samples such as blood may be introduced directly without prior dialysis treatments. Since nearly all particles possess a negative charge, the sample inlet tube is laterally offset to more fully utilize the entire electrophoresis area. A rotatable L-shaped tube is located in the center of the takeoff fixture to permit collection of any desired particle stream.

The two electrode chambers positioned at the rear of the cell

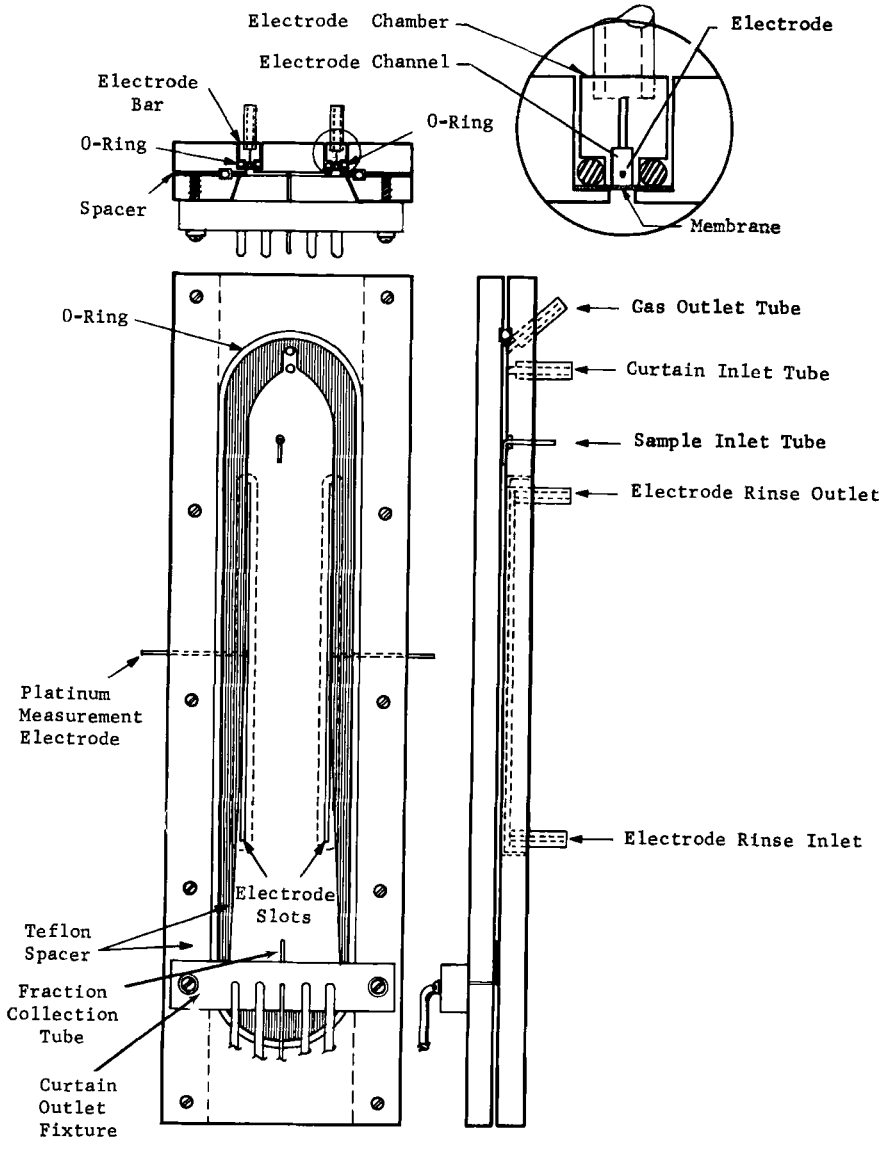

FIG. 1. Electrophoresis cell.

house platinum electrodes 13 cm long. These chambers are sealed by O-rings and physically isolated from the active electrophoresis area by means of cellophane dialysis membranes.

A buffered electrolyte, usually of the same composition as the curtain buffer, is rapidly circulated through the electrode channels to remove electrolysis products. Small platinum sensing electrodes at either side of the active electrophoresis area permit the actual field strength to be measured.

This construction method permits a precise and uniform cell spacing to be obtained even after repeated disassembly. This design differs from previous ones in that the electrode chambers can remain in place while the cell is being cleaned. An additional advantage is that different front plates may be substituted. Besides the front plate shown in Fig. 1, plates containing four fraction pickup tubes, 48 pickup tubes, and cooling fluid channels have been used.

EXPERIMENTAL

A modified Michaelis buffer consisting of 0.002 *M* Beckman B-2 buffer (sodium veronal) and 0.002 *M* sodium acetate was used in all tests. This buffer has a pH of 8.6, an ionic strength of 0.0036, and a conductivity of 250 μmho at 25°C. A colloidal suspension of Prussian blue (approximately 10-ppm concentration) was used as the test sample because of its good visibility. The sample flow rate was maintained constant for each test with a Sage Instruments metering pump. The position of the particle band was determined with the aid of a 10-power microscope mounted on a calibrated moving stage. All migration distance values reported herein represent the average of at least three determinations.

EFFECT OF ELECTRICAL FIELD STRENGTH

According to the Helmholtz–Smoluchowski equation, electrophoretic velocity is directly proportional to the electrical field strength (volts per centimeter). Conformity of the electrophoresis cell to this relationship was determined by varying the applied voltage in 100-V increments up to 600 V (this corresponded to field strengths between 6 and 37 V/cm). The corresponding electrophoretic migration distances of Prussian blue were measured.

The electrophoretic migration distance is plotted against the

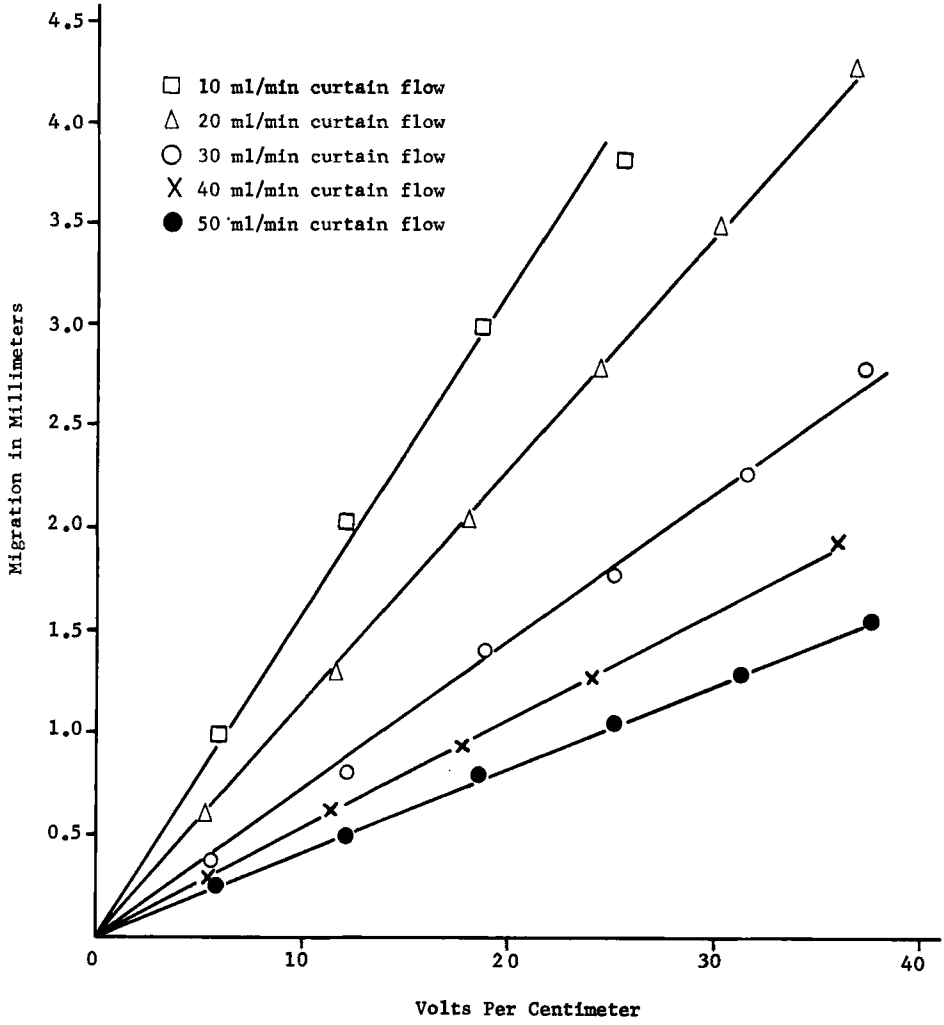

FIG. 2. Effect of field strength on migration.

measured field strength at various curtain flow rates in Fig. 2. It may be seen that a linear relationship between field strength and electrophoretic migration exists at all measured curtain flow rates. This verifies the uniform flow velocity distribution in the active area between the electrodes. This linear relationship makes it possible to normalize and compare migration values obtained at

different field strengths. A convenient method of normalization is to express electrophoretic migration in terms of microns per volt per centimeter.

EFFECT OF CURTAIN FLOW RATE

The data in Fig. 2 indicated that electrophoretic migration increases as the curtain flow rate is decreased. This is due to the increased residence time in the electrical field at lower curtain flow rates. The migration values obtained at various applied voltages for Prussian blue particles are plotted against the reciprocal of the curtain flow rate in Fig. 3. It may be seen that a linear relationship is evident at $1/F$ values of 0.06 or less. At $1/F$ values greater than 0.06 (flow rates below 17 ml/min), lower than expected migration values were obtained. This nonlinearity was found to be due to a change in the flow pattern near the lower end of the cell at low flow rates as a result of curtain narrowing at exit tubes. These data indicate that migration values obtained at different flow rates above 17 ml/min may be compared on an equivalent basis after normalization. Although migration distance is not exactly proportional to the reciprocal of flow rate at flows of less than 17 ml/min, low flow rates are not, of course, precluded from use.

REPRODUCIBILITY

Reproducibility of particle migration values is somewhat difficult to determine precisely because of the large number of factors affecting it. In addition to instrumental errors, measurement errors as well as variations in the ζ potential of the sample also must be considered. In experience gained with this cell over a 10-month period, it has been found that duplicate determinations of migration distance agree to within 3% in over 90% of the runs. On a week-to-week basis, where the cell is disassembled and reassembled, the error is usually less than 3% for inorganic particles and less than 6% for biological particles. Even when repeat measurements are made after a 6-month time interval, migration values have been found to agree within 6%. In no instance did the migration distance of any type of particle vary by more than 10%. Consequently, an error of 6% may be taken as a typical error if reasonable care is taken to prepare the samples in the same manner (use the same sample diluent, culture microorganisms under the same conditions, etc.).

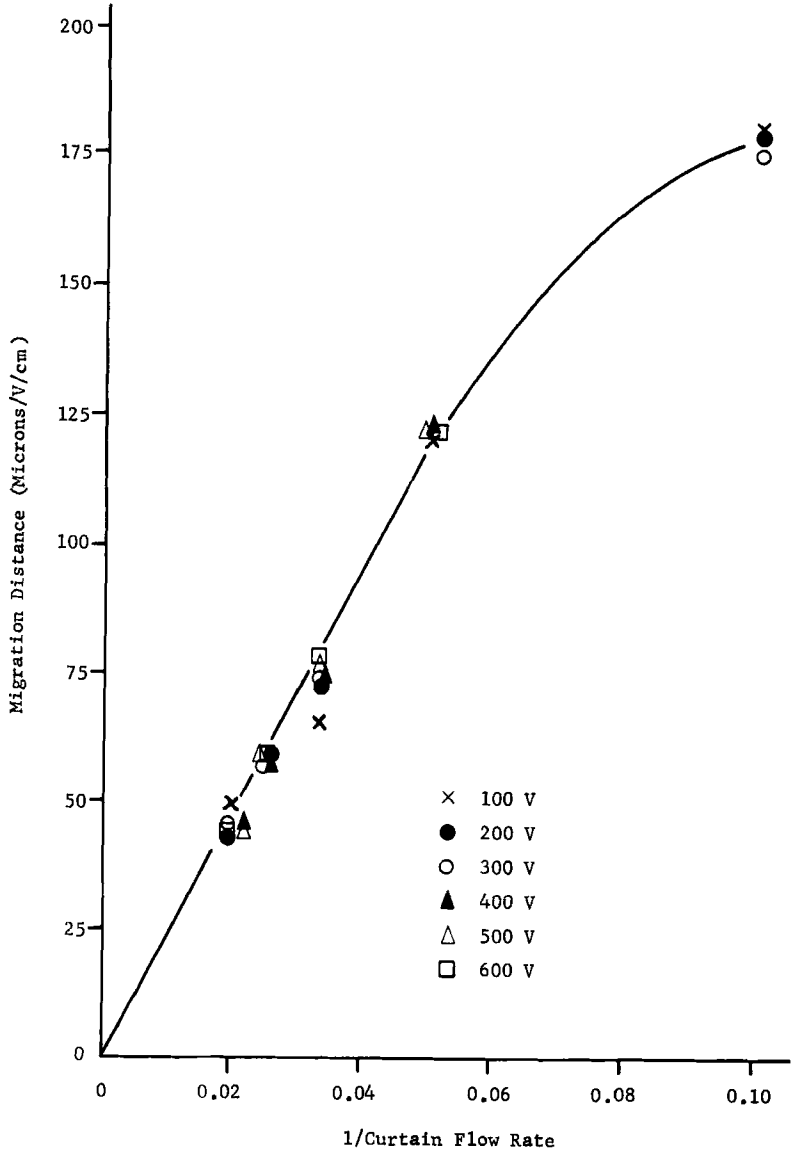

FIG. 3. Effect of flow rate on migration.

PARTICLE BAND WIDTH

The particle band width is primarily influenced by the sample flow rate. Greater sample input rates produce wider bands, thereby reducing resolution. In the absence of extraneous band-broadening effects, the band width theoretically should be proportional to the square root of the sample input rate, since hydrodynamic principles predict the formation of a cylindrical stream when one liquid is metered into a flowing body of another liquid. To determine the magnitude of this band-broadening effect, a colloidal dispersion of Prussian blue particles was metered into the electrophoresis cell with a motor-driven syringe at flow rates between 32 and 230 μl/min. The curtain flow rate was varied between 20 and 60 ml/min, and various applied voltages up to 600 V were used. Preliminary results indicated that higher sample flow rates did not increase the band width if the curtain flow rate also was increased proportionately. Consequently, the data obtained from these studies will be expressed in terms of sample-to-curtain-flow-rate ratio rather than sample flow rate per se.

The particle band width is plotted as a function of the square root of the sample-to-flow-rate ratio in Fig. 4. It may be seen that the square root of moderate-sample-to-curtain-flow-rate ratios is essentially proportional to the resultant particle band width. This is consistent with the assumption that the sample stream is cylindrically shaped and has a cross-sectional area which is proportional to the relative sample input rate. This proportionality appears valid either with or without an electrical field at sample-to-curtain-flow-rate ratios up to at least 4×10^{-3} (corresponding to a sample input rate of 120 μl/min at a curtain flow rate of 30 ml/min). Higher relative sample rates tend to increase the band width more rapidly. This may be due to the formation of an increasingly elliptical sample band cross section at higher sample flow rates.

Although the particle band-broadening trend at an applied potential of 600 V (37 V/cm) is similar to that without an electrical field, the actual band widths are greater. Intermediate voltages produced band widths intermediate in value between the 0 and 600 V values. The band width at 0 V is primarily determined by the relative sample flow rate. Band broadening under conditions of applied voltage is affected not only by the sample input rate, but also by particle charge inhomogeneity, variations in residence time within the electrical field, and electroosmotic effects in the cell. The data

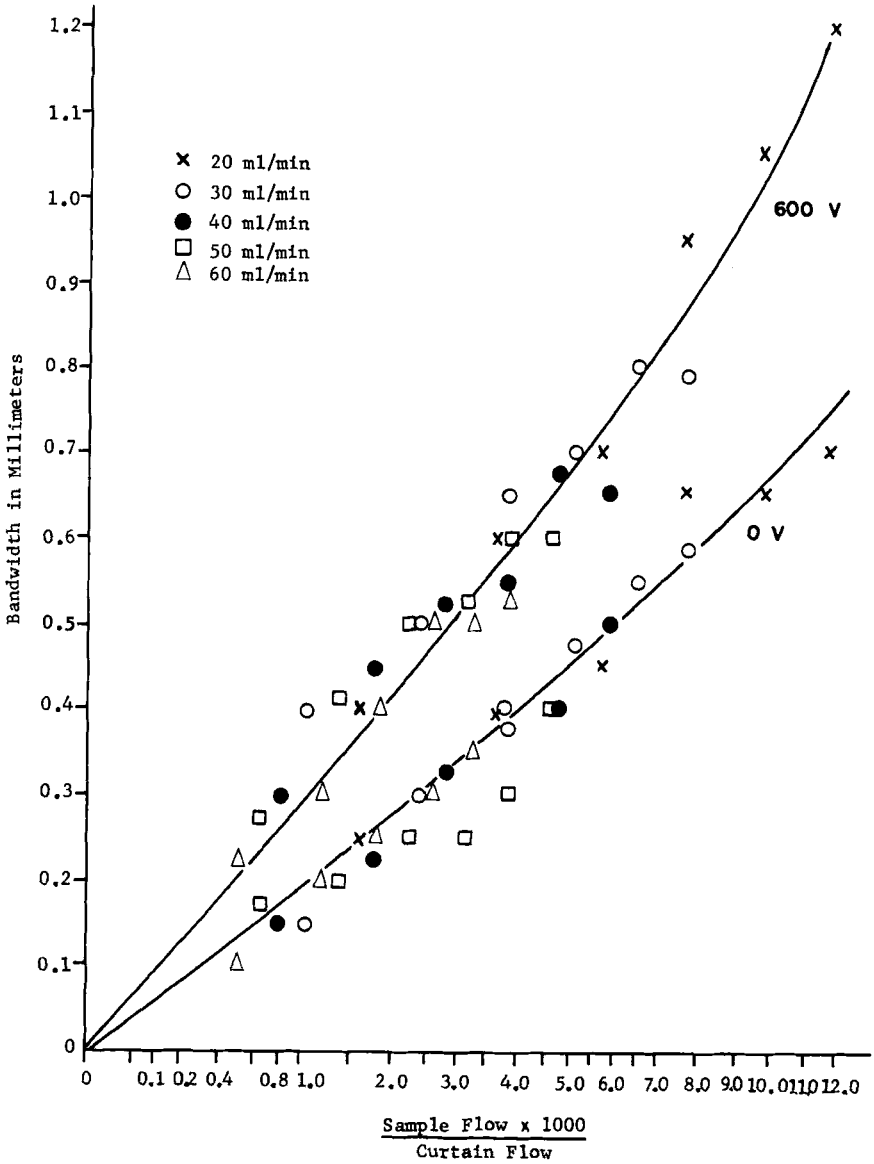

FIG. 4. Effect of sample-to-curtain-flow ratio on band width.

in Fig. 3 indicate that the application of a 600 V potential increases the band width by approximately 50% at a sample-to-curtain-flow ratio of 3×10^{-3}. Thus the combined electrical band-broadening factors are considerably less than the hydrodynamic band broadening attributable to the relative sample input rate. At very high relative sample flow ratios (above 7×10^{-3}) the electrical band-broadening factors increase rapidly. Since the flow–velocity profile between the two cell plates is parabolic, sample particles positioned closer to the walls will have longer residence times and correspondingly greater electrophoretic migration. This results in the larger electrical band-broadening effect at high sample input rates.

RESOLUTION

In electrophoretic separations, as with other separation techniques, it is desirable to have migration distances as great as possible and band widths as small as possible. For discussion purposes, the ratio of migration distance to band width will be termed resolution factor.

It was previously shown that migration distance varies inversely with the curtain flow rate (Fig. 3), whereas the band width varies inversely with the square root of the curtain flow rate (Fig. 4). Consequently, lower curtain flow rates will provide increased resolution factors. This is limited by the curtain flow rate–migration distance nonlinearity experienced at curtain flow rates below 17 ml/min. Thus, a curtain flow rate of approximately 20 ml/min will be optimum for most applications. Where high sample input rate (above 100 μl/min) is desired for some preparative applications, resolution may be increased by using higher curtain flow rates to minimize the velocity profile band-broadening effects experienced with high sample-to-curtain-flow ratios.

Since the particle band width is primarily determined by the sample-to-flow ratio, higher voltages will always permit higher resolution factors to be achieved. Higher voltages result in greater migration with only a small increase in band width if electrophoretically homogeneous particles are tested. At the same time, increased voltages result in higher power dissipation in the cell, resulting in thermal convective disturbances which limit the field strength that can practically be applied. Excessive thermal convection manifests itself by creating random thermal currents which grossly degrade particle band stability. When the cell described here is

operated at a curtain flow rate of 20 ml/min with 0.004 *M* Michaelis buffer, the effects of thermal convection become evident at field strengths of 50 V/cm or higher.

In the present study, Prussian blue particles migrated a total distance of 4.3 mm when a curtain flow rate of 20 ml/min and a field gradient of 37 V/cm were used. These conditions, which are much milder than those required for the onset of thermal convective disturbances, resulted in resolution factors of 3.7 to 10.7 when the sample flow rate was varied between 230 and 32 μl/min.

For preparative applications, it would be advantageous to increase the migration distance to overcome the resolution-degrading influence of high sample input rates. Lower concentration buffer media result in greater migration distances as well as permitting higher field strengths to be applied. The lower limit of buffer concentration is set by pH changes arising from intrusion of electrolysis products and from the sample itself. When the electrophoresis cell described herein is operated with 0.004 *M* Michaelis buffer, a curtain flow rate of 20 ml/min, and a field strength of 37 V/cm, the pH variation across the curtain is less than 0.02 units. One might conclude that the buffer concentration could be considerably reduced before any serious effects from electrolysis product intrusion would occur. In actual practice the effective buffer concentration is influenced by the ionic strength of the sample. When high ionic strength samples such as blood are analyzed, the use of buffer concentrations below 0.001 *M* does not result in increased migration distances, because the ionic constituents of the sample do not diffuse sufficiently to approach the ionic strength of the buffer.

A more effective method of increasing the maximum attainable migration distance is to remove the heat generated in the curtain. When the cell's Lucite front plate was replaced by an aluminum front plate coated with a polyethylene sheet 0.4 mm thick on the inner surface, much higher field strengths were possible before the onset of thermal convective disturbances. It was found possible to utilize a field gradient of 180 V/cm at a curtain flow rate of 20 ml/min without any evidence of thermal convective disturbances. Under these conditions a resolution factor of 25 was obtained when Prussian blue particles were metered into the cell at the rate of 35 μl/min. The increased resolution possible with this "heat sink" approach could, of course, be traded for a higher sample input rate if preparative collections were desired.

APPLICABILITY TO BIOLOGICAL CELLS

Without exception, the effects of curtain flow rate, sample flow rate, field strength, etc., on migration distance and band width previously discussed have been found to apply equally well to biological cells. Typical results are shown in Table 1. These studies were made with sample input rates of 30 μl/min and curtain flow rates of 20 ml/min. Since these studies were made with different buffers and special sample-handling procedures, the absolute values cannot be directly compared to the Prussian blue results. However, it may be seen that a useful degree of resolution is also achieved for biological cells. As expected, particles with lower migration also tend to have lower resolution factors. Although biological samples generally have somewhat lower resolution factors than do inorganic particles, presumably due to greater degree of surface charge heterogeneity, typical resolution factors compare favorably with other separation techniques. In view of the increasing interest in the study of whole cells, it is highly possible that particle electrophoresis may achieve the importance of contemporary separatory techniques such as chromatography, centrifugation, and paper electrophoresis.

TABLE 1

Typical Resolution Factors Obtained with Biological Particles

Type of cell	Field strength V/cm	Migration distance, mm	Band width, mm	Resolution factor
Rat liver nuclei	100	17	2.5	6.8
Human lymphocytes	100	18	3.2	5.6
Human monocytes	100	9	2.8	3.2
Ragweed pollen	30	1.3	0.41	3.2
E. coli	30	1.0	0.30	3.3
B. subtilis var. niger	30	3.5	0.45	7.8

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