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On-column detection in capillary zone electrophoresis with ion-selective microelectrodes in conical capillary apertures

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ABSTRACT

In capillary zone electrophoresis with an ion-selective microelectrode (ISME) as detector in an on-column position, drift and noise problems are encountered, mainly because the ISME is not decoupled from the electrophoretic field and because temporary instabilities in its position give rise to potential changes, which are superimposed on the Nernstian response. To stabilize the position of the ISME with a precision of at least ± 10 nm would be very costly. This paper describes a procedure for drastically reducing drift and noise by etching the detector-side capillary end with hydrofluoric acid to a conical aperture. The field strength at the tip of the ISME is considerably reduced compared with that of the remaining capillary.

INTRODUCTION

In the past few years, the use of capillary zone electrophoresis (CZE) has greatly increased, but so far only UV and fluorescent detectors are commercially available. In contrast to optical and conductivity detectors, electrochemical and potentiometric [ion-selective microelectrodes (ISMEs)] [1,2] on-column detectors must be decoupled from the electrophoretic field, otherwise meaningless or noisy signals are obtained. Decoupling from the electrophoretic current was first achieved by means of a porous glass joint [3], which allowed catecholamines to be detected amperometrically with a carbon fibre inserted in the capillary end and an external reference electrode. The major drawback of amperometric detectors is that they are applicable only to electroactive analytes. For electrochemically inert systems and very small detection volumes, ISMEs have certrain advantages. In a single run, it is possible to detect widely different ions, *e.g.*, of neurotransmitters and inorganic electrolytes [4]. It could even be possible to detect individually two co-migrating ions with the help of two ISMEs showing adequate selectivity behaviour. In addition, the detection limits for specially designed ISMEs may be lower than those for conductivity detectors.

When first using ISMEs as CZE detectors [1,2], the microelectrode tip was positioned several micrometres behind the capillary end (post-column) to avoid drifting and noisy potentials due to the electrophoretic field inside the capillary. If the buffer vessel is considered as a sphere of infinite radius, r, with its surface as the common electrode and the capillary end at its centre, the electrophoretic field decreases with r^3. Owing to irreproducible (thermal) turbulence, post-column detection leads to distorted peak shapes, so that conditions for quantitative analysis and maximum resolution are not fulfilled. With the aim of using ISMEs as on-column detectors in CZE, the capillary end was etched to a conical aperture in which the field strength is lower and thus allows the accurate measurement of ions.

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THEORY

For the following theoretical treatment, a system consisting of an ISME inserted in a buffer-filled capillary (Fig. 1) is considered electrically as a voltage divider with the capillary as a potentiometer and the ISME as its wiper. This allows a simple description of potential drift and noise of the ISME caused by instabilities in its position.

The resistance, R_l, of a cylindrical volume element with length l and radius r is given by

$$R_l = \rho l/\pi r^2 \quad (1)$$

where ρ is the specific resistance of the buffer solution. If a voltage V is applied over the total length, L, of a buffer-filled capillary (resistance R_L) and the ISME is inserted to a length l, the potential, U_l, sensed by the ISME corresponds to

$$U_l = V(R_l/R_L) \quad (2)$$

Substitution of eqn. 2 with eqn. 1 gives

$$U_l = V(l/L) \quad (3)$$

Discrete values of U_l are of interest only in so far as they are superimposed on the Nernstian response of the ISME, thus falsifying the e.m.f. values. Much more important are changes in U_l as a function of l, which describe the potential drift and noise caused by variations in l. On the other hand, the derivative dU/dl, corresponding to the field strength, E, is proportional to drift and noise at the tip of the ISME. Derivative of eqn. 3 leads to

$$dU/dl = V/L = E \quad (4)$$

which means that E at any point in the capillary is independent of l. This relationship is expected for a cylindrical conductor, but does not hold if the capillary has a conical aperture (Fig. 2). This aperture geometrically has the form of a frustum of a cone with height K and radii g and r of the base and top area, respectively. The corresponding cone is characterized by its height, H, and slope, m:

$$m = g/H \quad (5)$$

The resistance, dR, of a volume element with infinitesimal height, dh (h being the distance between the tip of the cone and any point on the axis of the frustum), is calculated as follows:

$$dR = \frac{\rho}{\pi m^2 h^2} \cdot dh \quad \text{with } (H - K) \leqslant h \leqslant H \quad (6)$$

Definite integration of eqn. 6 between the limits a and b leads to

$$R = \frac{\rho}{\pi m^2}\left[-\frac{1}{h}\right]_a^b \quad \text{with } (H - K) \leqslant a \leqslant b \leqslant H \quad (7)$$

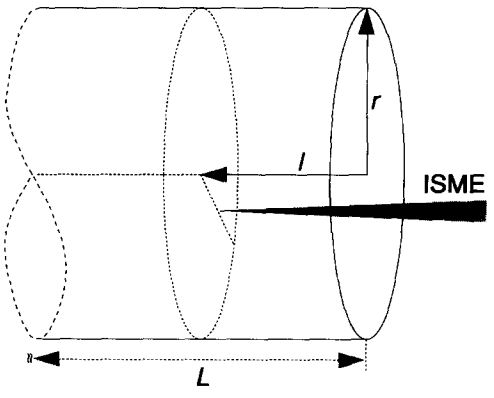

Fig. 1. On-column positioning of an ISME in a cylindrical capillary aperture. L = Total length of the capillary, l = distance from capillary end to ISME tip; r = inner radius of the capillary.

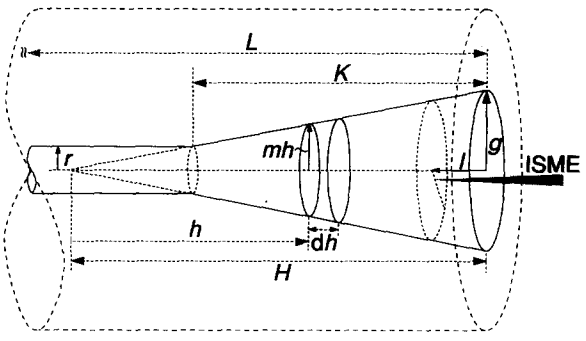

Fig. 2. On-column positioning of an ISME in a conical capillary aperture. g = Aperture radius at capillary end; H = height of conical aperture; h = distance from tip of the cone to any point on its axis; dh = infinitesimal element of h; K = height of conical frustum; L = total length of capillary; l = distance between tip of the ISME and capillary end; mh = radius of aperture at any point on cone axis; r = inner radius of unetched capillary.

Hence, the resistance R_l of a volume element of length l at the end of the capillary is given by

$$R_l = \frac{\rho}{\pi m^2}\left[\frac{1}{H-l} - \frac{1}{H}\right] \quad \text{with } 0 \leqslant l \leqslant K \quad (8)$$

Eqn. 8 only holds if $l < K$; if not, inaccurate values of R_l will result.

Using for R_L the same definition as for a cylindrical conductor (which entails an error $\ll 1\%$), U_l at the tip of the ISME in a capillary with a conical apertune is obtained by substituting eqn. 2 with eqns. 1 and 8:

$$U_l = \frac{Vr^2}{m^2 L}\left[\frac{1}{H-l} - \frac{1}{H}\right] \quad \text{with } 0 \leqslant l \leqslant K \quad (9)$$

Again, the derivative dU/dl yields the field strength, E_l:

$$E_l = \frac{Vr^2}{m^2 L} \cdot \frac{1}{(H-l)^2} \quad \text{with } 0 \leqslant l \leqslant K \quad (10)$$

Obviously, in this instance, the field strength is no longer independent of l, but decreases with $(H - l)^2$. This means that positioning the tip of the ISME in a conical aperture at a length, l from the end of the capillary yields lower field strengths than in a cylindrical aperture.

EXPERIMENTAL

Reagents

Chemicals of the highest purity available (Fluka, Buchs, Switzerland) and doubly quartz-distilled water were used.

CZE system and data acquisition

The CZE system used was similar to that described previously [1]. The electrophoretic field was generated by a Model 225-50 R high-voltage power supply (Bertan Associates, Hicksville, NY, USA). Fused-silica capillaries of I.D. 10 μm were purchased from Scientific Glass Engineering (Ringwood, Australia). Potentials were measured differentically, *i.e.*, the potential difference between the ISME and a reference electrode [Ag | AgCl | $MgCl_2$ (20 m*M*); tip diameter 1 mm] was determined with the aid of a platinum cathode as a common electrode. The platinum cathode, ISME, reference electrode and capillary end were placed in a small plastic vessel filled with buffer solution. On-column positioning of the ISME was achieved with micromanipulators and an inverse microscope (Narishige and Diaphot; Nikon, Chiyoda-ku, Tokyo, Japan). Capillaries were filled under pressure (80 bar) with buffer solution passed through a microfilter (0.2 μm Prep-Disk sample filter; Bio-Rad Labs., Richmond, CA, USA). For stabilizing the electrophoretic system, a high voltage was applied to the capillaries until a constant current was reached. The reference and the ion-selective electrodes were directly connected to Type AD 515 KH operational amplifiers (Analog Devices, Norwood, MA, USA), wired as voltage followers. Potentials were amplified with a laboratory-made electrode monitor.

Data were acquired with an Apple Macintosh IIx computer (Apple Computer, Cupertino, CA, USA) through a 16-bit NuBus A/D converter card (MacAdios II; GW Instruments, Somerville, MA, USA). Analysis of the electropherograms was performed with the program LabView (National Instruments, Austin, TX, USA).

Etching of capillaries

Conically shaped apertures were prepared by immersing the buffer-filled capillaries over a length of 3 mm in 40% hydrofluoric acid for 25 min. The dimensions (see Fig. 2) of the conical aperture were determined with a microruler under a microscope and typically were $g = 25$ μm and $H = 500$ μm.

Ion-selective microelectrodes

Preparation, pulling and silanization of the ISMEs have been described previously [5,6]. Under a microscope, tips were broken to a diameter of *ca.* 4 μm against a polished glass rod. By applying a slight overpressure with a syringe, the micropipettes were back-filled with 20 m*M* $MgCl_2$. The tips were then front-filled to a height of *ca.* 150 μm by dipping them into the ion-selective membrane phase, which consisted of potassium tetrakis(4-chlorophenyl)borate (10 mg) in 2-nitrophenyl octyl ether (500 mg). The ISMEs were conditioned on-column for 3 h, applying an electrophoretic voltage of 30 kV.

RESULTS AND DISCUSSION

Using unetched capillaries (see Fig. 1) of length 50 cm with an applied voltage of 30 kV, the field strength, E, at the tip of the ISME is 60 mV/μm according to eqn. 4. Through a microscope, it was observed that the ISME tip suffered slight variations of up to 5 μm/h in its axial position. Theoretically, this would lead to a drift of up to 50 mV in the time necessary for running an electropherogram (*ca.* 10 min). Accordingly, a noise of ± 0.4 mV would correspond to axial vibrations of about ± 7 nm, which of course are too small to be detected under the light microscope. On the other hand, theory shows that positional instabilities perpendicular to the capillary axis do not induce significant potential drift. This was confirmed experimentally by varying, within a single run, the position of the ISME perpendicularly to the capillary axis. The axial stabilization of the ISME within the maximum required range of ± 7 nm would be very costly and the use of such a detection system cumbersome. According to eqn. 10, E_t is expected to decrease significantly if on-column potentiometric detection is performed in a conically shaped capillary end. By inserting the ISME in the capillary aperture to a length of l = 10 μm (see Fig. 2), the field strength at the tip is only 2.5 mV/μm, which means that potential drift and noise are reduced by a factor of *ca.* 25.

TABLE I

TYPICAL POTENTIAL DRIFT AND NOISE VALUES FOR DIFFERENT CAPILLARY APERTURES

Parameter	Capillary aperture		Improvement factor
	Cylindrical	Conical	
Drift	≤5.0 mV/min	<0.17 mV/min	≈30
Noise [7]:			
Short-term	±0.4 mV	±0.06 mV	6
Long-term	±2.0 mV	±0.25 mV	8

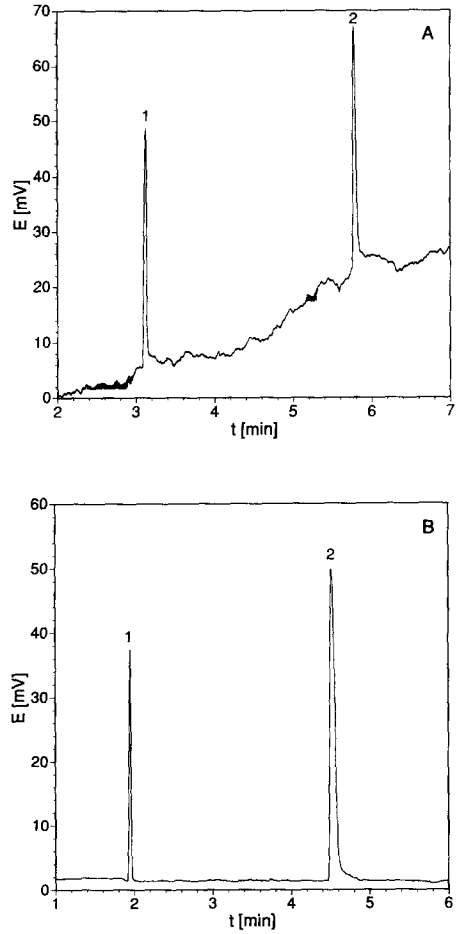

Fig. 3. CZE on a 50-cm capillary. Buffer, 20 mM magnesium acetate solution; voltage, 30 kV; injection, electrokinetic, 5 kV for 5 s. (1) 1 mM potassium chloride and (2) 3 mM dopamine hydrochloride in 10 mM magnesium acetate solution. (A) Cylindrical aperture, with ISME inserted 1 μm; (B) conical aperture, with ISME inserted 10 μm.

Fig. 3 shows two electropherograms obtained on CZE capillaries with a cylindrial and a conical aperture. The improvements in the baseline stability and the drift reduction are obvious. Table I gives typical results obtained with both detection systems. For the drift, the improvement factor is in agreement with the above theoretical calculation, whereas for the noise it is much lower. This could be due, *e.g.*, to noise from the electronic set-up or to environmental disturbances. Nevertheless, a straight baseline is achieved, which is of utmost importance for accurate peak integration and analyte quantification.

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