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Electrophoretotron: a new method for enhancing resolution in electrokinetic separations

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Abstract

Two capillaries, each of which have different surface preparations on their inside walls, are joined together to form a closed loop, and electrodes are placed inside the two capillaries. When the loop is filled with liquid and a potential difference is applied between the two electrodes, a circulating flow of liquid is established inside the loop because the resistance to flow is unequal in going from one electrode to another in a clockwise versus a counterclockwise direction. Consequently, a sample injected into this device, which we call an electrophoretotron, repeatedly circulates between the two electrodes and the capillary separation column becomes effectively one of unlimited length. On each cycle the separation between analytes with different mobilities increases, thus enhancing resolution of analytes having nearly the same mobilities. The operation of a prototype electrophoretotron is demonstrated. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

In capillary electrokinetic separations, it is desirable to increase the resolution to separate different species with nearly identical retention or migration times. For example, the resolution of capillary electrophoresis (CE) depends on several factors such as the separation voltage applied between electrodes, the electroosmotic flow velocity, the net velocity of analytes, the length of the injection volume, and the length of the separation capillary. In typical experimental arrangements for CE, two ends of a linear capillary are connected to a high-voltage power supply so that the electric field reaches a value of

100–500 V/cm. Theoretical considerations [1] indicate that the resolving power R should scale with the voltage V according to the relation of $R \propto (V)^{1/2}$. Consequently, a 4-fold increase in applied potential is needed to double the resolution. Theory also indicates that the best resolution is obtained when the electrophoretic mobility of one species is exactly counterbalanced by the electroosmotic flow (EOF) [1]. Heat generation and electrical breakdown, however, place practical bounds on the voltage that can be used for the separation. These considerations prevent the realization of the resolution improvement expected by simply increasing the length of the capillary without limit. In addition, precise control of EOF is difficult to achieve, posing a problem in obtaining better resolution in CE.

Several attempts have been reported in the literature to enhance the resolution in CE by controlling

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EOF. One of these attempts is the direct control of electroosmosis by using external (radial) electric fields [2–5]. In these experiments, however, several limitations were encountered involving the dependence of the flow on pH and buffer concentration, the external voltages applied, and the diameter of the capillary.

Culbertson and Jorgensen [6] explored with great success a different approach in which a pressure-induced flow is used to counterbalance analyte migration. They applied pressure to one end of the capillary to retard, halt, or move the analytes back and forth across the detection window. In this manner, a substantial increase of both the efficiency and the resolving power of the separation were obtained by keeping the analytes of interest in the separation field much longer than the use of a simple linear CE setup. For example, they demonstrated the ability to resolve compounds with electrophoretic mobility differences as little as 1×10^{-7} cm²/s. Nevertheless, several drawbacks exist for this arrangement, caused primarily by the need to time the application of the pressure to the capillary end, and by the broadening caused by the pressure-induced parabolic flow.

Buggraf et al. [7] have presented a different approach for ion separations in solution utilizing the concept of repeated column switching with synchronized cyclic capillary electrophoresis on a planar microstructure [7]. Micellar electrokinetic separations were also performed with this device [8]. It was essential once again to control precisely the timing in switching the voltage between electrodes.

We present an alternative approach that does not require special timing. It is loosely based on ion cyclotron resonance mass spectrometry. In ion cyclotron mass spectrometry, gaseous ions circulate in a strong magnetic field with a frequency depending on the mass-to-charge (m/z) ratio for a given species. The application of an ac voltage having this cyclotron resonance frequency affects only ions with the same m/z ratio, causing these ions to be moved relative to the others. Here we report a related method for enhancing the resolution in CE by using what we call an electrophoretron. In this approach, we join together the two ends of a capillary to form a closed loop in which two electrodes are located so that the flow resistance in the clockwise direction

differs from that in the counterclockwise direction. Upon application of a voltage between the two electrodes, electroosmosis causes the sample to circulate repeatedly inside the capillary. Provided that some separation of the analytes occurs on a single cycle, caused for example by electrophoresis or electrochromatography, then the electrophoretron acts to enhance the separation achieved by each cycle.

2. Experimental

2.1. Instrumentation

Fig. 1 presents a schematic diagram of the equipment required to perform cyclic electroosmotic flow in the electrophoretron. It consisted of a closed-loop capillary column. One part of the capillary loop was a regular fused-silica column (Polymicro Technologies, Phoenix, AZ, USA) of 75 μ m I.D. \times 365 μ m O.D. The other part of the loop was completed with a capillary of the same dimensions (inside and outside diameters) that had a positively charged coating (CElect-Amine column, Supelco, Bellefonte, PA, USA). The length of each capillary can be varied (a total length of approximately 50–100 cm was typically used in our case). The joints of the two columns were assembled inside tightly fitting PTFE tubes that were mounted in the middle of buffer reservoirs. Several holes were punched in the tubes to allow the buffer solution to enter and exit. The fused-silica capillary was preconditioned by flushing it with 1 M NaOH to make the uncoated capillary wall negatively charged. This structure was flushed by flowing phosphate buffer solution (2 mM NaH₂PO₄) for approximately 1 h before joining the capillaries to form a loop.

The separation potential was provided in the range 10–20 kV depending upon experimental conditions from a reversible high-voltage power supply (Model R50B, Hipotronics, Brewster, NY, USA). On-column detection was carried out through a window that was created in the capillary loop. The window was prepared by removing the polyimide coating from a section of the fused-silica capillary with heat. Absorption measurements were made using an ultraviolet absorbance detector (ISCO CV⁴) operated at

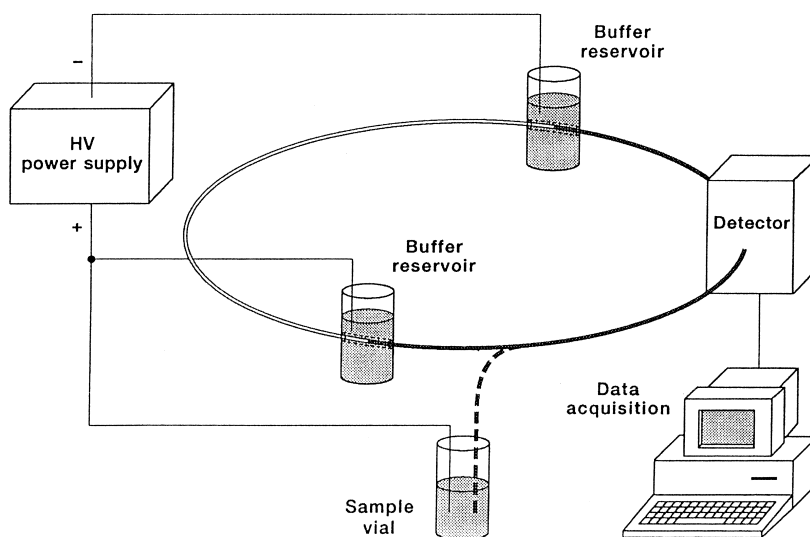


Fig. 1. Schematic representation of the electrophoretotron.

254 nm. In typical experimental runs, samples were introduced electrokinetically into the anodic end of the negatively charged capillary by placing the end in the sample reservoir and then by applying a voltage for approximately 3 s. Electropherograms were collected through a personal computer equipped with a multifunction data acquisition board and software (Lab Calc, Galactic Industries, Salem, NH, USA).

2.2. Chemicals

All chemicals were obtained from Sigma (St. Louis, MO, USA) and were used without further purification. The concentration and the pH of the buffer solution were systematically varied to find the optimal conditions for experimental runs. In the experiments reported here, 2 mM NaH_2PO_4 solution at pH 4.5 was employed as the buffer. Sample solutions of 1 mM benzyl alcohol or 1 mM guanosine were used as analytes for the studies. Freshly deionized and distilled water was used to prepare the solutions.

3. Results and discussion

The essence of the electrophoretotron is to generate

a cyclic electroosmotic flow so that the capillary separation column becomes effectively of unlimited length. The direction of the electroosmotic flow inside the closed-loop capillary is controlled primarily by the nature of the capillary walls. The magnitude of the electroosmotic flow depends upon the buffer solution used as well as the nature of the walls. In a bare fused-silica capillary, the negatively charged surface produces electroosmotic flow directed toward the negative electrode. On the other hand, when the capillary wall is positively coated the EOF is reversed in direction. By connecting the two capillaries with differently charged walls in a closed loop, such as a circle, electroosmosis causes overall flow in only one direction, resulting in circulation of the solution in the capillary.

Fig. 2 illustrates the repeated circulation of a sample of benzyl alcohol within the loop. This electropherogram is obtained by the sample passing through the detection zone multiple times. The time for the sample to travel each cycle can be determined by measuring the distance between subsequent peaks. A decrease in peak height is observed on each cycle and is discussed below.

To assure the performance of the electrophoretotron, it is necessary to separate and detect different components in a mixture. Thus, experiments were carried out with a mixture of two differently charged

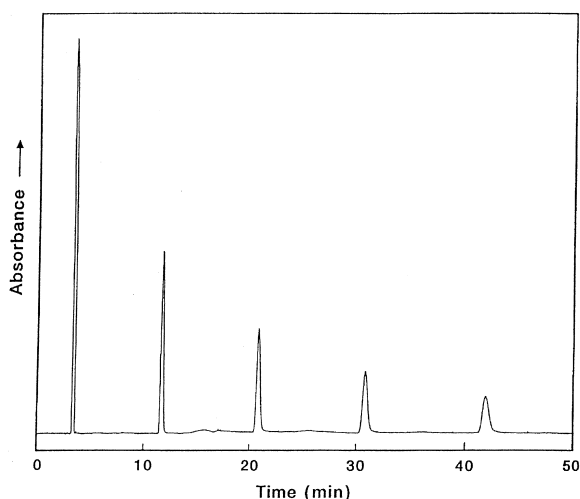


Fig. 2. A typical electropherogram of benzyl alcohol obtained by the electrophoretion representing the repeated circulation of the sample. A 1 mM sample was injected into the capillary. A potential of 10 kV was applied to the system. A buffer solution of 2 mM NaH_2PO_4 at pH 4.5 was used.

species: (1) benzyl alcohol as a neutral substance; and (2) guanosine as a cationic component (at pH 4.5). The separation voltage was 10 kV and the length of the negatively charged capillary segment was about 15 cm. Fig. 3 shows a typical electropherogram of the two analytes, guanosine (A) and benzyl alcohol (B). Analogous to Fig. 2, a set of peaks is observed whenever the electroosmotic flow causes the sample to make a complete revolution around the capillary loop. More importantly, as clearly observed in Fig. 3, the separation between the

two peaks increases as the circulation is repeated. In other words, in the electrophoretion the difference in net velocities between two differently charged components is amplified because of the increase in effective capillary length.

We also note in Figs. 2 and 3 that the peaks decrease in intensity and broaden with time. In general, the analyte loss, which was calculated by the peak areas, was determined to be approximately 20% for each cycle. This loss is similar to that observed in other studies, such as the flow counterbalanced CE [6] and the synchronized cyclic CE arrangements [8], in which the loss of the peak was reported to be 3–9%/h and 28% for each detection cycle, respectively. Some plausible reasons for this loss of sample include leakage through the two joints connecting the two different capillary segments and the adsorption of samples onto the column walls. It may be possible to reduce the loss of samples through further modifications of the experimental setup, such as the introduction of specially designed capillary unions and joints with zero dead volume. Peak broadening as a function of time results from diffusion and from flow disturbances in traversing the cyclic path.

Although we have utilized two different columns with opposing charges in this system, other methods may be applicable to create this cyclic flow. These include any method to induce differential flow rates in each of the capillary segments. The present study has employed a prototype design for an electrophoretion, and peak broadening and analyte loss presently impair its performance. Nevertheless, we expect that many design improvements are possible.

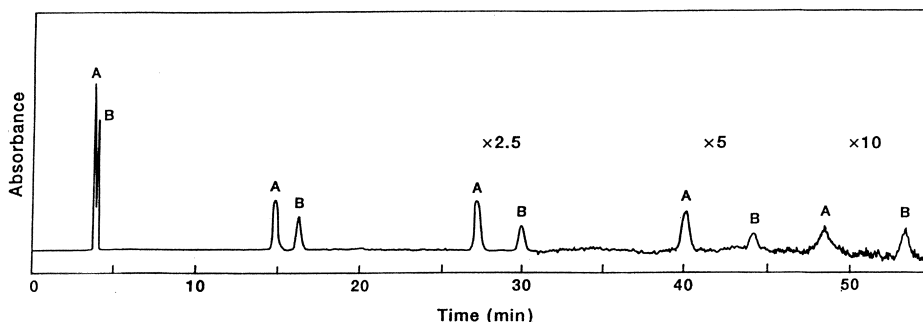


Fig. 3. An electropherogram of two analytes demonstrating better separation as they are repeatedly circulated in the loop. A mixture of 1 mM guanosine (A) and 1 mM benzyl alcohol (B) was injected into the capillary. Other conditions were the same as in Fig. 2.

The separation power of the electrophoretion can be described more quantitatively. Suppose that the two paths between the oppositely charged electrodes in the closed loop of total length L are equal (i.e. each of the two capillaries is of length $L/2$), and the applied voltage is V . Then the time t_1 that it takes an analyte 1 to make a full cycle is:

$$t_1 = (\mu_{eo}L^2/2V)/(\mu_{eo}^2 - \mu_1^2),$$

where μ_{eo} is the mobility of the electroosmotic flow (assumed to be equal in both capillaries) and μ_1 is the electrophoretic mobility of analyte 1. Note that the electroosmotic mobility must be greater than the electrophoretic mobilities of the individual analytes ($\mu_{eo} > \mu_1$) in order for the analytes to continue to migrate in the loop. Hence, we can calculate the time difference $\Delta t = t_2 - t_1$ between two species 1 and 2 with electrophoretic mobilities μ_1 and μ_2 , respectively.

This difference can be further explained by taking account of charges of the analytes. For example, suppose that one of the analyte is a neutral substance with the electrophoretic mobility of μ_1 which is zero, and the other is cationic component with the electrophoretic mobility of μ_2 . In the closed loop of total length L , the neutral analyte 1 migrates only with the electroosmotic mobility, μ_{eo} , while the analyte 2 migrates with $\mu_{eo} + \mu_2$ in a fused-silica column part, and then with $\mu_{eo} - \mu_2$ in the positively charged part. Simply considering the concept of the average velocity only, for one cycle, the time difference between two species, $\Delta t = t_2 - t_1$ is then given by:

$$\Delta t = \frac{L/2}{V} \left(\frac{L/2}{\mu_{eo} + \mu_2} + \frac{L/2}{\mu_{eo} - \mu_2} \right) - \left(\frac{L/2}{\mu_{eo}} + \frac{L/2}{\mu_{eo}} \right) = \frac{L^2}{2V} \left[\frac{\mu_2^2}{\mu_{eo}(\mu_{eo}^2 - \mu_2^2)} \right]$$

Because $\mu_{eo} > \mu_2$, Δt is always greater than zero during one cycle of the migration. Consequently, the total time difference, which is indicative of the resolution of the electrophoretion, can be increased as the number of cycle is increased. The same reasoning applies for the case that one analyte is an anion instead of a cation.

Another possible case is the migration of one anion and one cation in the same loop of two

differently charged capillaries. In the first half cycle the anion migrates with the mobility of $\mu_{eo} - \mu_3$ and in the other half cycle it migrates with $\mu_{eo} + \mu_3$, where the electrophoretic mobility of the anion is μ_3 and the mobility change of the cation is the same as the former example. When two analytes are located in the electrophoretion, the time difference between two different ionic species, Δt , is equal to:

$$\begin{aligned} \Delta t &= \frac{L/2}{V} \left(\frac{L/2}{\mu_{eo} + \mu_2} + \frac{L/2}{\mu_{eo} - \mu_2} \right) \\ &\quad - \left(\frac{L/2}{\mu_{eo} - \mu_3} + \frac{L/2}{\mu_{eo} + \mu_3} \right) \\ &= \frac{L^2 \mu_{eo}}{2V} \left[\frac{\mu_2^2 - \mu_3^2}{(\mu_{eo}^2 - \mu_2^2)(\mu_{eo}^2 - \mu_3^2)} \right] \end{aligned}$$

We notice that in above equation Δt is not zero because the magnitude between the electrophoretic mobilities for the mixed analytes of oppositely charges is different in general except when $|\mu_2|$ is accidentally equal to $|\mu_3|$. In some cases, the migration order can be reversed for the case of $|\mu_3| > |\mu_2|$. However, it is obvious that as the analytes circulate through the closed loop repeatedly, the magnitude of the time difference between two analytes is increased, thus resulting in enhanced resolution of the electrophoretion. Of course, beyond some point, the resolution decreases again as the separation distance is modulo the total length of the closed loop.

We tested the above consideration for enhanced resolution by calculating the migration time difference of benzyl alcohol as a neutral species, benzyltrimethylammonium chloride cation, and acetylsalicylate anion with the data shown in Ref. [1]. The simulation is carried out with the assumption that the electroosmotic flow is identical in a total capillary column and the electrophoretic mobility of the same analyte in a positively coated column is just reverse to the data obtained in fused-silica column. These results have clearly demonstrated that the separation between different analytes is enhanced as they repeatedly circulate in the loop. Although this calculation considers a purely electrokinetic basis for separation, other mechanisms for separation (e.g. micellar electrokinetic chromatography, capillary electrochromatography) are also adaptable to the electrophoretion.

4. Conclusion

We have demonstrated the construction of a device we call an electrophoretion in which the sample travels around a closed loop under the action of electroosmotic flow. On each cycle, the separation between analytes increases. This feature becomes particularly important when the analytes have nearly identical mobilities. As a consequence, the electrophoretion can be used to improve the separation efficiency (resolving power). In this sense, the electrophoretion for the separation of analytes in liquid samples is analogous to ion cyclotron mass spectrometry for the separation of charged ions in the gas phase. The electrophoretion possesses several potential advantages over other methods. Using only a capillary channel of modest length, a significant increase of effective capillary length can be achieved with this simple experimental setup. This advantage might be best realized on a microchip. The electrophoretion also has the advantage of generality. It can be used with various detection schemes and with various separation methods in the two segments that form the closed loop.

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