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Moving boundary capillary electrophoresis with concentration gradient detection

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ABSTRACT

A rugged and inexpensive Schlieren optics detection system consisting of a laser, focusing lens and light beam position sensor was combined with moving boundary electrophoresis performed in a 10 cm × 20 μm I.D. capillary. This system facilitated rapid analysis and universal detection of sample components. The laser beam, which is focused directly into the separation capillary, probes the refractive index gradients produced by the arrival of new boundaries. The frontal injection method used in this approach introduces samples into the capillaries without discrimination and provides higher sensitivities because of the lack of dilution during the separation process compared with the zone technique. A specially designed cartridge accommodates a short piece of the separation capillary and reservoirs for buffer and sample. The separation in this method is completed usually within 30 s. During this short separation time only a small dispersion associated with diffusion of analytes along the flow direction occurs, which ensures that high gradients are generated in the detection volume. The detection limits in this approach are sub-micromolar concentrations of analyte injected for low-molecular-weight compounds such as sucrose. This system has been applied to the separation of amino acids and carbohydrates.

INTRODUCTION

Recent advances in electrophoretic methods have produced dramatic improvements in the efficiency of these techniques. For example, capillary zone electrophoresis achieves efficiencies of close to 10^6 theoretical plates [1–3]. This technique holds promise for increasing the speed of analysis, not only for ions, but also for neutral species. Electroosmotic flow which is present in electrophoretic separations is uniform across the diameter of the capillary, unlike the hydrodynamic flow associated with chromatographic methods characterized by parabolic velocity profiles [4]. Therefore, the dispersion process in capillary electrophoretic techniques is governed primarily by diffusion along the flow direction [5,6]. This results in sharp peaks and high efficiencies of the electrophoretic methods.

The narrow peaks produced in capillary electrophoretic separations generate high concentration gradients in the detection volume which can be effectively monitored by using the concentration gradient detector based on Schlieren optics [7–9]. The low detection volume, derivative response and universal nature of the technique make it very attractive for this application. In this method a laser beam is

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focused directly into the separation capillary. The refractive index gradients (dn/dx) associated with high concentration gradients (dC/dx) of eluting analytes deflect the beam towards higher refractive indexes [9]:

$$\theta = \frac{l}{n} \cdot \frac{dn}{dx} = \frac{l}{n} \cdot \frac{dn}{dC} \cdot \frac{dC}{dx} \qquad (1)$$

where θ is the deflection angle in rad and l is the separation capillary diameter. This deflection is monitored using a silicon position sensor [10]. This detection method is most effective in rapid short-column separations [9], as under the conditions used the dispersion is small.

Investigations of capillary electrophoretic methods are focused on zone electrophoretic separations [9]. In this method, in the injection step, a narrow sample plug is introduced at the front of the capillary. This task is usually accomplished by the electromigration technique, which is slow and discriminates between analytes [3]. In addition, it is very difficult to introduce sub-millimetre plugs which are required to perform successful short-time separations.

In this paper we discuss the use of another technique, moving boundary capillary electrophoresis, to facilitate rapid analysis. In this method a sample mixture is introduced continuously throughout the separation [2]. The injection in this technique is very rapid and convenient as the separation begins as soon as the contact is made between the buffer in the capillary and the sample. We describe the application of moving boundary capillary electrophoresis with concentration gradient detection for the rapid separation of amino acids and sugars. The method is in principle similar to the early experiments reported by Tiselius *et al.* [11] almost a half century ago, but the performance of the system has increased significantly with the use of modern optical components to design the detector, and by using capillaries for electrophoresis.

EXPERIMENTAL

Instrumental

A 20 μm I.D. × 0.35 mm O.D. fused-silica capillary (Polymicro Technologies, Tucson, AZ, USA) was used for separations. The total length of the capillary was 12.5 cm and the effective separation length, from the anodic end to the detection point, was about 10 cm. Fig. 1 shows the separation cell used in the experiments. The capillary was fixed on a Plexiglas plate using epoxy glue, and its two ends were connected to small buffer reservoirs made of polyethylene. First, both reservoirs were filled with buffer. The injection of the sample was performed by emptying and then filling the reservoir at the anodic end with sample mixture. This step was accomplished by using a syringe and a small-diameter tube made of a copolymer of tetrafluoroethylene and hexafluoropropylene (Cole Parmer, Chicago, IL, USA). The separation was driven by a high-voltage d.c. power supply (Spellman, Plainview, NY, USA). The current passing through the capillary was monitored at the cathodic end of the capillary. After each run the capillary was rinsed with 0.1 *M* sodium hydroxide solution for 30 s, with water for 30 s and finally with buffer for 2 min. The capillary electrophoresis separation cell and detection system are also shown in Fig. 1. The whole system was mounted on a vibration isolation table.

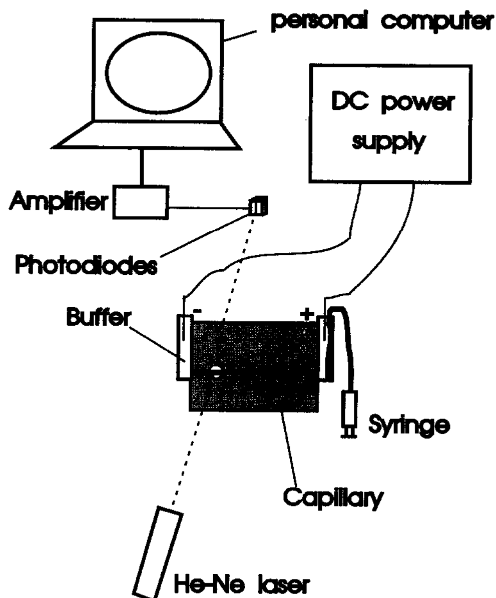

Fig. 1. Experimental arrangement.

A beam from a helium–neon laser (Uniphase, San Jose, CA, USA) or a laser diode (MWK Industries, Pomona, CA, USA) was focused by a 30-mm focal length lens directly into the capillary. The beam deflection was monitored by a position sensor consisting of a dual silicon detector (United Detector Technologies) with appropriate electronics. The probe beam is arranged so that the far-field intensity profile points to the centre between two photodiodes placed close together in the bicell configuration. When irradiated uniformly, the photodiodes generate equal amounts of photocurrent. On encountering a concentration gradient the laser probe baem is deflected and the amount of light reaching the diode is not equal. The difference in photocurrent associated with the two diodes corresponds to the magnitude of deflection of the laser beam. The difference in photocurrent genrrated by the diodes is converted to a voltage. The data is collected by an IBM DACA board, in a PC-AT compatible personal computer, using the software ASYST (Asyst Software Technology, Rochester, NY, USA).

Reagents

All chemicals were of analytical-reagent grade, and solutions were prepared using deionized water. Borate or phosphate buffers were used to perform separations. All samples were dissolved in buffers before use. All solutions were filtered through 200-nm pore size filters and degassed by purging with helium.

RESULTS AND DISCUSSION

Rapid moving boundary capillary electrophoretic separations were conveniently performed in the separation cell shown in Fig. 1. First, both reservoirs and the capillary

were filled with buffer, and the high d.c. voltage was turned on. Then buffer in the reservoir of the anodic end was removed with a syringe and filled with sample. In our experiments the separation begins to occur immediately after the sample solution reaches the separation capillary. Various sample components migrate with different velocities related to their mobilities and applied electric field gradient. The analytes are never fully separated from one another as in zone electrophoresis, but rather they form boundaries which move with characteristic velocities. Fig. 2a illustrates the situation in the capillary after the boundaries have reached the detector volume. The migration direction of ions is from right to left. The electrolyte composition varies along the separation capillary because the analytes have different velocities. The first boundary is formed when the front associated with the fastest ion, alanine, has reached the detector. Immediately afterwards histidine arrives, which produces a zone consisting of buffer constituents, alanine and histidine. Finally, the tryptophan, with the slowest migration velocity, begins to elute and forms the third boundary.

The composition of the electrolyte varies along the separation capillary. Along with the composition change, properties such as refractive index will also vary. For example, a refractive index detector could be applied to the separation shown in Fig. 2a to yield a hypothetical trace as given in Fig. 2b. The moving boundary electropherogram would consist of "steps" indicating the position of the boundary. The height of the step will be proportional to the analyte concentration corresponding to the given boundary. The exact concentration profile of the boundary (Fig. 3a) can be described as [12]

$$C = \frac{1}{2} C^0 \left[\mathrm{erf}\left(\frac{x - x_r}{2\sqrt{Dt_r}} \right) + 1 \right] \tag{2}$$

where C^0 is the concentration of analyte in the sample, D is the diffusion coefficient of the analyte in the electrolyte, x_r is the position of the centre of the boundary and t_r is the retention time. In this expression we assume that the longitudinal diffusion is the primary cause of the boundary broadening. In that situation the width of the boundary can be defined by the parameter σ_x (Fig. 3):

$$\sigma_x = \sqrt{2Dt_r} \tag{3}$$

Eqn. 3 indicates that, assuming "ideal" injection, the width of the zone depends on the separation time, t_r. For rapid separations using very short capillaries as discussed in this paper, the concentration "steps" are expected to be very sharp. Under these conditions high concentration gradients ($\mathrm{d}C/\mathrm{d}x$) are produced at the boundaries (Fig. 3b):

$$\frac{\partial C}{\partial x} = \frac{1}{2} C^0 \exp\left[- \frac{(x - x_r)^2}{4Dt_r} \right] \Big/ \sqrt{\pi D t_r} \tag{4}$$

Fig. 3b and eqn. 4 show that the gradient signal consists of a single Gaussian peak per boundary with height proportional to the concentration of the analyte in the

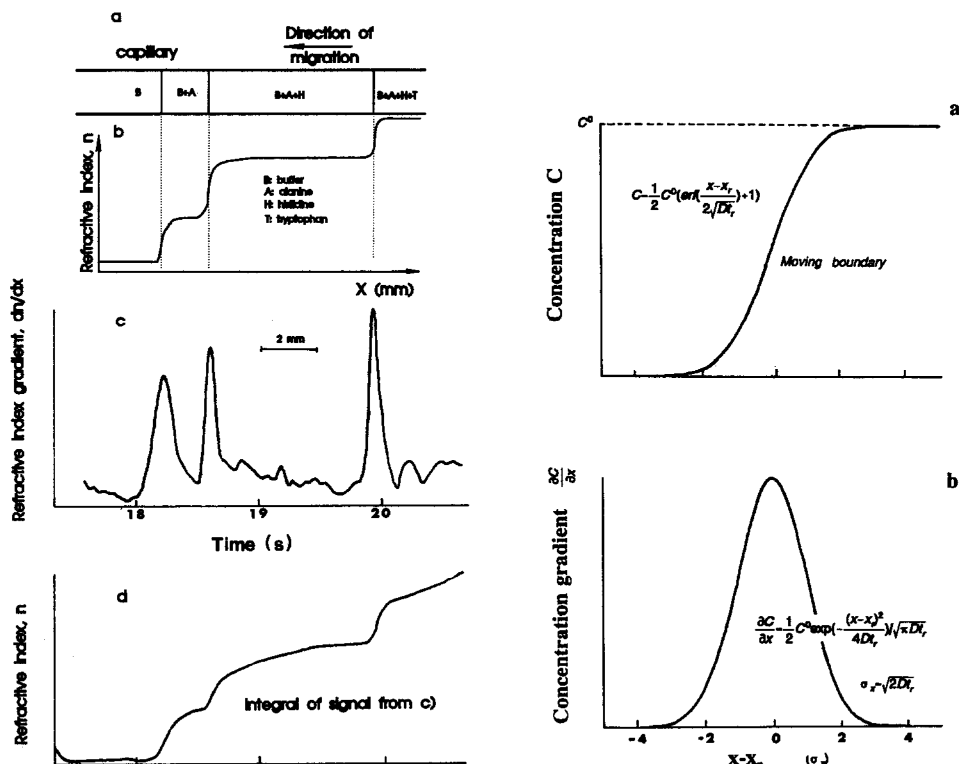

Fig. 2. (a) The moving boundaries inside a capillary; the three boundaries are generated by alanine, histidine and tryptophan; (b) the refractive index steps corresponding to these boundaries; (c) the deflection signals of the probe beam generated by the refractive index steps; (d) integral of signal from (c).

Fig. 3. (a) Moving boundary in capillary; (b) its first derivative.

sample. Indeed, as shown in Fig. 2c, three peaks corresponding to the boundaries associated with alanine, histidine and tryptophan are present in the moving boundary capillary electropherogram detected by the concentration gradient detector.

The clearly visible peaks produced by the gradient detector are in direct contrast to the small "steps", difficult to recognize from drifts, which would be produced by the refractive index detector as illustrated in Fig. 2d. Fig. 2d is generated by integrating the refractive index gradient trace from Fig. 2c. In contrast to the hypothetical trace in Fig. 2b, actual refractive index detection is subject to drifts most likely associated with temperature fluctuations.

The peak corresponding to tryptophan from Fig. 2c is about 200 ms wide. Therefore, the dimension of the corresponding boundary is about 1 mm as the total length of the capillary is 10 cm and the separation time is about 20 s. The standard deviation (σ_x) of the Gaussian is therefore about 0.2 mm. This value is in agreement with the calculated value obtained from eqn. 3 assuming a diffusion coefficient of 10^{-5} cm^2/s [13]. Diffusion of the analytes along the capillary determines the dispersion in this separation.

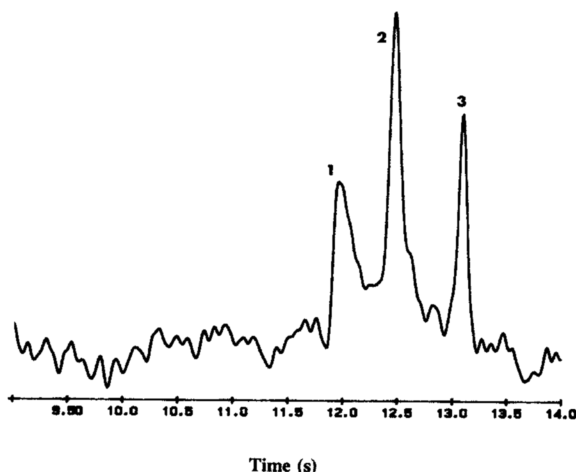

Fig. 4. Rapid separation and detection of amino acids. Buffer, 50 m*M* borate (pH 10); applied voltage, 12 kV. 1 = 2.5 m*M* alanine; 2 = 1 m*M* histidine; 3 = 1 m*M* tryptophan.

The number of theoretical plates generated in the separation illustrated in Fig. 2c is about 25 000 for the 11-cm separation capillary. This short capillary is sufficient to separate the three amino acids completely. In fact, the capillary can be shortened by about 20% and still produce a successful separation, as shown in Fig. 4. Now the total analysis time is reduced to 13 s because of the shorter capillary length and higher electric field gradients. Also, the sensitivity of detection is improved since the boundaries are narrower. The limit of detection estimated from Fig. 4 is in the sub-millimolar range and is similar to the theoretical limits that can be calculated using eqns. 1 and 4 [9].

The unique feature of the Schlieren optics detection is its universal nature. Therefore, all eluted analytes are detected and derivatization procedures are unnecessary. This is particularly beneficial in the case of sugars which are difficult to tag. It has been shown that sugars can be successfully separated by electrophoresis using borate

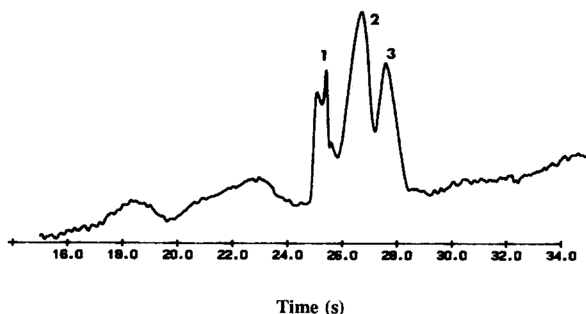

Fig. 5. Separation and detection of sugars as the borate complexes. Buffer, 50 m*M* borare (pH 10); applied voltage, 9 kV. 1 = 5 m*M* glucose; 2 = 5 m*M* sucrose; 3 = 2 m*M* raffinose.

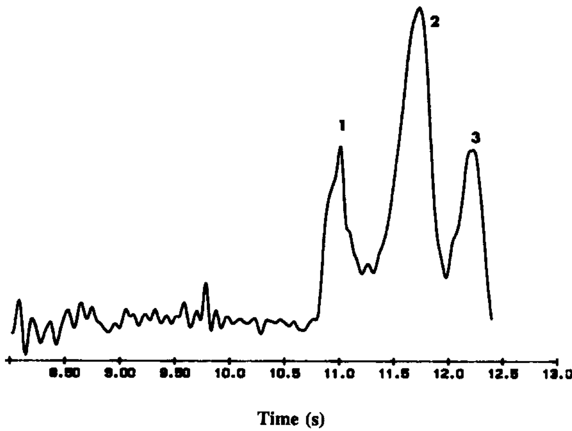

Fig. 6. Separation and detection of sugars as the borate complexes. Buffers, 50 m*M* borate (pH 10); applied voltage, 15 kV. 1 = 5 m*M* glucose; 2 = 5 m*M* sucrose; 3 = 2 m*M* raffinose.

buffer as these molecules form charged borate complexes [14,15]. Figs. 5 and 6 show the separation of underivatized glucose, sucrose and raffinose. The total time of the analysis is about 30 s. This time can be substantially decreased by increasing the separation voltage (Fig. 6). The detection limit is estimated to be in the sub-millimolar range from the signal-noise ratio in Figs. 5 and 6. This sensitivity is of the same order of magnitude as that of the indirect fluorescence method [16], which needs an expensive helium–cadmium laser, a photomultiplier and carefully selected buffer systems. The detection of glucose and sucrose by the direct spectrophotometric method is impossible because of the lack of a chromophore in their structure. It can be noted that the width of the peaks is similar in both instances, about 3 mm, much higher than expected when assuming the diffusion model discussed above. It appears that the heterogeneity of the complexes formed plays an important role in the contribution to peak broadening. This mechanism was discussed extensively when considering

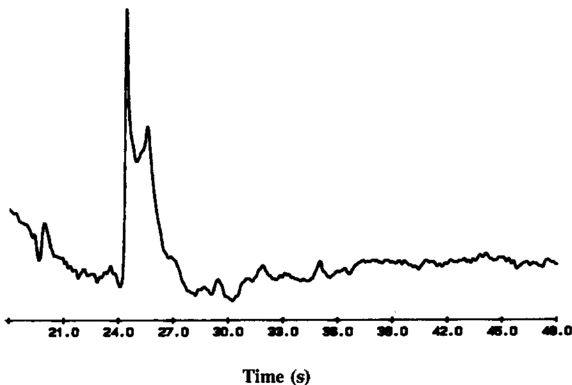

Fig. 7. Rapid separation and detection of sugars in a sample of corn syrup. Buffer, 50 m*M* borate (pH 10); applied voltage, 9 kV; sample concentration. 4 mg/ml.

micellar capillary electrophoresis [17]. The efficiency of the separation process is still over 10 000 theoretical plates. Fig. 7 shows the rapid separation of sugars in a sample of corn syrup. The individual components are not fully resolved because of the many components present. However, such a separation has application in rapid quality control by examining the electrophoretic trace as is commonly done in polymer analysis with size-exclusion chromatography. To improve separations for complicated samples, the length of the capillary needs to be substantially increased. This, however, will result in longer separation times and lower sensitivities of the concentration gradient detection method.

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