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Free Flow Electrophoresis with Multiple Gating Electrodes

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Abstract

A method of continuous free flow electrophoresis is proposed in which a mixture of particles with different electrophoretic mobilities is fractionated in an alternating electric field between multiple electrodes. Neighboring electrodes form fractionation compartments in which faster migrating species are able to move from one compartment on to the next. If a species with a certain electrophoretic mobility is extracted from a compartment, then slower migrating species remain in the previous compartment and faster migrating species move on to the following compartment.

INTRODUCTION

Continuous free flow electrophoresis is used for fractionating cells, organelles, membrane proteins, and other species with distinct electrophoretic mobilities which are difficult to fractionate by other techniques.

The most often employed method is a "thin-film" system where a narrow streak of a heterogeneous mixture is injected into a continuous film of carrier electrolyte flowing in a narrow gap between two plates with two parallel electrodes at opposite ends (*1*). Other methods include rotary electrophoresis (*2*) and field flow fractionation (*3–7*).

All continuous free flow electrophoresis methods have two things in common (*1–7*). One is that the feed mixture is introduced to the separation chamber as a thin ribbon, thus allowing at best a limited feed throughput. Another is that a typical feed mixture contains many different charged species, and only one species needs to be extracted. Unfortunately, all particles with different electrophoretic mobilities have to be separated throughout the width of the separation chamber. Therefore, free flow electrophoresis chambers are usually quite wide (i.e., 10–30 cm or more). This causes difficulty in mechanical design and frequently leads to excessive feed dilution with a carrier electrolyte.

Free flow electrophoresis with multiple gating electrodes is designed to overcome these limitations. It is achieved by a feed inlet which flows through a compartment of a large cross-sectional area with separate compartments for the flow outlets. In the simplest apparatus, there are only three outlet streams. One contains all species having electrophoretic mobilities lower than the species to be extracted. The second contains the species that need to be extracted. The third contains all species with electrophoretic mobilities larger than the species to be extracted. If there are N types of species that need to be extracted, the simplest fractionation apparatus will contain $N + 2$ outlet flow compartments.

A schematic drawing of the separation chamber is shown in Fig. 1. Dashed lines are the electrode screens which separate one compartment from another. A mixture of three species, 1, 2, 3, is introduced to the first compartment. Driven by an alternating electric field, species migrate from one compartment to another until they flow out of the separation chamber. The separation is achieved by increasing the migration time necessary to travel across each consecutive compartment from the left to the right. Therefore, only the fastest migrating particles are able to cross into the extreme right compartment. A dual configuration of the electrode screens

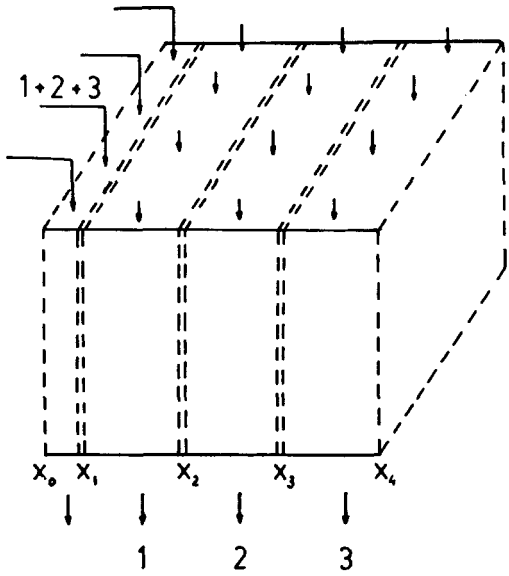

FIG. 1. A schematic drawing of the separation chamber with twin electodes.

with the narrow "trap" spaces between them is used to move the faster migrating particles from one compartment to the next.

THEORETICAL ANALYSIS

A. Main Trajectories

The fractionation chamber is comprised of parallel electrode screens shown with dashed lines in Fig. 1. Openings in the screens are sufficiently wide to allow any fractionated particle to pass through. Multiple arrows indicate the continuous downward flow of the carrier electrolyte between the electrode screens. Coordinates x_0, x_1, x_2, x_3, and x_4 indicate the positions of the parallel electrode screens. The polarity of electrodes is changed periodically at intervals, T. If, at time t, electrodes x_0, x_2, and x_4 were cathodes and electrodes x_1 and x_3 were anodes, then, at time $t + T$, electrodes x_0, and x_2, and x_4 would become anodes and electrodes x_1 and x_3 would become cathodes, respectively. T is therefore the length of a single time cycle. The electric field E between the alternating electrodes is shown in Fig. 2 with solid lines at time t and with dashed lines at time $t + T$.

To fractionate a mixture of three negatively charged particles 1, 2, and 3 with mobilities $\mu_1 < \mu_2 < \mu_3$, the mixture is injected into the separation chamber between electrodes x_0 and x_1. All three particles have sufficient electrophoretic mobility to migrate from electrode x_0 to electrode x_1 within time period T. As the particles flow downward between electrodes x_0 and x_1, they reach anode x_1. The feed compartment between electrodes x_0 and x_1 is needed to align all particles along electrode x_1 before they start migrating toward electrode x_2. After the electrode polarities are reversed,

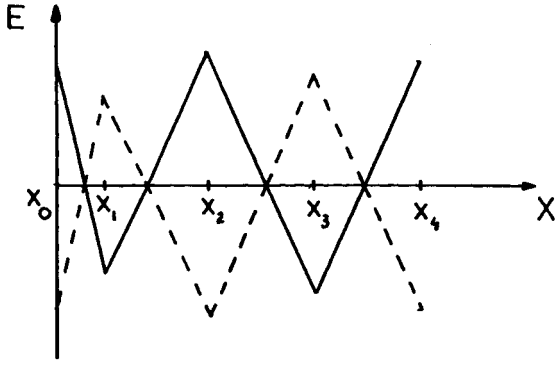

FIG. 2. Electric field diagram.

negatively charged particles start migrating from cathode x_1 toward anode x_2.

The applied magnitude of the electric field E_{12} between electrodes x_1 and x_2 is such that the slowest migrating particles 1 never reach anode x_2 and particles 2 reach anode x_2 before the end of the time period. Because particles 3 are migrating faster than particles 2 they also reach electrode x_2. Having reached electrode x_2, particles 2 and 3 stay there until the electrode polarities are alternated; they then start migrating toward anode x_3. Particles 1 keep migrating back and forth between electrodes x_1 and x_2 until they flow out of the separation chamber. The distance $(x_3 - x_2)$, electric field E_{23}, and time T are preset for particles 3 to reach anode x_3. Particles 2 keep migrating between electrodes x_2 and x_3 until they flow out of the fractionation chamber. Having passed electrode x_3, particles 3 migrate between electrodes 3 and 4 until they flow out of the fractionation chamber.

In a general situation, a heterogeneous feed contains N species with electromobilities μ_j:

$$\mu_{j+1} > \mu_j, \qquad j = 1, \ldots, N - 1 \tag{1}$$

There are two more electrodes than the total number of different species to be separated. Coordinates x_i, where $i = 0, \ldots, N + 1$, indicate the positions of the parallel electrode screens. Species with electrophoretic mobility μ_j should flow out of the separation chamber between electrodes x_j and x_{j+1}:

$$\text{at } i > j, \qquad \mu_j E_i T > x_{i+1} - x_i, \qquad j = 1, \ldots, N; \qquad i = 0, \ldots, N + 1 \tag{2}$$

$$\text{at } i = j, \qquad \mu_j E_i T > x_{i+1} - x_i, \qquad j = 1, \ldots, N \tag{3}$$

where E_i is the electric field between electrodes i and $i + 1$.

The electrophoretic chamber should be sufficiently long for any particle j to reach the compartment (x_j, x_{j+1}) before it flows out of the system.

$$L \geq \max \left(2 \int_{x_0}^{x_1} V_{zi} dt + \sum_{i=1}^{j+1} \int_{x_i}^{x_{i+1}} V_{zi} dt \right), \qquad j = 1, \ldots, N \tag{4}$$

where L is the length of the fractionation chamber and V_{zi} is the downward carrier electrolyte velocity in the (x_i, x_{i+1}) compartment. A symmetrical

Poiseuille velocity profile is formed in each compartment during the flow of liquid through the separation chamber.

$$L \geq \max \left(\sum_{i=1}^{j+1} \frac{2V_{0zi}}{3\mu_j E_i} (x_{i+1} - x_i) + \frac{4V_{0z0}}{3\mu_j E_0} (x_1 - x_0) \right), \quad j = 1, \ldots, N \quad (5)$$

where V_{0zi} is the maximum velocity in the compartment (x_i, x_{i+1}).

Species j and $j + 1$ are separated from each other across electrode x_{j+1}. For optimal fractionation, species j should stop migrating as far away as possible from anode x_{j+1}, at the end of time period T, and species $j + 1$ should have reached electrode x_{j+1} as early as possible before the end of the same time period T. An equal distance of species j and $j + 1$ from electrode x_{j+1} is assumed for calculating the fractionation parameters.

$$E_j T = \frac{2(x_{j+1} - x_j)}{\mu_{j+1} + \mu_j}, \quad j = 1, \ldots, N - 1 \quad (6)$$

The electric field differential, E_j, can be calculated from Eq. (6) if the positions of the electrodes and the time period T are known.

B. Gating across the Electrodes

The critical part of the separation is that after the particles reach the electrode, they should move from it onto the next electrode, passing over the potential peaks shown in Fig. 2. Unfortunately, the particles never reach the peaks due to repulsion from the electric field in the next compartment. Therefore, an additional maneuver is required for the faster migrating particles to migrate across the electrode after the electrode charges are reversed.

A dual configuration of the electrode screens with narrow "trap" spaces between them is used to transport the faster migrating particles from one compartment to the next as shown in Fig. 3(a). The dual electrode coordinates are x_{ic} and x_{ia}, where c and a stand for the cathode and anode, respectively. A dual electrode operates in two modes, entrapment and ejection.

In the entrapment mode, anode x_{ia} causes negatively charged particles to migrate toward it, into the "trap" space between electrodes x_{ia} and x_{ic}. Electrode x_{ic} is disconnected during that time. At the end of time period T, electrode x_{ia} is disconnected and electrode x_{ic} is charged negatively.

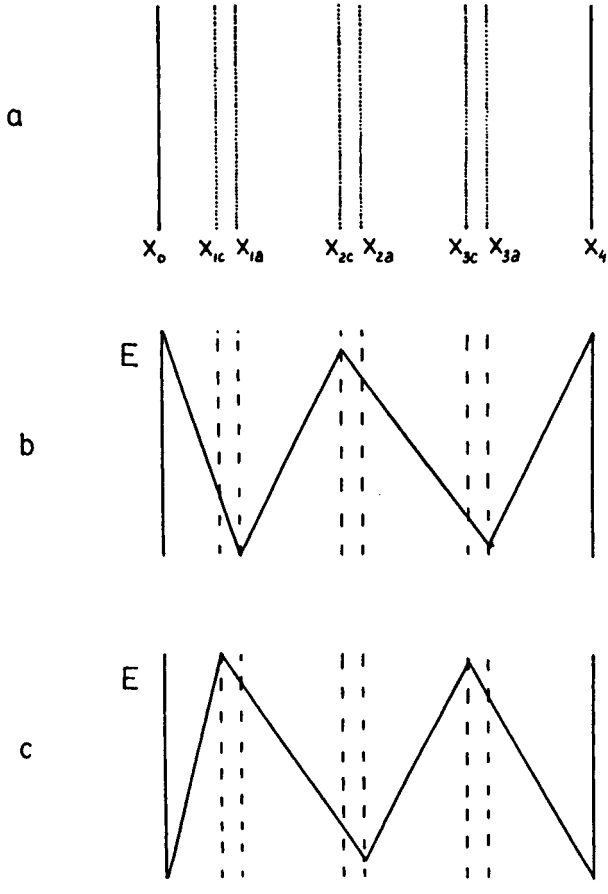

FIG. 3. Electric field diagram for the separation chamber with twin electrodes at different times.

Then, all negatively charged particles between electrodes x_{ic} and x_{ia} start migrating across electrode x_{ia} to the next compartment.

The direction of the electric field at time t and at time $t + T$ is shown in Figs. 3(b) and 3(c), respectively.

To limit dispersion, both the width of the fractionation compartment $(x_{j+1c} - x_{ja})$ and the relative separation between fractionated species $(\mu_{j+1} - \mu_j)E_jT$ should be much greater than the width of the dual electrode $(x_{ja} - x_{jc})$.

$$\frac{x_{ja} - x_{jc}}{x_{j+1c} - x_{ja}} < 1, \qquad j = 1, \ldots, N \tag{7}$$

$$\frac{x_{ja} - x_{jc}}{(\mu_{j+1} - \mu_j)ET} < 1, \qquad j = 1, \ldots, N \tag{8}$$

RESULTS AND DISCUSSION

An electrophoretic separation chamber was designed to fractionate a mixture of three particles with electrophoretic mobilities $\mu_1 = 1.0$ μm·cm/V·s, $\mu_2 = 1.1$, and $\mu_3 = 1.2$, respectively. The feed inlet compartment was 0.5 cm wide and the other three compartments were each 1 cm wide. The time period T was 100 s and $V_{0z} = 0.2$ cm/s.

The electric field was calculated from Eq. (6): $E_0 = 90$ V/cm, $E_1 = 95$ V/cm, $E_2 = 87$ V/cm, and $E_3 = 80$ V/cm.

The length of the fractionation chamber was calculated from Eq. (5): $L = 50$ cm.

The feed throughput for a 20 cm by 0.5 cm feed inlet compartment was calculated to be 4.8 L/H, almost twice as much as in existing systems (*1*).

By this example we have shown that in theory it should be possible to fractionate species with electrophoretic mobilities that differ by as little as 0.1 μm·cm/V·s.

To transport different species from one compartment to another across the electrode screens, screen openings should be sufficiently large to allow the passage of all species. The dual trapping electrodes should also be sufficiently thin and protected by semipermeable membranes to keep by-products of the electrode reactions from the fractionated species. For example, the electrode screens may comprise a closely spaced grid of thin wire electrodes wherein each electrode is inside a lumena of a thin (less than 50 μm outer diameter) hollow membrane fiber and the electrolyte solution is recirculated through the annuluses inside the hollow fiber.

CONCLUSIONS

A new electrophoretic method with multiple gating electrodes is shown to have significant advantages in throughput and selectivity over existing electrophoresis methods. This type of electrophoretic separator is particularly advantageous for extracting a single component out of a heterogeneous mixture with a high feed throughput and minimal carrier electrolyte dilution.

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