and one must rely on the less specific but still powerful predictions of theory.

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Use of an On-Column Frit in Capillary Zone Electrophoresis: Sample Collection

Xiaohua Huang and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, California 94305

The design of a simple, on-column frit for capillaries is described. The frit allows electrical connection 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) is free of applied electric field, facilitating its use either for electrochemical detection or for sample collection. The latter is illustrated, and a quantitative study is made of its performance as a fraction collector.

Capillary zone electrophoresis (CZE) as it is normally practiced has both the inlet and outlet of the capillary submerged in electrolyte reservoirs to complete a closed circuit (*1-3*). Consequently, sample zones are directly discharged into the outlet reservoir. This severely hinders sample collection. One way to overcome this problem has been introduced by Rose and Jorgenson (*4*), who move the capillary outlet from one fraction collector to another in a programmed manner. A similar technique involving interruption of the current flow has been employed by Cohen et al. (*5*). Both these methods have the drawback that the capillary outlet must contact electrolyte in the fraction collector to complete a circuit. This causes the collected sample to be diluted. There is also a possible problem from electrochemical reactions at the outlet electrode, which is typically submerged in a small amount of electrolyte in the fraction collector.

A superior approach would be to complete the electrical circuit in the capillary prior to its outlet. One method to achieve this has been introduced by Wallingford and Ewing (*6*), who use a porous glass junction. This junction is prepared by cutting the capillary tubing into two segments and placing both inside a custom-made porous glass sleeve that makes electrical contact to complete the circuit. Dead volume effects are minimized by carefully matching the capillary outside diameter to the inside diameter of the porous glass sleeve. The separation potential may be applied over the first segment of the capillary without significantly affecting the second segment. The porous glass sleeve permits ion movement but not bulk electrolyte flow, allowing its use for electrochemical detection (*6*) or sample collection (*7*).

We describe an alternative method for achieving the same objectives as those of the porous glass junction. We present the use of an on-column frit structure that can fulfill the need to complete the electrical circuit prior to the outlet of the capillary. Such a device offers all the advantages of being able to collect sample with no external dilution caused by the collection procedure. Also, it allows the use of detection schemes in which the detector is at ground potential, such as electrochemical detection (*6*).

EXPERIMENTAL SECTION

Construction of Frit Structure. We use a focused CO_2 laser to make a hole (about 40 μm in diameter) on the side of the capillary wall (*8*, *9*). The capillary is fused silica (Polymicro Technologies, Phoenix, AZ) having an inside diameter of 75 μm. We insert a tungsten wire (50-μm o.d.) inside the capillary in order to cover the capillary hole. Then a mixture of solder glass (7723, Corning Glassworks, Corning, NY) and powdered fused silica (Thermal American Fused Quartz Co., Montville, NJ, used as received) with particle size between 1 and 10 μm is added to amyl acetate to make a slurry, which is used to paste over the hole in the capillary. Once the hole structure has set, which is aided by gentle heating, the tungsten wire is removed. The proportion of Corning solder glass 7723 to fused silica is about 4:1. Next, the hole structure is placed inside a miniature, 1-cm-long heater, made of wound nichrome wire. The sintering temperature of solder glass is about 700 C and that of fused silica is about 2000 C. It has been found that satisfactory frit structures can be made by heating the mixture to about 1000 C for approximately 30 s. This procedure is illustrated in Figure 1.

The capillary is fragile in the region of the frit structure where the polyimide coating has been removed. To reduce its fragility, we have built a protective jacket, shown in Figure 2a. This jacket is made of Delrin with Teflon washers cushioning the capillary. The ends of the jacket are sealed with epoxy (Torrseal, Varian, Lexington, MA). There is a hole in this jacket that allows it to

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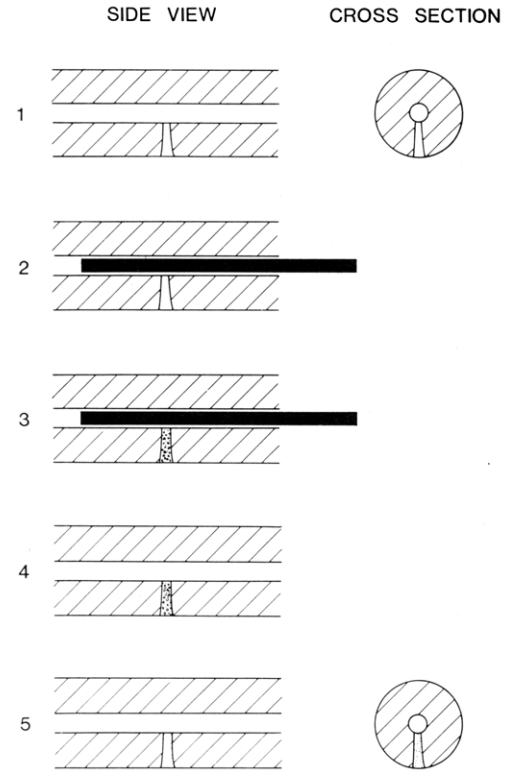

Figure 1. Construction steps for fabricating the on-column capillary frit: (1) prepare hole in wall of capillary; (2) insert tungsten wire to cover hole; (3) add slurry of glass solder and fused silica powder, and heat gently until set; (4) remove tungsten wire; and (5) heat locally frit structure to cause particles to sinter.

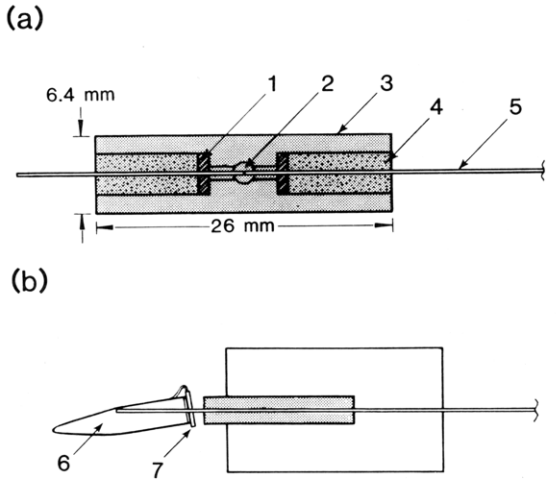

Figure 2. (a) Protective jacket for frit structure: 1, Teflon washer; 2, hole in side of jacket lining up with frit so that electrolyte can contact frit structure; 3, jacket body, made from Delrin; 4, epoxy seal; and 5, capillary. (b) Simple device for collecting sample: 6, disposable microcentrifuge tube; and 7, cap with hole allowing insertion of the capillary.

be filled with electrolyte so that the frit structure becomes part of the electrical circuit. This is shown in Figure 3.

We find that we can make this frit structure on a routine basis with a yield of 50%. The major problem is that some frits leak, i.e., do not seal satisfactorily. There seems to be a small variation from one frit to another, discounting those that seal improperly. This variation manifests itself by the observation of different electroosmotic flow rates (see below). Once an acceptable frit structure has been fabricated, no degradation in performance has been observed during several months of operation.

Previous studies by Wallingford and Ewing (*6*) showed that the separation efficiency decreased as the distance between the grounding structure and the outlet was increased. They found

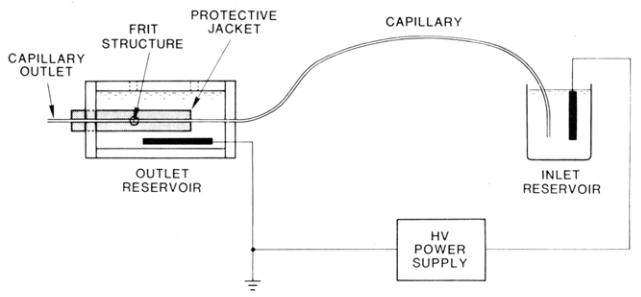

Figure 3. CZE setup with on-column frit.

that when this distance is shorter than 2.0 cm the efficiency is vritually no different than that of a standard CZE system employing the same electric field strength and detection conditions. Consequently, we chose to place the frit structure about 1.5 cm from the outlet. If desired, this distance can be easily decreased to as short as 0.5 cm.

When one compares the frit structure with the porous glass junction for on-column electrical contact in CZE, it appears that the frit structure may have a longer lifetime and less dead volume, and its fabrication may be readily automated. However, its construction requires that a small hole be made in the side of the capillary wall, a task that is routine with a focused CO_2 laser but difficult otherwise. On the other hand, the porous glass joint requires "thirsty glass" with dimensions matching the capillary. Unfortunately, this material is not readily available.

Instrumentation. The CZE system has been described elsewhere (*2*). Samples are introduced by gravity at the cathodic or the anodic end of the capillary by raising the inlet a known height (7–12 cm) with respect to the outlet for a fixed period of time. The injection volume is determined in continuous fill mode either by observing the time it takes for the sample to reach the detector or by weighing the amount of eluent collected for a fixed time period. Gravitational injection avoids biases associated with electrokinetic sampling (*10*). On-column detection is accomplished using UV absorption (Model UVIDEC-100 V, Japan Spectroscopic Co., Tokyo). A reversible high-voltage power supply (Model R50B, Hipotronics, Inc., Brewster, NY) provides a variable separation voltage of 0–30 kV. For sample collection we used disposable microcentrifuge tubes (Applied Scientific, San Francisco, CA).

Chemicals. All chemicals are from Sigma Chemical Corp. (St. Louis, MO) and are used without further purification. Different buffers are used, mostly phosphate buffers at pH = 6.8 with a concentration of 10 mM for collection studies and 20 mM for quantitation of collected samples. 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).

RESULTS AND DISCUSSION

Characterization of Capillaries with On-Column Frits. It is necessary to ascertain that the frit structure does not adversely affect the operation of the capillary. CZE separations were run for a frit-free capillary and for a frit capillary of the same length. It is found that the value of the current is about the same (<2% deviation in 10 runs) in both capillaries for the same applied electric field strength and buffer. As a further check, the electroosmotic flow rate was measured using the current-monitoring method (*11*). The grounding was made either through the frit structure or through the capillary outlet, which is about 1.5 cm from the frit structure. It was observed that the electroosmotic flow rate was essentially the same within a single capillary.

We also used the weighing method (*12*) to calculate the electroosmotic flow for a capillary having a frit. Here the electrolyte leaving the capillary outlet is collected in a 0.5-mL disposable microcentrifuge tube with attached cap that has a 0.8-mm-diameter hole drilled in its center (see Figure 2b). The capillary outlet is inserted inside this hole, making a loose fit. Because there is only a small gap between the capillary (360-μm o.d.) and the hole, evaporation of liquid inside the

collector tube is very slow. A control test was done on 10 tubes filled with 3 μL of electrolyte and having the capillary inside the hole in the cap for 30 min. The weight loss during this period was less than 0.1 mg in each case. Because collection normally takes less than 30 min, evaporation losses may be considered to be negligible. With this procedure, four capillaries with on-column frits were tested. The results of 10 repeat runs of each capillary show that the CV (coefficient of variation) for each capillary is less than 6%. Between capillaries there is a much larger variation in the electroosmotic flow rate (~30%). This variation likely represents the differences between the frit structures, which may pass electrolyte to different extents. We have also checked whether the electroosmotic flow rate depends linearly on the applied voltage for a capillary with an on-column frit. We find that a linear relationship holds in the range 100–500 V/cm (r = 0.98). In another study we hold the applied electric field strength fixed and vary the collection time from 5 to 30 min. The amount of sample collected shows good linearity with collection time, indicating that the electroosmotic flow rate is stable.

These results encourage us to believe that the on-column frit structure is a useful means for separating the capillary column into two segments, one in which electrokinetic separation takes place, another in which the sample may be detected or collected without an external electric field being applied.

Sample Collection. One advantage of the on-column frit structure is that it enables us to collect eluent continuously. It is not necessary to interrupt the electrokinetic separation because the capillary outlet is not part of the electrical circuit. The outlet is at or near ground potential so that it can be manipulated without any safety precautions. Therefore, it is easy to couple it to various collection (or detection) systems.

Two methods are used to collect sample. In the first, we collect the eluent for a fixed period of time in a fraction collector. However, this method may be unsatisfactory when two analytes have nearly identical electrokinetic migration times. In the second method, a detector is placed very close to the capillary outlet and the detector signal is used to trigger the collection procedure. We have implemented the second method with a conductivity detector, fabricated as described previously (*8*).

To demonstrate that we are able to collect different samples, we carried out the following study. First we prepared a mixture of pyridoxamine dihydrochloride ($C_8H_{12}N_2O_2 \cdot 2HCl$) and adenosine ($C_{10}H_{13}N_5O_4$) in 10 mM phosphate buffer so that the concentration of pyridoxamine was 2 mM and that of adenosine 1 mM. Then 45 nL of this mixture was injected into a capillary with an on-column frit. Eluent was collected during the period 0–6 min. The total volume collected was about 2.8 μL, which is greater than the total volume of the capillary used (~2.4 μL). We reinjected 20 nL of the collected sample in a second CZE setup having a UV detector. This setup allowed us to quantitate what had been collected. The resulting electropherogram is shown in Figure 4a. Two peaks are observed, corresponding to pyridoxamine and adenosine, as expected. Under the experimental conditions, pyridoxamine is positively charged and elutes before adenosine, which is almost neutral. The concentration of these species has been reduced by about a factor of 100 by this separation and collection procedure, but is still sufficient for CZE/UV detection.

We have repeated this experiment but collected the eluent into three fractions, one during the period 0–3 min, another during the period 3–6 min, and a third during the period 6–9 min. Each fraction has a total volume of about 1.4 μL. We reinjected 20 nL of each fraction, and the resulting electropherograms are shown in Figure 4b–d. We observe that fraction 1 contained only pyridoxamine, fraction 2 adenosine, and fraction 3 neither pyridoxamine nor adenosine. We conclude that the on-column frit structure allows us to collect different sample species individually.

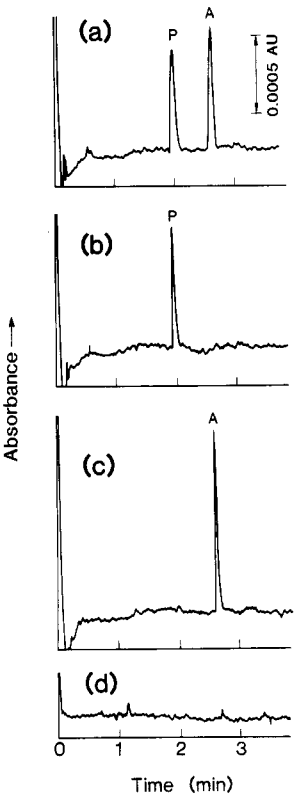

Figure 4. Electropherograms of sample fractions containing pyridoxamine (P) and adenosine (A): sample collected (a) 0–6, (b) 0–3, (c) 3–6, and (d) 6–9 min. Operating conditions for collection were 10 mM phosphate buffer at pH = 6.8, capillary length 50 cm from inlet to frit and 1.5 cm from frit to outlet, applied voltage 20 kV, and current 20 μA. The electroosmotic flow rate was about 7.5 nL/s. Operating conditions for detection were 20 mM phosphate buffer at pH = 6.8, capillary length 42 cm from inlet to UV detector window and 19 cm from there to outlet, applied voltage 25 kV, and current 32 μA. The UV wavelength is 254 nm.

Next we establish the reproducibility of the collection procedure for small fractions. In these studies we collected only about 0.4 μL of eluent. Some additional buffer was added to the make the total volume 0.8 μL. The collection sample tube was centrifuged 5 s to make the liquid stay at the bottom. Care must be taken in injecting sample from such a small volume. We did an experiment to examine the variance of peak height in 10 runs in 0.8 μL of total sample volume. The CV is less than 11% for the species injected.

The frit structure may be operated with the electroosmotic flow either from the anode to the cathode (usual procedure) or from the cathode to the anode (reversed electroosmotic flow). The latter is readily achieved by adding a cationic surfactant to the buffer (*13*). We find that such surfactants do not interfere with the operation of the frit junction.

We have determined the dilution factor for our sample collector by studying the CZE separation of adenosine diphosphate (ADP) at different concentrations. The results are shown in Figure 5. The initial injection volume is about 40 nL (30-s gravitational injection at a height difference of 10 cm). The collected sample was reinjected on a CZE setup with a UV detector. The concentration of the collected sample was determined from the absorbance signal, which was calibrated against an ADP sample of known concentration. Figure 5 shows that the concentration of sample collected is directly proportional to the concentration of sample injected for the

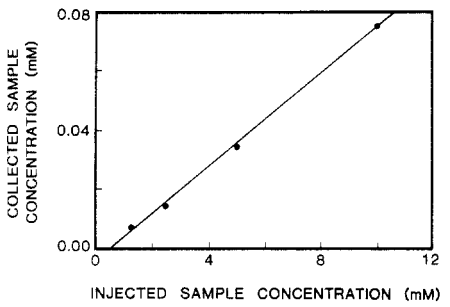

Figure 5. Plot of collected sample concentration verus injected sample concentration for adenosine diphosphate.

concentration range studied (r = 0.99). The inverse of the slope gives the dilution factor. For this study we have determined the dilution factor for ADP to be 1:130. Rose and Jorgenson (*4*) reported that their fraction collector has a dilution factor of about 1:3000 for adenosine, some 25 times larger than that of the present study. It is possible to reduce further our dilution factor by injecting larger amounts of sample, if desired. We have been able to find conditions whereby the dilution factor is only 1:10. However, this decrease in the dilution factor is only obtained by also sacrificing resolution through the use of large sample plug lengths (*14*).

One possible source of the observed dilution is leakage of injected sample through the frit structure. We have determined the percentage of sample collected by injecting fluorescent sample (dansyl-L-leucine) and determining the ratio of the sample in the reservoir attached to the frit structure to that in the sample collector. We find that the leakage is about 10%, 8%, and 7% for three on-column frit-structure capillaries that we tested. Thus, more than 90% of the injected sample can be collected.

The small dilution factor combined with the high recovery factor for the on-column frit structure represents a particular advantage of this method. In this collection procedure, it is not necessary to place an electrode with a certain amount of electrolyte in the fraction collector. We therefore avoid external dilution by the electrolyte in the fraction collector (and we also avoid possible interference from electrochemical reactions at the electrode). Thus, the on-column frit structure has many favorable features that recommend its use as a sample collector. Moreover, this frit structure may allow CZE to be readily interfaced to other hyphenated separation schemes.

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