Current-Monitoring Method for Measuring the Electroosmotic Flow Rate in Capillary Zone Electrophoresis

Xiaohua Huang, Manuel J. Gordon, and Richard N. Zare*

Department of Chemistry, Stanford University, Stanford, California 94305

Capillary zone electrophoresis (CZE) is attracting much attention (*1–4*) as a new separation technique that can complement high-performance liquid chromatography (HPLC). In CZE a migration channel of capillary dimensions is filled with electrolyte; a sample to be analyzed is injected at one end of the channel; and a high voltage is applied across the channel. When the electrolyte contacts the walls of the capillary, the inner surface of the capillary becomes charged, either through the ionization of surface groups on the capillary walls or through the adsorption of charged species from the electrolyte onto the inner surface. In either case, the electrolyte inside the capillary is no longer electroneutral but acquires a net charge, which may be positive or negative. Under the action of the applied electric field, the electrolyte moves toward one end of the capillary, and this movement is referred to as electroosmotic (electroendosmotic) flow. In addition to the bulk flow of the electrolyte, electrophoresis also takes place; that is, the applied electric field exerts a force on positively charged species to cause them to move to the negatively charged electrode and on negatively charged species to cause them to move to the positively charged electrode. As a result, the components in the injected sample separate into distinct zones, based on their different mobilities. However, in many cases, the rate of electrophoretic flow is typically less than the rate of electroosmotic flow. Consequently, species in the injected sample move in one direction—the direction of the electroosmotic flow—and thus the different species can be detected as each zone passes through some suitable detector located downstream from the capillary inlet.

Clearly, a precise characterization of the electroosmotic flow is highly desirable not only for understanding CZE but also for optimizing the operation of CZE in analyzing a given sample. One way to measure the electroosmotic flow rate is to record the elution time of an injected uncharged marker solute, which will be carried through the capillary under the action of only electroosmotic flow (*4–6*). For this purpose it is necessary that the marker solute be truly neutral, that it have negligible interaction with the capillary walls, and that it be readily detected. Another way is to weigh the mass of electrolyte transferred from the capillary inlet to the capillary outlet over a timed interval (*7*). For this purpose, losses caused by evaporation must be eliminated and the use of a digital balance appears to be recommended. Both of these procedures have been demonstrated to give reliable measurements of the electroosmotic flow rate, provided that some care is taken. We describe here what we believe might be a simpler procedure for measuring the electroosmotic flow rate. It is based on recording the time history of the current during CZE operation. Thus the new method requires no special type of injected solute or detector, and it can be used by anyone carrying out CZE separations.

EXPERIMENTAL SECTION

Figure 1 shows the experimental setup used for measuring the electroosmotic flow rate by monitoring the current in the CZE system. The polarity of the power supply is chosen so that the electroosmotic flow is from electrolyte reservoir 1 to electrolyte reservoir 2 through the capillary tube T. The procedure is to fill capillary tube T and reservoir 2 with electrolyte at a concentration C and to fill reservoir 1 with the same electrolyte but at a different concentration C'. As the electrolyte at concentration C' in reservoir 1 migrates into capillary tube T during CZE, it displaces an equal volume of electrolyte at concentration C in the tubing. As a consequence, the total resistance of the fluid in the capillary tube changes, and this change can be followed by recording the current I during the CZE operation. A resistor, R (metal film, 10.0 kΩ, 2W), is inserted between the reservoir 2 electrode and ground. The choice of 10.0 kΩ for this resistor means that a 1-μA current change would produce a 10-mV potential drop across the resistor. A chart recorder (Linear, Model 585) is connected directly across the resistor R. In this manner I is recorded as a function of time during the CZE operation. The concentrations C and C' need not differ greatly for the electroosmotic flow rate to be measured. As will be shown below, it is sufficient for C and C' to differ by about 5%.

In our studies we used an electrolyte consisting of 20 mM sodium phosphate buffer with a pH of about 7.0. This solution is said to have the concentration C. The same electrolyte mixture at concentration C' is prepared by diluting the electrolyte at concentration C with water (19:1) so that $C' = 0.95C$.

The bipolar power supply delivers 19–21 kV, and the untreated fused silica capillary (Scientific Glass Engineering, Austin, TX) has an inside diameter of 75 μm and is 63 cm long. Under these conditions, with the current I at about 40 μA, the electroosmotic flow is in the direction indicated in Figure 1 when the polarity of the power supply is positive.

RESULTS AND DISCUSSION

Figure 2, trace a, presents the CZE current I as a function of time when reservoir 1 has phosphate buffer at concentration C' and capillary tube T and reservoir 2 have phosphate buffer at concentration C (where $C' = 0.95C$). Note that the current falls with time as the electrolyte of concentration C' displaces continuously the electrolyte of concentration C in the capillary tube. This current drop continues until the entire capillary becomes filled with electrolyte of concentration C'. If the direction of electroosmotic flow is reversed from what is shown in Figure 1, then there would be no current change. Hence this experimental setup readily determines the direction of electroosmotic flow. The time interval Δt is the time required to complete the filling of capillary tube T by the electroosmotic flow of the electrolyte in reservoir 1 into the tube. Thus with a knowledge of the capillary length L between reservoirs 1 and 2, the electroosmotic flow rate, v_{eo}, is given by $L/\Delta t$.

Because two different concentrations of the same electrolyte were employed in the above measurement, the question arises whether the electroosmotic flow rate is a strong function of electrolyte concentration, and if so, what does our measured value of v_{eo} mean. To investigate this question, we reversed the procedure; namely, we filled reservoir 1 with phosphate buffer at concentration C and capillary tube T and reservoir 2 with phosphate buffer at concentration C'. The resulting current versus time plot is presented in trace c of Figure 2. We observe no significant difference between the values of Δt for trace a and trace c. An unpaired comparison t test was applied to two groups of runs resulting in $t = 0.833$, which shows that the differences are not statistically significant. Therefore we conclude that the electroosmotic flow rate can be accurately determined by this simple current measurement method.

As a further check on this conclusion, we used an on-column conductivity detector (*8*) to measure the electroosmotic flow rate by observing the conductivity change as a function of time. The results are shown in traces b and d of Figure 2. The

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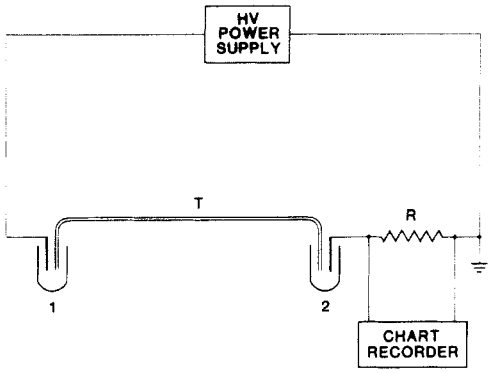

Figure 1. Schematic diagram of the current measurement for determining the electroosmotic flow rate. Here 1 and 2 denote electrolyte reservoirs, which are connected by the capillary tube T.

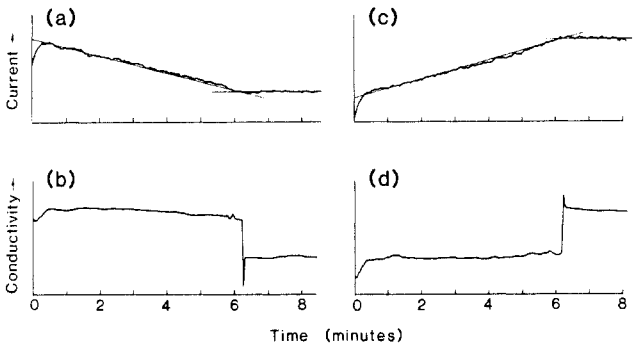

Figure 2. Electropherograms showing the measurement of the electroosmotic flow rate. Trace a shows the CZE current versus time for 19 mM phosphate buffer replacing 20 mM phosphate buffer in the capillary tube, and trace b shows, under the same conditions, the conductivity change. Traces c and d are the corresponding electropherograms when 20 mM phosphate buffer replaces 19 mM phosphate buffer in the capillary tube. The actual operating conditions are given in the text. In traces a and c regression lines are indicated to aid identification of the slope change.

conductivity detector is located only 5 mm from the outlet so that the distance to the detector is almost the same as the total length of the capillary tube, i.e., $l \approx L$. Consequently, the conductivity change should take place at almost the same time the slope in the current changes. This is observed to hold by comparing in Figure 2 trace a to trace b and trace c to trace d.

We also compared the current-monitoring method with the neutral marker method (*4–6*) for determining the electroosmotic flow rate. Following Tsuda, Nomura, and Nakagawa (*9*), we used pyridine as the neutral marker, and we employed a UV absorption detector. Three different experiments were carried out at an electric field strength of 300 V/cm in a capillary tube of 75-μm i.d. and 70-cm length (50 cm to the detector). First we filled reservoir 1, capillary tube T, and reservoir 2 with the same concentration of phosphate buffer (20 mM). By measuring the time of appearance of the UV absorption peak corresponding to pyridine and by knowing the length of the capillary to the UV absorption detector, we determined the electroosmotic flow rate to be v_{eo} = 0.149 43 ± 0.001 763 cm/s where the uncertainty is one standard deviation (eight runs). The second and third experiments are for the same concentration conditions as in traces a and c of Figure 2, respectively. In the second experiment, we find v_{eo} = 0.150 06 ± 0.001 198 cm/s for the neutral marker method and v_{eo} = 0.014 904 ± 0.002 655 cm/s for the current-monitoring method. In the third experiment, the corresponding values are v_{eo} = 0.149 72 ± 0.001 279 and v_{eo} = 0.014 861 ± 0.002 468 cm/s, respectively. We used a paired t test to determine if these measurements have a systematic relationship. We found for the second experiment t = 1.184 and for the third experiment t = 1.033. These values of t indicate that there is no significant difference between these two methods. The current-monitoring method is generally applicable and often more convenient. We recommend its use.

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Aperture-Based Thermospray Vaporizer

John A. Koropchak* and Henry Aryamanya-Mugisha

Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, Illinois 62901-4409

Thermospray is a means of aerosol generation that has gained great popularity as an interface between high-pressure liquid chromatography (HPLC) and mass spectrometry (MS) (*1*, *2*). Aerosols generated by thermospray have also been found to be advantageous for liquid sample introduction to inductively coupled plasma atomic emission spectrometry (ICP-AES) (*3–5*). Although the operating conditions of thermospray for HPLC/MS and ICP-AES differ significantly, the fundamental requirements of the thermospray vaporizer are similar for either experiment.

With thermospray, liquid is pumped through a capillary tube that is heated, partially vaporizing the solvent. The interaction of the hot gas (solvent vapor) with remaining liquid ultimately results in the formation of aerosol (*1*, *5*). Commercial designs of thermospray vaporizers consist of an electrothermally heated stainless steel capillary (1.6 mm o.d., 150 μm i.d.) that is brazed at the outlet end to a length of 6.4-mm stainless steel tubing. In order to provide a more chemically robust vaporizer tip for the corrosive environments encountered with ICP-AES samples, we have recently described the use of a stainless steel reducing union (6.4 mm to 1.6 mm) as a replacement for the brazed tip of the commercial designs (*4*).

A periodic problem arising with the use of thermospray has been clogging of the capillary due to deposition of nonvolatiles. Since operating temperatures for the ICP-AES experiment

* Author to whom correspondence should be sent.

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