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Potentiometric detection of anions separated by capillary electrophoresis using an ion-selective microelectrode

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Abstract

Capillary electrophoresis in fused-silica capillaries of 10 μm I.D. coupled to an anion-selective microelectrode as on-column detector allows inorganic and organic anions, especially lipophilic ones, to be separated with high resolution and sensitivity of detection. For perchlorate, plate numbers of up to 10^7/h and a detection limit of $5 \cdot 10^{-8}$ M have been achieved. After delogarithmizing the response function of the microelectrode, quantitative results are obtained as described previously.

1. Introduction

Capillary electrophoresis (CE) has two major advantages as compared with conventional ion chromatography. On the one hand, there are almost no limits to the miniaturization of electrophoretic separation systems [1], allowing the injection of sample volumes down to the 100-fl range [2]. On the other hand, CE methods yield a flat elution profile providing much higher separation efficiency. To fully take advantage of these two facts, the detector must meet the following requirements: the detection must take place on-column since the elution profile outside the electric field changes to a parabola (as in conventional ion chromatography), which causes a drastic drop in the maximum resolution to be reached [3]. Furthermore, the detection device must be so sensitive as to respond to even a few ions only. For a CE analysis performed on an analyte solution of 10^{-10} M with a total run time of 200 s in a capillary of 50 cm × 10 μm I.D., the detector must respond to 20 zmol (ca. 10 000 ions)/s. Up to now, on-column detection of such low quantities was only possible with fluorescence spectroscopy. As an example, the detection of rhodamine 6G with axial-beam laser-excited fluorescence in a capillary of 75 μm I.D. has been reported with a limit of detection (LOD) in the 10^{-11} M region [4]. However, the sample, apparently prepared with distilled water, was injected electrokinetically which entails an enormous on-column analyte concentration [5] and makes it difficult to compare the result with others. Wu and Dovichi [6] detected amino acids labelled with fluoresceine isothiocyanate, obtaining a LOD in the pM region but give no details about the specific resistance or total ionic strength of the sample solutions. The major drawback of fluorescence detection is its limita-

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tion to fluorescent ions, although this is of minor importance when dealing with organic and easily derivatizable analytes. In the case of small organic and of inorganic ions, derivatization is not possible. Nevertheless, Milofsky and Yeung [7] separated non-derivatized, i.e. unlabelled, amino acids and performed native fluorescence detection achieving a LOD up to four orders of magnitude higher than for the corresponding derivatized compounds. Jones and Jandik [8] succeeded in separating 36 inorganic and organic anions with excellent resolution in a total analysis time of only 3 min, using indirect UV absorption as detection method and a chromate buffer as background electrolyte. The analyte solutions were prepared with distilled water to obtain samples of high specific resistance. On-column analyte enrichment was obtained by electrokinetic injection and for certain ions, LODs in the sub-ppt region were achieved. This means that the specific LOD of the detection system may be up to some orders of magnitude higher than the concentrations of the samples injected. Recently, CE systems with suppressed conductivity detection were described [9,10] for which detection limits in the range of 1–10 ppb were found with buffered sample solutions.

In view of achieving as low a LOD as possible, it seems more promising to concentrate on the development of direct detection methods. Indirect procedures have the disadvantage of implying the subtraction of two high values to give a very small difference thus limiting the LOD. For example, with indirect fluorescence detection [11,12], no significant improvement in sensitivity is to be expected over indirect UV.

In previous work, the use of ion-selective microelectrodes (ISMEs) as on-column detectors for cations in a CZE separation system has been reported [13,14]. The present paper describes the application of this technique to the separation of anions, using ISMEs based on anion-exchanger liquid membranes [15]. Their selectivity coefficients depend on the free enthalpy of hydration of the analyte anions [16], i.e. the more lipophilic the anion, the higher is the sensitivity of the ISME. Several inorganic anions plus an organic one have been separated in a total analysis time of 4 min. For perchlorate, a plate number of 300 000 and a LOD $< 10^{-7}$ M have been achieved. In analogy to the procedure described for the separation of cations [14], a calibration plot allows quantitative determinations of perchlorate.

2. Experimental

2.1. Reagents

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

2.2. CZE–ISME system with data acquisition

Except for minor modifications, the CZE apparatus with fused-silica capillary of conical aperture and an ISME as potentiometric on-column detector is similar to that described earlier [13,14]. The electrophoretic field was generated by a high-voltage power supply, Model 225-50 R (Bertan Associates, Hicksville, NY, USA). Fused-silica capillaries of 10 μm I.D. were purchased from Scientific Glass Engineering (Ringwood, Australia) and cut to a length of 50 cm. Poly(N,N,N′,N′-tetramethyl-N-trimethylenehexamethylenediammonium dibromide) (Polybrene)-coated capillaries were prepared by purging them with a 0.01% aqueous polybrene solution for 30 min. Afterwards, they were rinsed under pressure (50 bar) with buffer solution passed through a microfilter (0.2-μm Nalgene syringe filter; Nalge Company, NY, USA). The conically shaped aperture at the detection end was then obtained by immersing the buffer-filled capillary over a length of 3 mm in 40% hydrofluoric acid for 20 min. Potentials were measured differentially, i.e., the potential difference between ISME and reference electrode (Ag|AgCl| electrophoretic buffer solution saturated with AgCl; 1 mm tip diameter) was determined with a platinum wire serving at the same time as common electrode and electrophoretic ground (anode). The reference and anion-selective electrodes were directly con-

nected to operational amplifiers (type AD 515 KH, Analog Devices, Norwood, MA, USA) wired as voltage followers. Potentials were monitored with a laboratory-made electrode amplifier. The ISME, reference electrode, platinum anode and conically etched capillary end were placed in a small Plexiglass vessel filled with buffer solution. On-column positioning of the ISME was achieved with the aid of micromanipulators and an inverse microscope (Narishige and Diaphot; Nikon, Tokyo, Japan).

For data acquisition, an Apple Macintosh IIfx computer (Cupertino, CA, USA) equipped with a 16-bit NuBus A/D converter card (MacAdios II; GW Instruments, Somerville, MA, USA) was used. Electropherograms were displayed using the program LabView (National Instruments, Austin, TX, USA) and hard copies of the acquired data were generated with the graphic program DeltaGraph (Delta Point, Monterey, CA, USA).

2.3. *Anion-selective microelectrodes*

Glass micropipettes were pulled from clean borosilicate glass tubes (GC 150T-15, Clark Electromedical Instruments, Pangbourne, Reading, UK) with the help of a vertical pipette puller (Model 700C, David Kopf Instruments, Tujunga, CA, USA). Under a microscope, their tips were broken to a diameter of ca. 1 μm against a polished glass rod. The micropipettes were put vertically in a glass desiccator (without drying agent), flushed with nitrogen, placed in an oven with the desiccator valve left open and predried at 180°C for 1 h. After reflushing the desiccator with nitrogen and increasing the temperature to 200°C, N,N-dimethyltrimethylsilylamine (0.1 ml) was injected into it and allowed to react in the vapour phase with the micropipettes for 30 min. The hot silanized micropipettes were then transferred to another desiccator. They can be stored over silica gel for some weeks. Back-filling of the micropipette with electrophoretic buffer solution (saturated with silver chloride) was performed by applying a slight overpressure (ca. 5 bar) with a syringe. The tip was then front-filled to a height of ca. 100 μm by dipping it into the anion-selective membrane phase which consisted of 10% tridodecylmethylammonium chloride in 2-nitrophenyl octyl ether. After inserting a chloridized silver wire (prepared by immersing a 1 mm silver wire in 10% iron trichloride dissolved in 0.1 *M* hydrochloric acid for 24 h), the microelectrode was completed by fixing the silver chloride electrode at the top with insulating tape.

3. Results and discussion

Generally, the electrolyte solution in a fused-silica capillary is driven by the electroosmotic force from the anode towards the cathode. This can have an adverse influence on the separation, particularly when the absolute mobilities of the analyte anions are smaller than the electroosmotic mobility. It must be attempted, therefore, to reverse, stop or at least slow down the electroosmotic flow (EOF). This can be achieved by different techniques, e.g. by applying an external electric field to the outer capillary wall [17], adding a surfactant [18–20] or a viscosity modifier to the running buffer [21] or coating the inner capillary wall with a polymer [22]. With ISMEs as detectors in CE, surfactants (especially lipophilic additives, e.g. cetyltrimethylammonium salts, CTMA) proved to be useless. Although the addition of $5 \cdot 10^{-4}$ *M* CTMA to the background buffer causes a reversal of the EOF, the anion-selective liquid membrane phase was spontaneously washed out of the microelectrode. A further disadvantage of CTMA and similar quaternary ammonium salts is their affinity to lipophilic anions (as perchlorate, salicylate and others), causing precipitates or bulky agglomerates. Polybrene has proved to be a good surfactant, covering the glass surface with a positively charged layer [23], and specially useful when employing ISMEs as CE detectors. There is no need to add it to the background buffer. To obtain a robust film providing a stable EOF in the reverse direction, it is sufficient to rinse the capillary with a 0.01% aqueous Polybrene solution. From run to run, an average slowing down in EOF of only 0.02% has been observed (n =

500). This is significantly lower than the statistic variation in EOF for a single measurement, even without reversal.

Another possibility to decrease the EOF or bring it to a halt consists in lowering the pH of the background buffer to 2–3 [24]. This method can only be used if the pK values of the anionic analytes are smaller, or at least not significantly higher than the pH of the buffer. Otherwise, the elution times will become unacceptably long due to a drastic drop in effective charge number of the analyte ions.

To ensure that the results, particularly the LODs, can be compared with those obtained with other detection methods, on-column enrichment (as a consequence of electrokinetic injection) was avoided by preparing the analyte samples from 10^{-2} M stock solutions diluted with background buffer.

Fig. 1 shows a CZE–ISME analysis at pH 7.0 of six anions (each 10^{-4} M) in a Polybrene-coated capillary. Evidently, the peak heights are a function of the lipophilicity of the analyte anions. While the signal for ClO_4^- has a height of ca. 110 mV, that for Br^- reaches only ca. 7 mV. In the delogarithmized form (B), i.e. for $\mathcal{D}$ vs. t (where $\mathcal{D} = 10^{E/s} - 1$, with E as the potential measured and $s = -2.303\ RT/F$, the slope of the ISME response function for monovalent anions [14], R is the universal gas constant and F the Faraday constant), the effect of these differences in selectivity is even more obvious. The delogarithmized response allows to compare the CZE–ISME system with other ones, e.g. CE with UV, fluorescence or amperometric [2] detection or with ion chromatography. As in the last-mentioned method, the resolution of the CZE separation can be evaluated directly from the delogarithmized form. For the ClO_4^- peak, a plate number of 300 000 has been achieved. With regard to routine analysis, this means almost 10^7 theoretical plates/h. Besides, it is noteworthy that the resolution of peaks 4 (ClO_4^-) and 5 (SCN^-) is better, with less tailing, than the logarithmic form (A) of the electropherogram might suggest.

Similar electropherograms (Fig. 2) are obtained with an uncoated capillary when the EOF is greatly reduced or even brought to a standstill by lowering the pH to 2.5. Even at this low pH, the ISME shows the same characteristics as in neutral background buffer.

Fig. 3 shows the detection of 0.1 μM ClO_4^-. The peak height is of the same order of magnitude as the amplitude of the long-term noise [13], so that proper peak identification becomes difficult, whereas a signal/short-term noise ratio of 10 was obtained. Considering these results, the LOD was established at $5 \cdot 10^{-8}$ M.

A ClO_4^- concentration as low as 10^{-8} M in tap

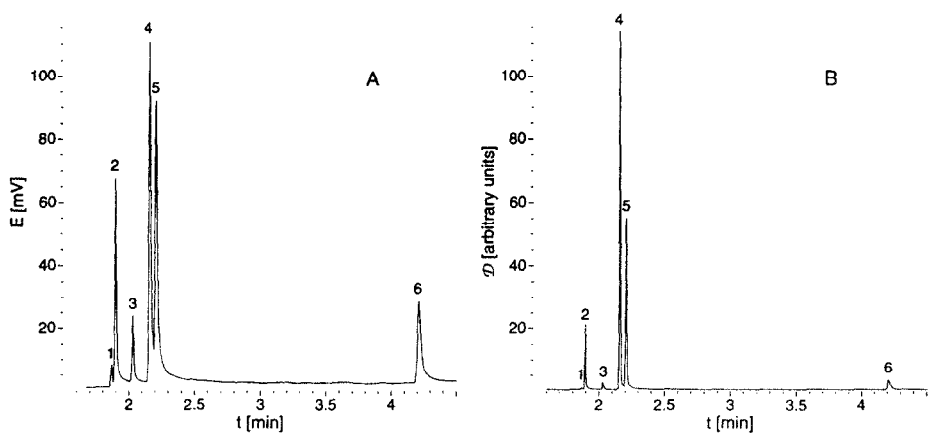

Fig. 1. CZE–ISME analysis of the Na^+ salts of (1) Br^-, (2) I^-, (3) NO_3^-, (4) ClO_4^-, (5) SCN^- and (6) salicylate, each 10^{-4} M in background buffer. Buffer: 20 mM Tris–formate, pH 7.0; separation voltage: 25 kV; electrokinetic injection: 3 kV for 3 s. Polybrene-coated capillary. (A) As recorded by ISME detector, (B) delogarithmized form (see text and [14]).

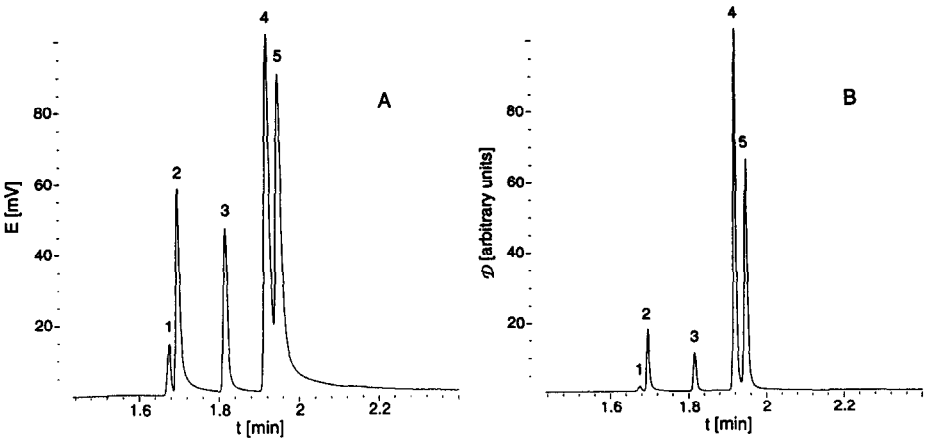

Fig. 2. CZE–ISME analysis of the Na^+ salts of (1) $5 \cdot 10^{-4}$ *M* Br^-, (2) 10^{-4} *M* I^-, (3) $5 \cdot 10^{-4}$ *M* NO_3^-, (4) 10^{-4} *M* ClO_4^- and (5) 10^{-4} *M* SCN^- in background buffer. Buffer: 20 m*M* Na_2SO_4, adjusted to pH 2.5 with H_2SO_4; separation voltage: 30 kV; electrokinetic injection: 2 kV for 2 s. Uncoated capillary. (A) As recorded by ISME detector, (B) delogarithmized form (see text and [14]).

water can be detected owing to the on-column concentration effect achieved by electrokinetically injecting a sample of high specific resistance (Fig. 4). If the total ionic strength of the sample is adjusted with running buffer salt, no enrichment occurs and only NO_3^- gives an appreciable signal.

From Fig. 5, it can be gathered that quantitative CZE–ISME analysis of anions can be carried out in the same way as that of cations, using the integrals $\mathcal{J} = \int \mathcal{D}\, dt$ [14]. In the case of ClO_4^-, a mean error (residual S.D. divided by

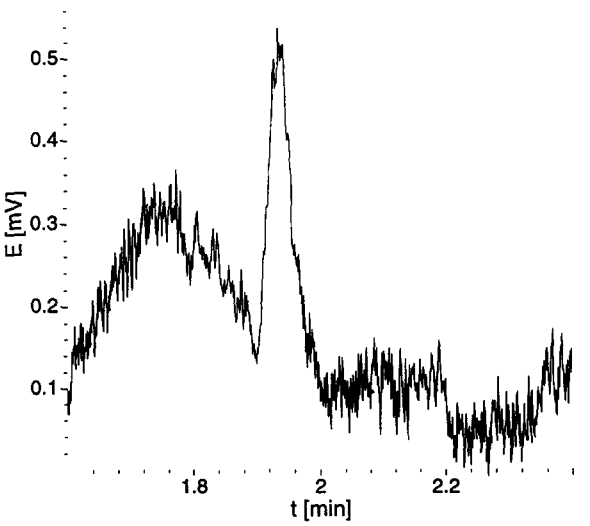

Fig. 3. CZE–ISME analysis of ClO_4^- (10^{-7} *M*) near the detection limit. Buffer: 20 m*M* Na_2SO_4, adjusted to pH 2.5 with H_2SO_4; separation voltage: 30 kV; electrokinetic injection: 5 kV for 10 s. Uncoated capillary.

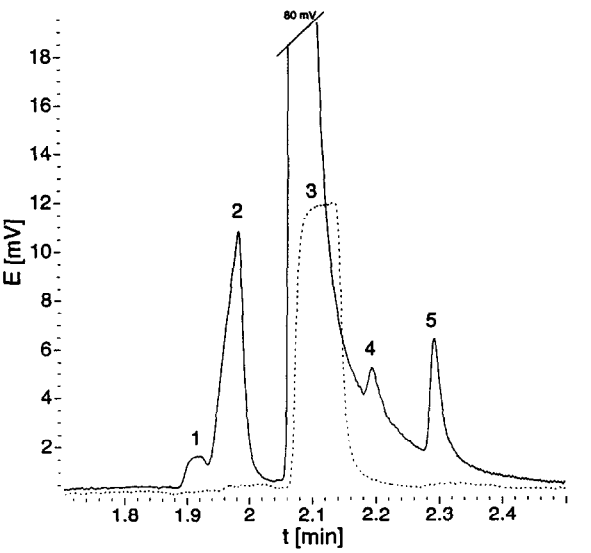

Fig. 4. Determination of 10^{-8} *M* (ca. 1 ppb, w/w) ClO_4^- in tap water as recorded by IMSE. Buffer: 20 m*M* Na_2SO_4, adjusted to pH 2.5 with H_2SO_4; separation voltage: 30 kV; electrokinetic injection: 10 kV for 10 s. Uncoated capillary. Solid line = 10^{-8} *M* $NaClO_4$ in tap water; broken line = 10^{-8} *M* ClO_4^- + 20 m*M* Na_2SO_4 in tap water. Peaks: 1 = Br^-; 2 = Cl^-; 3 = NO_3^-; 4 = ClO_4^-; 5 = unknown.

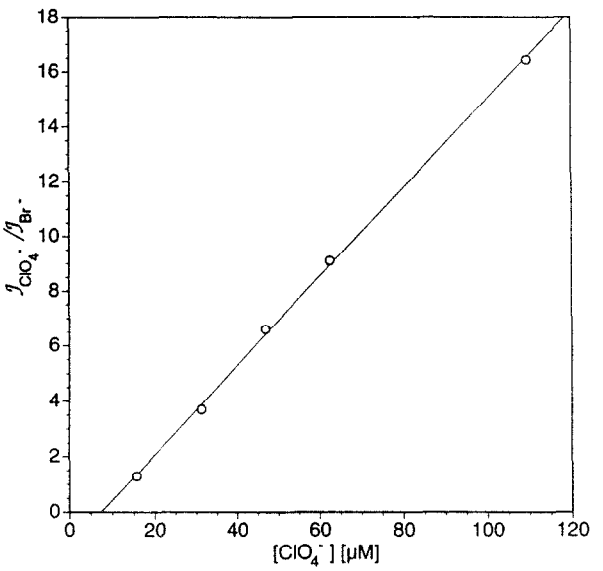

Fig. 5. Linear calibration plot (see text and [14]) for (1.5 to 11)·10^{-5} *M* ClO_4^- ($n = 5$) with 10^{-3} *M* Br^- as internal standard (Na^+ as counter ion). Buffer: 20 m*M* Tris–formate, pH 7.0; separation voltage: 25 kV; electrokinetic injection: 2 kV for 2 s. Polybrene-coated capillary.

the mean ordinate value) of 3% was obtained from the calibration plot.

4. Conclusions

It is shown that anion-selective microelectrodes can be used as powerful detectors in CE. Considering the detection limits and the possibility of miniaturizing the separation systems, even better results are achievable than with conventional detection methods [25].

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