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A NEW APPROACH TO SCALING UP ELECTROPHORESIS

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Abstract

Free Flow Electrophoresis (FFE) has been utilized for the separation of proteins and cells for many years, and has evolved into the most promising method of continuous separation. One of the major drawbacks inherent with FFE, however, is the thermal convection due to Joule heating which occurs whenever current is passed through a conducting solution. To provide efficient heat dissipation, the size of FFE units is restricted, which limits sample throughput. A new type of FFE design, which internally cools the separation unit by passing water through capillary tubes, has been developed and tested. Results of separations of dyes are presented, using a bed 1/4 inch thick which maintains efficient cooling.

INTRODUCTION

Free flow electrophoresis (FFE), which was introduced by Barrolier et al. in 1958 (1) and Hannig in 1961 (2), is one of the few methods of continuous multi-component separation. A sample which contains two or more components is continuously fed into the apparatus, separated, and the separated components collected. Recently, there has been a new wave of interest in FFE, especially in the area of biotechnology. FFE has been demonstrated to be

useful for the separation of complex protein mixtures (3-5), cells (6-9), and amino acids (10).

Scaling up FFE, however, runs into the problem of dissipating the Joule heat which is generated by the electrical current passing through the conducting solution. The build up of Joule heat creates thermal convective currents which distort the separation. Several methods have been developed which attempt to overcome this problem. The most successful approach utilizes a carrier solution which is continuously cooled and recycled outside the separation unit (11,12). This method, however, does not allow for continuous multi-component separations, only batch separations.

Capillary zone electrophoresis (CZE) has recently gained popularity as a high performance analytical method which can both complement and compete with HPLC. It is, probably, the most univeral and efficient method for quantitative analytical separations of proteins, peptides, polynucleotides, and inorganic ions (13).

The problem of heat dissipation in CZE is solved by conducting electrophoresis in a capillary tube, which reduces the electric current, provides efficient heat dissipation, and prevents convective mixing. While CZE is an excellent analytical technique, however, two drawbacks preclude it from preparative scale separations.

First, unlike FFE, CZE is a batch method instead of a continuous separation method. Second, the conventional logic of scaling up an analytical method is to increase the dimensions of the separation unit. To scale up a capillary, however, seems to be a contradiction in terms.

The starting point of our approach is the idea that a capillary is not necessarily a tube. A capillary can be thought of as any volume which is open to fluid flow, and where the distance from any point inside the volume to the surface does not exceed a sufficiently small distance δ such that capillarity exists. By aligning a multitude of capillary tubes close enough together, two types of capillary spaces are created: one is formed inside the

independent capillary tubes themselves, and the other is formed in the interstitial space between the tubes where individual capillary channels communicate with each other.

Using this idea, therefore, we have conducted electrophoresis in the interstitial spaces outside a bundle of Teflon capillaries, rather than inside the capillary tubes themselves. The capillaries tubes are used only for cooling (14).

In this paper, we describe preliminary experiments with our modification of FFE, which bridges the gap between CZE and traditional FFE. We call our method Capillary Free Flow Electrophoresis (CFFE).

EXPERIMENTAL

Design of the Apparatus

The separation bed consists of 150 Teflon capillary tubes (0.4 mm I.D., 0.7 mm O.D.) aligned and spaced by woven Teflon mesh, filament diameter 0.27 mm. There are 5 layers of tubes separated and spaced by 6 layers of mesh. The bed is stitched across the capillaries by 4 rows of thin nylon thread to keep the tubes aligned and the mesh in place (Fig. 1A). The bed is 12 mm thick, 136 mm long, and 62 mm wide. As a comparison, a typical FFE chamber is usually 0.5 - 1.0 mm thick; therefore our separation chamber is more than 20 times thicker than a conventional FFE chamber.

The ends of the capillaries are sealed in two chambers (Fig. 1B), so that the assembly of the capillary bed and the chambers forms a heat exchanger. Cooling water enters one chamber, flows through the capillaries, and exits the heat exchanger through the other chamber.

The carrier solution, which can be a buffer or any electrolyte solution, flows in the interstitial space parallel to the capillaries. The flow of the carrier changes direction twice: once when it enters the bed and once when it leaves the bed.

The general design of the apparatus is shown in Fig. 2. The carrier enters the separation chamber through seven carrier inlets.

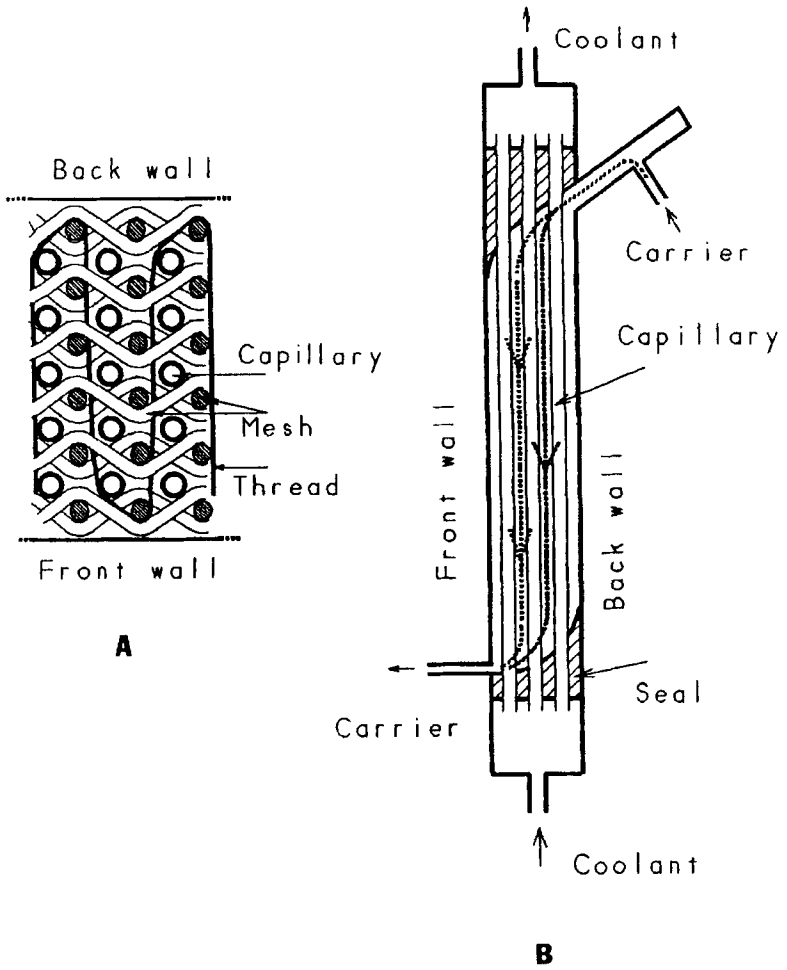

FIGURE 1. A - Cross section of cooling bed showing arrangement of teflon capillaries, nylon mesh, and thread. B - Cross section of CFFE instrument. Only three layers of capillary tubes are shown.

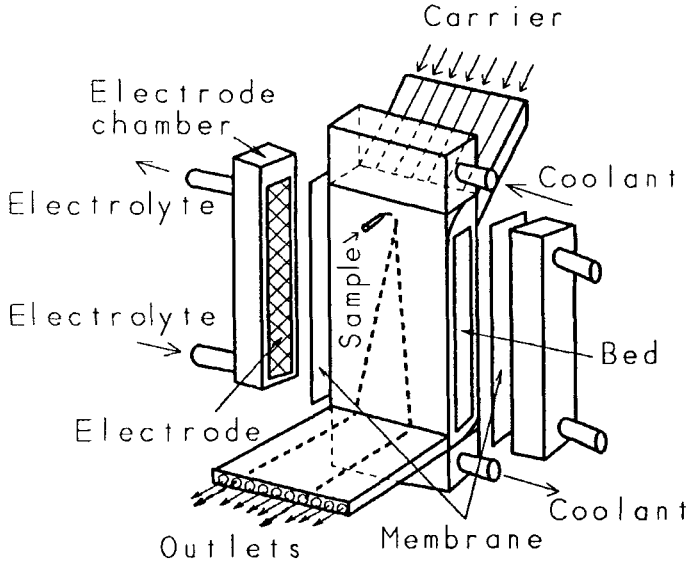

FIGURE 2. Exploded diagram of the CFFE instrument.

Partitions inside the inlet chamber allow stepwise gradients of pH and/or conductivity across the flow. Since the flow is descending, the sloped carrier inlet chamber serves as a bubble trap.

There are five sample inlet positions placed at intervals along the top of the separation bed where the sample can be injected. The sample inlet tube is a piece of narrow diameter teflon tubing with a diagonal cut at the tip, which can be placed in any of the inlets. The tip is positioned so that it is midway between the front and back walls of the separation unit.

The carrier with separated components exits the unit through 44 outlets made from Teflon tubes. All 44 fractions are collected separately and simultaneously. The ends of the outlet tubes are positioned at the same level, slightly above the separation chamber. Therefore, the apparatus is always under some back pressure from the carrier liquid.

The coolant enters through the bottom coolant chamber and exits through the top coolant chamber. Two identical electrode chambers contain electrodes made of stainless steel mesh, and are constantly washed by an electrolyte solution to remove products of electrolysis.

The walls of the apparatus are made of polycarbonate. The separation of colored compounds can be conveniently observed when the bed is illuminated from behind.

Carrier solutions, rinsing electrolytes for the electrodes, and the sample were supplied by three separate peristaltic pumps. The tap water used for cooling was regulated by a flow meter.

Chemicals & Equipment

Amaranth and Patent Blue VF were purchased from Aldrich Chemical Co. Sodium Sulfate was purchased from Fisher. The pH was determined using a Cole-Parmer pH meter with a combination working-reference electrode. Conductivity was determined using a YSI Instruments conductivity meter with a 1 cm conductivity cell. Visible absorbance measurements were made at 635 nm and 521 nm using a Varian Cary 210 UV-Vis Spectrophotometer.

Separation Conditions

Two sample dyes, Amaranth (λ_{max}=521 nm) and Patent Blue VF (λ_{max}=635 nm) were separated under various conditions by CFFE. Patent Blue also has a slight absorption at 521 nm. The sample solution consisted of 0.05% Amaranth and 0.035% Patent Blue VF in deionized water. The sample was injected at the rate of 0.3 mL/min in the center of the carrier flow.

Na_2SO_4 was used as the carrier electrolyte; the conductivity and flow rate was varied for different experiments. The electrode rinsing electrolyte was also Na_2SO_4 with a conductivity of 1500 µmhOs/cm pumped at a rate of 7.5 mL/min through each electrode chamber for all experiments.

Equilibration time was 15 min after the voltage was turned on for each experiment. The time of fraction collection was 7 min.

RESULTS AND DISCUSSION

A typical separation profile at 150 volts is shown in Fig. 3A. The carrier had a conductivity of 500 μmhOs/cm and a flow rate of 15.6 mL/min.

The two dye components are separated as well shaped symmetrical peaks. Such peaks are rarely reported in the literature on FFE. The slight absorption at 521 nm between outlets 18-22 is due to absorption of the Patent Blue at this wavelength. Electroosmotic distortions in the flow profile which occur near the wall (15) are virtually eliminated due to the width of the unit. The plot of separation distance vs. voltage is linear (Fig. 3B); thus the separation occurs in the mode of zone electrophoresis (16). The interference of the bed with the flow, therefore, appears to be very limited.

The conductivity of the buffer solution was then varied while maintaining a constant voltage (Fig. 4A and 4B). As expected for zone electrophoresis, the separation did not depend on conductivity. The current at 250, 500, 1000, and 2000 μmhOs/cm was 0.06, 0.15, 0.3, and 0.65 A, respectively.

The separation was also studied at a constant voltage of 150 V and conductivity of 500 μmhOs/cm at four different flow rates: 10.0, 12.0, 15.6, and 20.0 mL/min. The separation distance is shown to be linear with the inverse of the flow rate (Fig. 5). Therefore, in the zone electrophoresis mode, the behavior of the dyes is completely predictable and agrees with previous FFE theory (17).

The behavior of the carrier itself in the electric field, however, is quite complex. Figure 6 shows the pH and conductivity profile for the separation of the dyes at 150 V, 500 μmhOs/cm, and a flow of 15.6 mL/min. These profiles were not completely predictable and reproducible; nevertheless, the separation was predictable and reproducible. Using phosphate buffers for the carrier electrolytes did not change the situation. When the electrode rinsing electrolytes were changed to acid and alkali for the anode and cathode, respectively, however, the pH profile looked

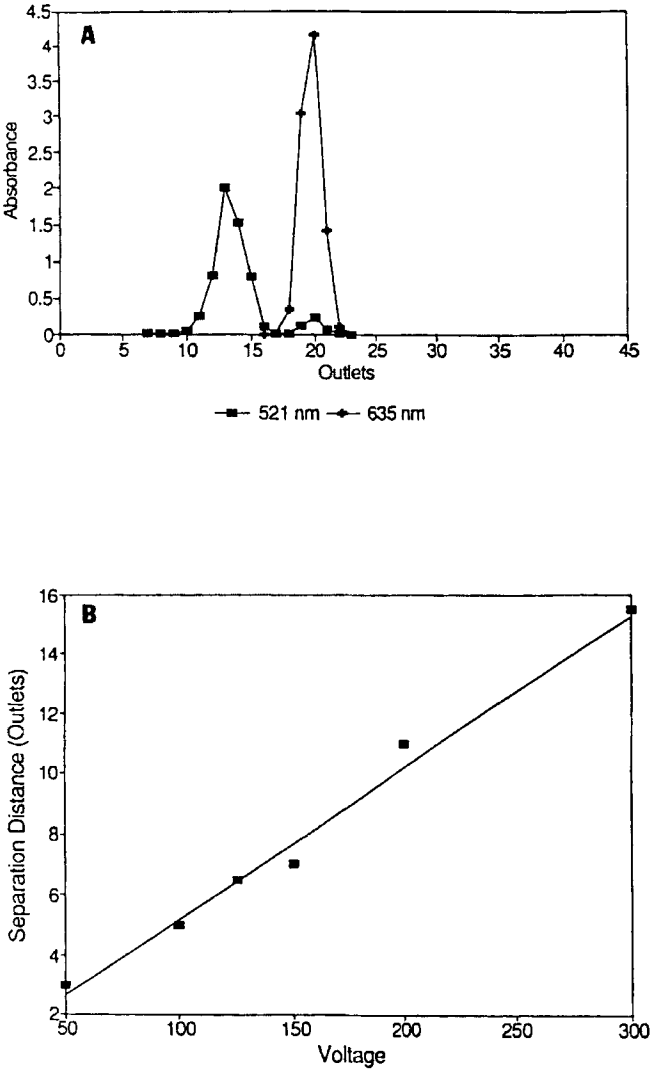

FIGURE 3A. Separation profile of amaranth and patent blue. Conditions: 150 V; carrier flow, 15.6 ml/min; conductivity of buffer solution, 500 μmh0/cm. B - Plot of separation distance vs. voltage for amaranth and patent blue.

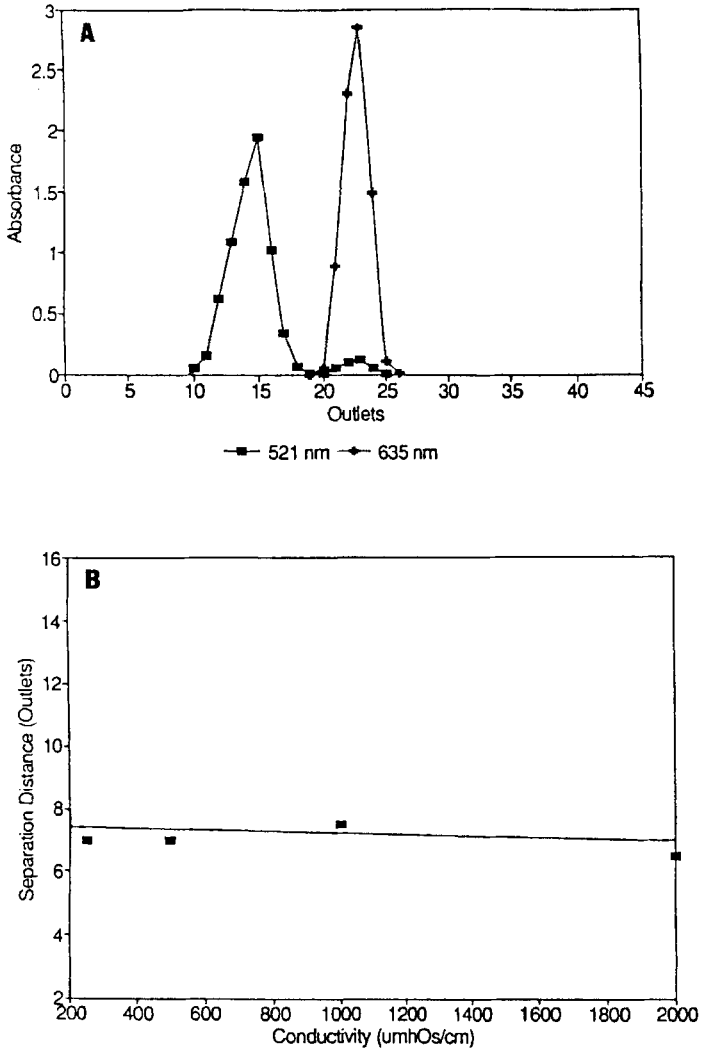

FIGURE 4A. Separation profile of amaranth and patent blue. Conditions: 150 V; carrier flow, 15.6 ml/min; conductivity of buffer solution, 1000 µmhO/cm. B - Plot of separation distance vs. conductivity for amaranth and patent blue.

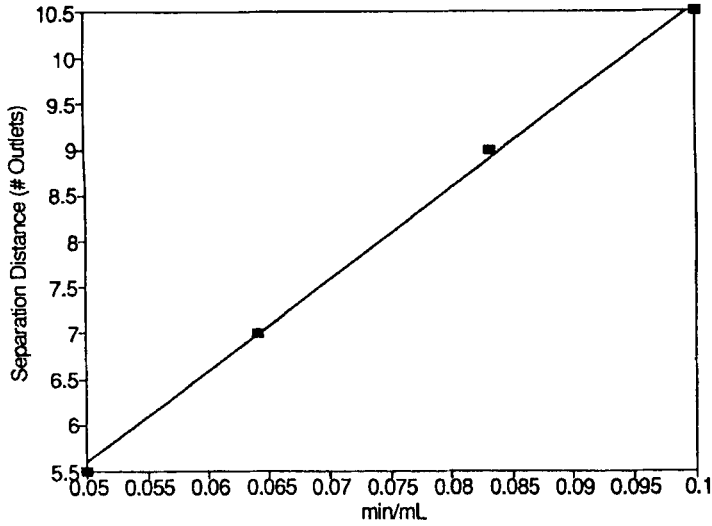

FIGURE 5. Plot of separation distance vs. flow rate^{-1} for amaranth and patent blue.

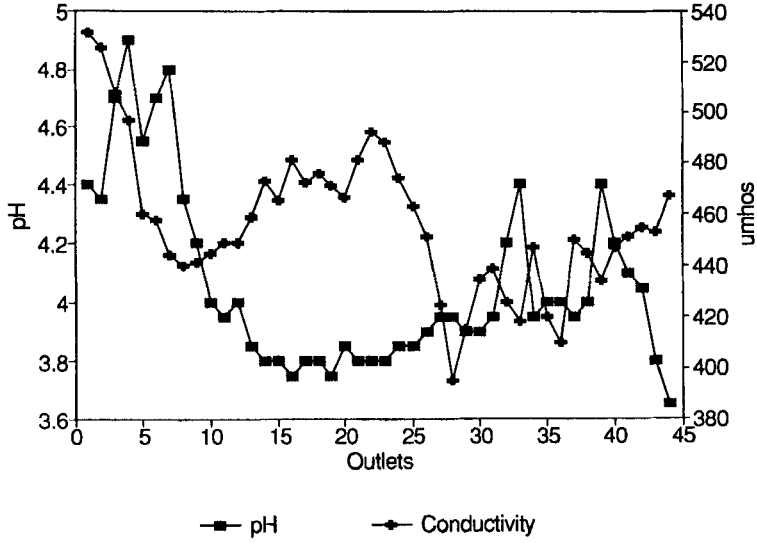

FIGURE 6. Plot of pH and conductivity vs. fraction # for separation of amaranth and patent blue. Same conditions as in figure 3A.

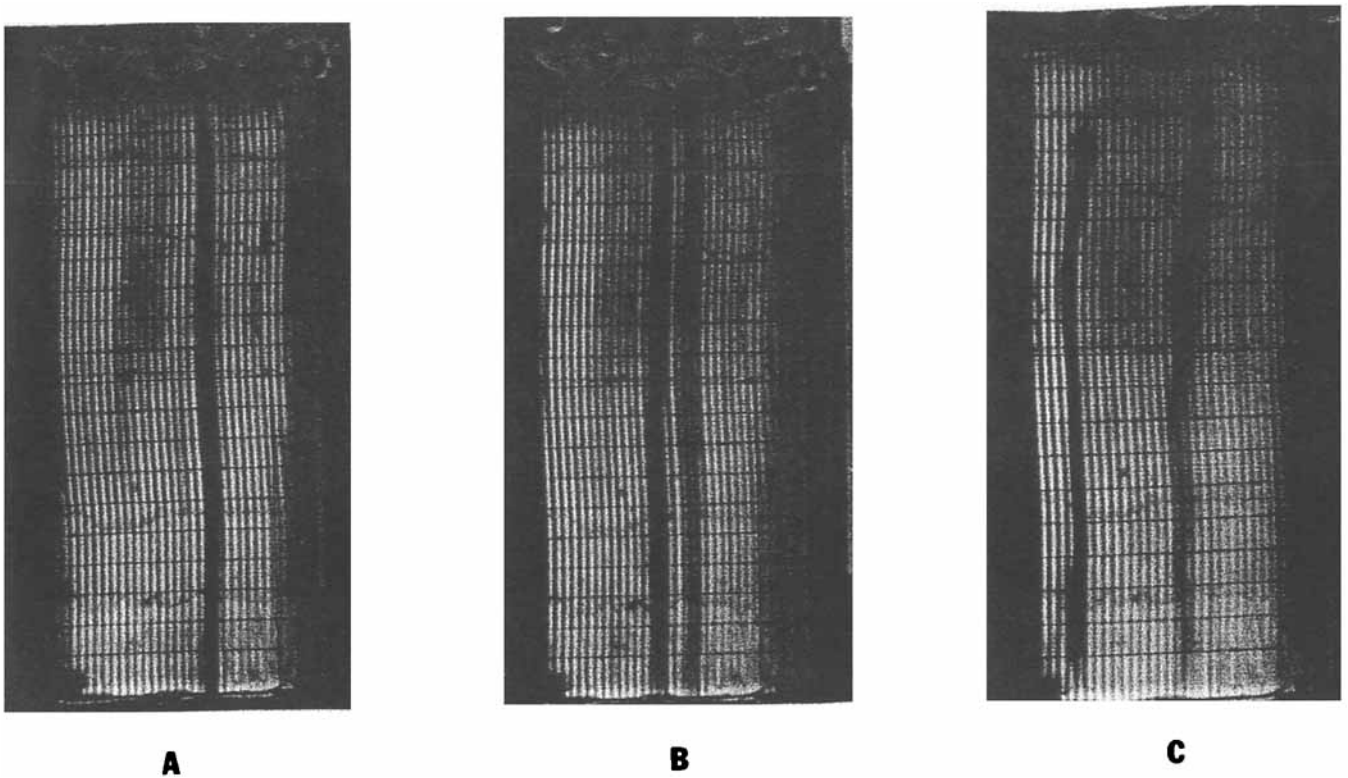

FIGURE 7. Separation of amaranth and patent blue under stopped-flow conditions at 300 V. A: time=0 min. B: time=1 min. C: time=4 min.

like a normal acid/base titration curve and the conductivity profile was much more stable. This area needs to be studied more thoroughly.

In the absence of the coolant flow, the separation underwent severe degradation within one minute. Flow turbulence caused significant mixing of the dyes all across the bed. Therefore, the capillary cooling was essential for the performance of our apparatus.

Finally, we studied the behavior of the dyes at the condition of zero carrier flow, which can be compared with the conditions of gel electrophoresis. We introduced the sample at zero voltage and then simultaneously stopped the sample and the carrier flows while maintaining the cooling flow. A voltage of 300 V was then applied across the electrodes, and the dyes were allowed to separate for several minutes. The narrow vertical zone of dye sample split into two narrow parallel bands of components (Fig. 7). After one minute the distance between the bands was 5 mm; after 4 min the distance increased to 20 mm. Only the upper and lower portions of the bands showed some degradation by that time.

Therefore, due to its fine regular structure and the presence of the mesh, the bed performs not only the cooling function, but most likely also an independent anti-convective function. The structure of the bed can be compared with a coarse granular packing or a macrogel which is cooled throughout its entire volume. No such structures have previously been reported.

Preliminary results with the separation of proteins have been positive. These results will be reported elsewhere in the future.

CONCLUSION

The concept of Capillary Free Flow Electrophoresis (CFFE) is introduced. It consists of conducting free flow electrophoresis in the interstices of aligned cooling capillaries. It combines the continuous nature of FFE with the heat dissipation capabilities of CZE and the anti-convective properties of gel electrophoresis. CFFE

showed encouraging results with model dyes even in the absence of the carrier flow.

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