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Continuous sample collection in capillary zone electrophoresis by coupling the outlet of a capillary to a moving surface

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ABSTRACT

Use of an on-column frit structure, constructed by sintering a mixture of glass powders, makes it possible to ground a fused-silica capillary on its side prior to its outlet. Electroosmotic pressure permits convenient sample collection. We illustrate the use of this device by depositing the eluent in a continuous manner on a moving surface. This provides a permanent record of the separated species in a mixture.

INTRODUCTION

Capillary zone electrophoresis (CZE) is rapidly becoming a powerful separation method for the analysis of complex mixtures, particularly those involving biomolecules in aqueous media[1–4]. Most applications involve on-column detection of the species of interest as a function of time to produce an electropherogram. Collection is made difficult because the capillary outlet is usually placed in a large reservoir containing milliliters of electrolyte and one of the electrodes. The volumes of the sample zones are typically nanoliters, so that the dilution factor is on the order of 10^6. A second problem is the remixing of the separated species in the outlet reservoir.

Several different approaches have been taken to overcome these problems so that the eluent can be collected. Rose and Jorgenson[5] move the capillary outlet from one small fraction collector to another in a programmed manner. A similar technique involving interruption of the applied voltage has been employed by Cohen *et al.*[6]. Both these methods have the disadvantage that the capillary outlet must contact electrolyte in the fraction collector to complete an electrical circuit. This causes some dilution of the sample as well as raises the problem of possible interference from electrochemical reaction at the outlet electrode inside the fraction collector. However, it is a simple matter to reconcentrate the fraction collected by evaporation, and electrochemical reactions are often minimal.

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Recently, we have developed an alternative approach for sample collection. In this method the electrical circuit is completed in the capillary prior to its outlet[7]. This is achieved by making a frit structure in the sidewall of the capillary about 1–2 cm before its outlet. This frit allows electrical connections to be made to the capillary so that the first segment of the capillary (inlet to frit) may be used for electrokinetic separations while the second segment (frit to outlet) may be used for field-free eluent collection because the electroosmotic flow in the first segment pumps eluent through the second segment. Measurements show that the leakage of eluent through the frit is nearly negligible; more than 90% of an injected sample can be collected[7].

In this paper we illustrate the use of a capillary with an on-column frit for sample collections in which we couple the capillary outlet to a moving surface (filter paper placed on the periphery of a plastic disc). In this way we are able to deposit continuously the eluent along a track on the filter paper, which becomes a recording of the separation. This record may be subsequently read by some detection scheme, such as fluorescence, or it may be interfaced to another separation method, such as paper or thin-layer chromatography.

EXPERIMENTAL

Construction of apparatus

The description of how to fabricate the frit structure has been presented elsewhere[7]. Briefly, a focused CO_2 laser makes a slightly tapered hole about 40 μm in diameter on the side of the fused-silica capillary wall (75 μm I.D., 360 μm O.D., Polymicro Technologies, Phoenix, AZ, U.S.A.). Next, a mixture of glass powders is used to cover this hole and is sintered in place. Finally, a protective jacket is carefully attached, and the entire frit structure is surrounded by a reservoir of electrolyte containing a Pt electrode. The yield of acceptable frit structures is presently about 50%. Once an acceptable frit structure has been made, no degradation in performance has been observed during months of operation.

Fig. 1 shows how the capillary outlet is interfaced to a moving surface. The particular configuration we have chosen is a rotating wheel whose speed of revolution

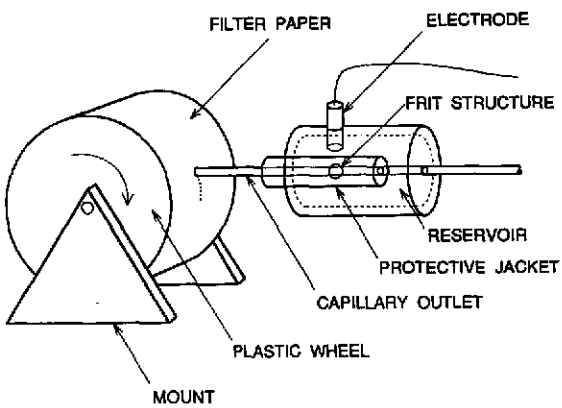

Fig. 1. Experimental setup for continuously depositing eluent onto a moving surface.

is controlled by a variable gear train attached to an electric motor. The periphery of the wheel is covered with a strip of filter paper (No. 41-01870; Schleicher & Schüll, Keene, NH, U.S.A.). The rate of linear displacement is variable but we typically operate at 0.1 mm/s. The position of the capillary outlet is adjusted so that it lightly touches the moving surface.

In order to view the capillary outlet during CZE separation, we use a 50× microscope (5× objective, 10× eyepiece) attached to a 35 mm SLR camera (Nikon FE). We also use UV fluorescence to view the eluent track on the filter strip. A photograph is made by irradiating the filter strip with a portable UV lamp (Model UV6-11; UVP, San Gabriel, CA, U.S.A.). For some runs it is convenient to use on-column detection. This is accomplished using UV absorption (Model UVIDEX-100 V; Japan Spectroscopic Co., Tokyo, Japan).

Chemicals

All chemicals are from Sigma (St. Louis, MO, U.S.A.) and are used without further purification. Buffer consists of 10 m*M* phosphate at a pH of 6.8. Water used to prepare solutions is freshly deionized and distilled with a water purifier (Model LD-2A coupled with a Mega-Pure automatic distiller, Corning Glassworks, Corning, NY, U.S.A.).

RESULTS AND DISCUSSION

Fig. 2. shows the droplet formation at the capillary outlet during CZE operation. The conditions were phosphate buffer with no sample injection. The electric field strength was 300 V/cm. Fig. 2a shows the capillary outlet before starting a run. Fig. 2b is a picture of the outlet *ca.* 1 s later, and Fig. 2c is *ca.* 3 s later. This last figure illustrates the formation of an eluent droplet with a volume of *ca.* 15 nl. Thus, we are able to visualize directly the pumping action of the electroosmotic flow in the first segment of the capillary (between the inlet and the ground through the frit structure).

In previous work[7], we collected the eluent for a fixed period of time in a 0.5-ml disposable microcentrifuge tube. By weighing the contents of the microcentrifuge tube we were able to determine that the flow-rate is linear with time. Moreover, we could reinject the collected sample to demonstrate that we were able to collect separate fractions of a mixture. We investigated the reproducibility of this collection scheme and found a coefficient of variance of less than 11%.

In this paper we report the "direct writing" of the eluent onto a moving surface, using the experimental setup described in Fig. 1. A three-component mixture of dansylated amino acids, *ca.* $1 \cdot 10^{-3}$ *M* each, was injected by raising the inlet of the capillary about 7 cm higher than the outlet for 10 s. This procedure gives an injection volume of about 20 nl. The electric field strength was again 300 V/cm. Fig. 3 shows a picture of the resulting filter paper strip under UV irradiation. For convenience, a ruler has been placed in this picture the origin of which is located at the start of electrophoresis. Three fluorescent spots are clearly visible in Fig. 3. The spot at shortest distance from the origin is dansyl-Arg, the next spot is dansyl-Ser, and the last spot is dansyl-Glu. Each spot corresponds to a few picomoles of the dansylated amino acid.

In a subsequent study, we have cut out one of the three spots, eluted the spot with

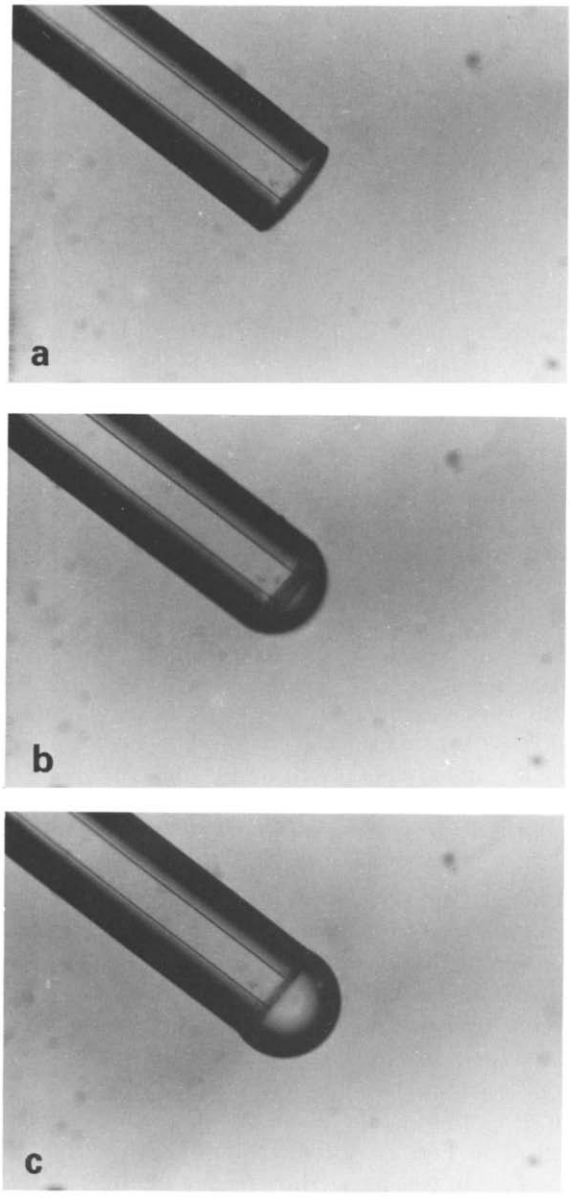

Fig. 2. Microphotographs (50 ×) of droplet formation at the outlet of a capillary having an on-column frit structure: (a) at start of electrophoresis, (b) *ca.* 1 s after start of electrophoresis, and (c) *ca.* 3 s after start of electrophoresis.

methanol, dried the methanol extract, redissolved the residue with 1 μl of buffer, and reinjected this sample. Using the on-column UV detector, we could easily see a single peak at a migration time corresponding to the dansyl amino acid injected. This simple procedure is able to recover more than 90% of the originally injected analyte.

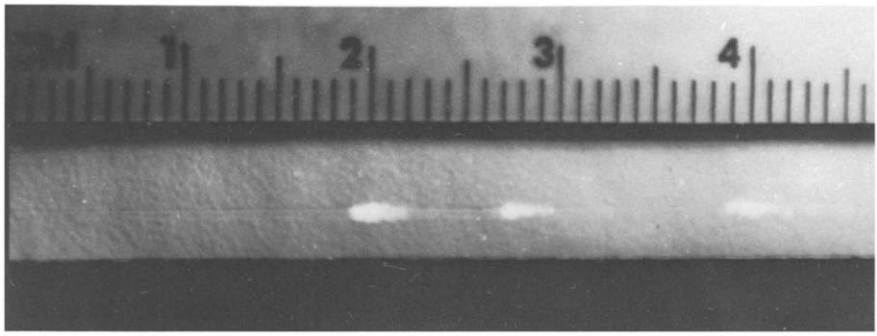

Fig. 3. Photograph of filter paper strip under UV irradiation (254 mm), showing fluorescence from the deposition of three dansylated amino acids. A ruler has been included to indicate length along track; the origin of the ruler is located at the start of the electrophoretic run.

This collection procedure has many advantages compared to writing with the outlet of a normal capillary on wet filter paper placed on a metal plate to complete the circuit. We found that the latter procedure suffered from the difficulty of maintaining the filter paper sufficiently wet to make good electrical contact but sufficiently dry to avoid extensive spreading of the spot. In addition to providing a convenient permanent record of the separated species in a mixture, the present device can also serve as the interface to another separation scheme, such as paper or thin-layer chromatography, so that "two-dimensional separations" are obtained. It is also possible to use this procedure to improve detection, such as taking autoradiographic exposures of the record track after radiolabels[8] are used to tag components in the mixture.

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