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On-line measurement of electroosmosis in capillary electrophoresis using a conductivity cell

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ABSTRACT

A common phenomenon in capillary electrophoresis is electroendosmotic flow (EOF). Although often useful, EOF fluctuations introduce extra variances in the overall reproducibility of the analysis. In order to correct for these fluctuations, an on-line EOF monitoring method is required. The method presented utilizes a conductivity cell as the ground electrolyte reservoir, filled with dilute running buffer. During the separation the EOF will cause a continuous flow of non-diluted buffer into this reservoir. From the resulting continuously rising conductivity signal, the EOF can be calculated and used for, *e.g.*, migration time and peak area corrections.

INTRODUCTION

Capillary electrophoresis (CE) is a separation method in which species are separated in a fused-silica capillary tube based on the different velocities obtained in an electric field [1]. A phenomenon often occurring in these capillaries is electroosmotic flow (EOF). This is a buffer flow which originates when an electric field is applied over a capillary having a charged inner surface. Under most separation conditions in CE this is the case, although the extent can vary considerably. Often the silica surface is negatively charged, compensated for by a diffuse positive charge in the electrolyte. This charge separation is called the electric double layer. When an electric field is applied the negative surface charge is immobile, while the positive ions in solution are able to move in the field. These ions drag solvent along, resulting in a flow of the liquid directed towards the cathode [2].

Owing to the EOF it is possible to determine both anions and cations in a single experiment using electrophoresis. The conditions, however, should be such that the mobility of the EOF is higher than that of any of the anions. Tuning of the flow can be important in optimizing the separation conditions because optimum resolution is achieved when the mobility of electrophoresis and electroosmosis are equal but of opposite sign [1].

The EOF can be influenced by many parameters. Adsorption of compounds on the capillary wall or pH changes can cause shifts from run to run. To compensate, a marker molecule (often a neutral compound) is co-migrated in the analysis so that the magnitude of the flow can be established [3–5].

Several methods have been developed to measure the EOF under various electrolyte conditions. Reijenga *et al.* [6] measured the EOF in PTFE tubing using the streaming potential method. This method was later automated and ap-

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plied to fused silica by Van de Goor *et al.* [7]. Altria and Simpson [8] used a balance to measure the EOF by mass. Huang *et al.* [9] used the current monitoring method to obtain information on the EOF by replacing the buffer in the capillary tube. Wanders *et al.* [10] proposed the addition of a UV-absorbing compound to obtain EOF information. Both the weighing and the UV addition methods could be used in an on-line set-up [7,10].

The aim of measuring the EOF is always to determine whether the desired flow has been achieved. Several ways to change the flow can be used, such as capillary coating, the use of a surfactant [11], viscosity changes in the buffer [12] or the addition of a radial external field [13–15] to influence the zeta potential on the inner surface and hence the EOF.

The direct coupling of an EOF flow detector to an EOF flow modification method would be the ultimate goal [16]. Here, we investigated the use of a conductivity cell to measure accurately the electroosmotic flow without extensive modification to the separation set-up, based on osmotic flow.

EXPERIMENTAL

The conductivity cell used was laboratory made in the following way. A hole of 1 cm was drilled in a cylindrical Perspex rod. In the centre of this hole a stainless steel rod of 5 mm diameter was positioned. Two platinum wires were wrapped tightly around this rod and the ends were passed through a hole in the side, shown in Fig. 1. The empty space was filled with Insulcast 510 (Permagil Industries, Plainview, NY, USA) and subsequently polymerized. After hardening, the stainless steel rod was removed, leaving a cylindrical space with platinum wires mounted in the wall. This conductivity cell was mounted on a stirring mechanism, consisting of either a small magnetic stirrer or an air-driven rotor.

The electrodes were connected to a digital conductivity meter (CDM 83; Radiometer, Copenhagen, Denmark) isolated electrically by a d.c. shield (Philips DC250V, No. 2222-344-41225
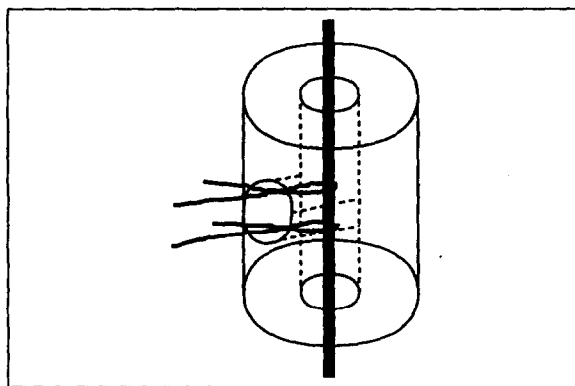

Fig. 1. Schematic diagram of the construction of the flow cell.

MKT metallized film capacitor). The set-up is shown in Fig. 2; a magnetic stirrer is used. The conductivity cell was also used as the ground electrolyte reservoir in the electrophoresis scheme. At the beginning of the experiment the capillary (Siemens, 50 cm total length × 75 μm I.D.) and the inlet/high-voltage reservoir were filled with running buffer (*e.g.*, 100 mM boric acid at pH 8.3). In the ground reservoir/conductivity cell a dilute buffer/deionized water is used. Then the high voltage is applied and EOF starts to occur, transporting buffer to the ground reservoir. Owing to this
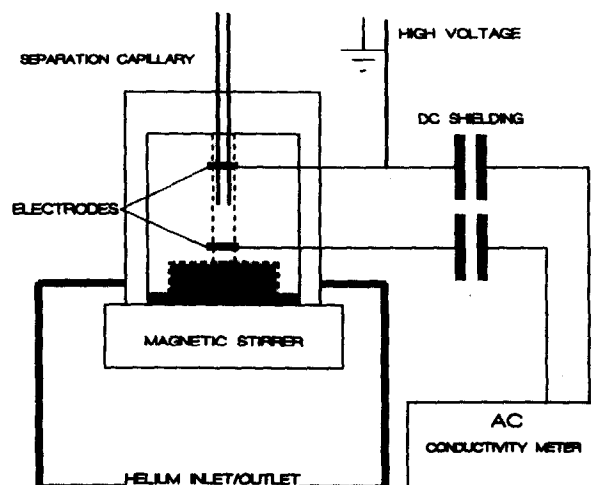

Fig. 2. Set-up of the conductivity cell and the measurement system.

flow the conductivity in the cell increases and the signal is measured.

RESULTS AND DISCUSSION

Fig. 3 shows the result from the conductivity measurements as a function of time. Line I shows the increase in conductivity when a gravity flow of buffer is used. To induce this flow the injection end of the capillary was lifted with respect to the ground end. Using the microbalance method [7], this flow was measured to correspond to 6 nl/s. This value was further used to calibrate the conductivity measurements.

Line II indicates the increase in conductivity due to the electroosmotic flow using different potential differences over the capillary. At 0 kV a constant value was measured as no buffer was transported. Then about every 60 s, the power supply setting was increased by 5 kV and the conductivity in the cell monitored. At 10 kV an increase in signal was observed having a slope comparable to the gravity flow. At higher voltages an even steeper curve was found. Using the calibration value, the EOF for these different situations can be calculated. The values are given in Table I.

A clear increase in EOF is observed when the applied voltage is increased, according to the

TABLE I

MEASURED CHANGE IN CONDUCTIVITY PER SECOND AND CALCULATED ELECTROOSMOTIC FLOW PER SECOND AS A FUNCTION OF APPLIED VOLTAGE

Voltage (kV)	Conductivity ($\mu S/s$)	EOF (nl/s)
0	0	0
5	0.019	3.4
10	0.034	6.1
15	0.049	8.8
20	0.082	14.7
25	0.161	23.2

theory. It shows that about every 20 s sufficient data are collected to calculate accurately the EOF. The linearity is fairly poor, however, especially for the higher voltages. This may be due to the fact that a non-thermostatted system is used, so a temperature increase can lead to an increased EOF.

Another problem which can occur is the influence of the electrode reactions on the increase in the conductivity in the cell. As electrode reactions must occur to allow current to flow, this can contribute to the conductivity in the cell. Even a change in pH is observed in the buffer reservoirs during longer runs. In a long run, at a high electric current this was found to disturb the accuracy of the method.

An advantage of the system, however, is that continuous information about the EOF is obtained and that the original situation can easily be restored by rinsing the ground reservoir. The set-up can easily be implemented in existing CE instrumentation without major modifications. The accuracy of the method is comparable to that of previously described methods.
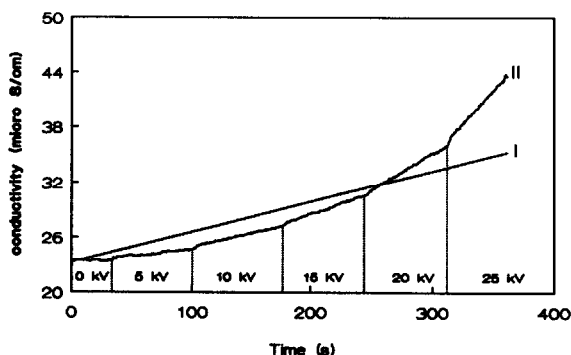

Fig. 3. Measured increase in conductivity in the cell as a function of time. Line I is for gravity flow (6 nl/s = 1 mm/s) and line II is for electroosmotic flow generated using different voltages across the capillary tube as indicated.

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