Potentiometric Detector for Capillary Zone Electrophoresis

Carsten Haber, Ivo Silvestri, Stephan Rössli, and Wilhelm Simon*

Abstract. The use of a potentiometric microelectrode as an end-column detector in capillary zone electrophoresis (CZE) is described. Uncoated fused silica capillaries with an internal diameter (I. D.) of 25 μm are used. The microelectrode is placed a few micrometers behind the capillary end. Due to its high internal resistance (10^12–10^14 Ω), special devices to decouple the potentiometric detector from the electrophoretic current are not necessary. The composition of the liquid membrane of the microelectrode was especially designed to show a good response for most cations except magnesium, which was used as a background electrolyte. Separations are carried out at potentials ranging from 15 to 20 kV. With this method, alkali and alkalai earth metals are successfully separated and directly detected down to concentrations of ca. 10^{-10} mol/l.

1. Introduction

Since the early 1980’s, capillary zone electrophoresis has received considerable attention in the domain of analytical separations. A major area of interest within this technique is in the development of new detectors. Since the use of very narrow capillaries is advantageous due to lower Joule heat production, higher separation efficiency and speed of analysis [1], good selectivity in small detection volumes is desired. Column I.D.’s typically range between 25 and 130 μm, resulting in detection volumes from 3 nl to 10 pl. Using a path width of one capillary I.D. results in a great number of separated molecules absorbing UV radiation, more than 90% of all capillary electrophoresis applications are based on photometric detection methods at present. The UV detection follows Lambert-Beer’s law and is strongly dependent on the pathlength of absorbing material. The detection limit of a strongly absorbing substance in a 100-μm capillary (signal-to-noise ratio 2) reaches 10^{-4} mol/l [2] but deteriorates drastically, as the column I. D. drops. Fluorescence detection is a more sensitive and selective detection method, but is limited to molecules that have the ability to emit fluorescence light. The highest sensitivities are reported to be at a level of 10^{-11} mol/l of methotrexate in a 75-μm capillary (signal-to-noise ratio 3) [3]. Recently, Zare and coworkers reported detection limits of 10^{-11} mol/l for amino acids labeled with fluorescein isothiocyanate [18]. Wallingford and Ewing describe an amperometric detector consisting of an ultraminiature carbon fiber inserted into the end of the column [4]. Detection is limited to electroactive molecules. Measurable signals for serotonin at 8.5 × 10^{-10} mol/l in a 12.7-μm capillary are reported [5]. Zare and coworkers [6] introduced an on-column conductivity detector, consisting of two Pt wires (25 μm O.D.) fixed in diametrically opposite holes in 50 or 75 μm I.D. fused silica capillary tubing. The apparatus reaches detection limits of ca. 10^{-13} mol/l for Li [6].

A major drawback in using electrochemical as opposed to optical detectors in CZE is the difficulty of electrical isolation from the high voltage power supply. Special devices for fused silica capillaries like porous glass joints [4] and on-column frits [7] have to be applied to create a current-free zone at the detection end, where the electrochemical sensor is placed. Ewing and Zare
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A major drawback in using electrochemical as opposed to optical detectors in CZE is the difficulty of electrical isolation from the high voltage power supply. Special devices for fused silica capillaries like porous glass joints [4] and on-column frits [7] have to be applied to create a current-free zone at the detection end, where the electrochemical sensor is placed. Ewing and Zare
selectivity and $K_{ij}^{\text{se}} > 1$ describes a higher preference of the sample ion $j$ against the background ion $i$ (with $K_{ij}^{\text{se}} = 1/K_{ji}^{\text{se}}$). In a real electrophoretic measurement, the height of a peak arises from the relative difference of the electromotive forces between the eluting component and the background electrolyte (Fig. 1).

$$E = E_{\text{ref}} + 2.303 \frac{RT}{zF} \log(a_i + K_{ij}^{\text{se}} a_j)$$  \(\text{(1)}\)

where $E$ is the emf of the cell assembly [mV], $E_{\text{ref}}$ is a potential difference comprising a constant plus the liquid junction potential at the reference electrolyte/sample solution interface [mV], $2.303 \frac{RT}{zF} = 59.16$ mV at 25°C, $z$ is the charge of the ions $i$ and $j$, $a_i$ is the activity of the sample ion $j$ [mol/l], $a_i$ is the activity of the background ion $i$ [mol/l], and $K_{ij}^{\text{se}}$ is the selectivity factor. $K_{ij}^{\text{se}} < 1$ means that the background ion $i$ is preferred over sample ion $j$. $K_{ij}^{\text{se}} = 1$ is the case of equal

2. Theory

In recent years, we published several articles describing the use of the potentiometric microelectrode as an on-column detector in HPLC [9-12]. The major advantage of this sensor is its ability to respond to extremely small amounts of ions. Tip diameters of easily pulled micropipettes are usually in the range of 1 μm, resulting in tip areas of $8 \times 10^{-13}$ m². Assuming a spherical diffusion layer with a thickness of approximately one tip diameter (1 μm), a detection volume of $26 \times 10^{-11}$ l is obtained [12].

The electromotive force (emf) of a potentiometric cell assembly of the type with two monovalent ions $i$ and $j$ of the same charge in the sample solution can be approximated by the Nernst-Eisenman equation [13][14]

$$E = E_{\text{ref}} + 2.303 \frac{RT}{zF} \log(a_i + K_{ij}^{\text{se}} a_j)$$  \(\text{(1)}\)

Fig. 1. Sample zone with activity $a_j$ in a buffer electrolyte of activity $a_i$.
3. Experimental

3.1. Electrophoretic System

The electrophoretic system is shown in Fig. 4. It consists of a high-voltage power supply (model 205A 50R, Bertin Associates Inc., Hicksville, NY 11801) providing a voltage up to 50 kV and a remote autosampler (model 624 and control unit 643; Fa. Metrohm AG, Herisau, Switzerland) to switch between sample and buffer solns. Some metallic pieces of the autosampler were replaced by plastic, to ensure the absence of metallic material near the high-voltage end. The high-voltage electrode (Pt wire) and the autosampler were placed in a plexiglass box, to protect the operator.

Separations were carried out in uncoated fused silica capillaries of 25 μm I.D. (Scientific Glass Engineering, Ringwood, Australia). The microelectrode, the Pt wire and the end of the fused silica capillary are placed in a small plexiglass vessel that contained the buffer solns. The whole detection cell was housed in a Faraday cage to reduce external noise. For the movement of the microelectrode, a mechanical micromanipulator (Wild Leitz AG, CH-8021 Zurich) was used and for the position control a microscope (Invertoskop H, Carl Zeiss AG, CH-8032 Zurich) together with a TV camera and monitor (Carl Zeiss AG, Philips AG, CH-8021 Zurich). The position control in the horizontal plane was carried out by comparing the sharpness of focus of the electrode tip and the capillary end. The best position of the tip of the microelectrode was found to be some microns beyond the end of the capillary (Fig. 5). The potential difference was measured between the ion-selective microelectrode and a Pt wire that served also to ground the detection vessel. To reduce the noise to a minimum, the signal from the high-impedance sensor had to be transformed into a low-impedance signal as close as possible to the source. Therefore, an impedance converter (AD 515KH, Analog Devices, Norwood, MA, USA; input-impedance 10^10Ω/1.6 pF differential, 10^9Ω/0.8 pF common mode; leak current at the input <100 fA; capacity elimination) was coupled directly to the internal reference half-cell of the potentiometric microelectrode. The signal was transmitted via special isolated cables to an FD 223 dual electrometer (World Precision Instruments). The signals were recorded with a four channel strip chart recorder (W+W recorder model 114, Kontron AG, CH-8040 Zurich). The current was constantly monitored by putting a resistor of 10^9Ω between the Pt wire and ground, and the IR drop was measured with a multimeter (179 TRMS Digital Multimeter, Keithley Instruments, Taunton, MA, USA).

3.2. Membrane Phase of the Ion-Selective Microelectrode

The membrane cocktail consists of a 1% (v/v) soln of the neutral ionophore bis(N,N-diphenyl)-1,2-phenylenebis(oxy-2,1-ethanediyl)bis(oxyacetamide) (Fig. 6, see also [15]) together with 68.5 mol-% (relative to the ionophore, 100% of potassium tetrakis(4-chlorophenyl) borate in 2-nitrophenyl octyl ether.

Fig. 4. Capillary electrophoresis system with potentiometric detection

Fig. 5. Position of the microelectrode and the Pt wire at the column end

Fig. 6. Selectivity coefficients and constitution of bis(N,N-diphenyl)-1,2-phenylenebis(oxy-2,1-ethanediyl)bis(oxyacetamide) as obtained by separate solution method [17] (0.1 M solutions). Composition of the membrane cocktail: 15% ionophore (100 mol-%), 68.5 mol-% of potassium tetrakis(4-chlorophenyl) borate in 2-nitrophenyl octyl ether.

Fig. 7. Electropherogram of a solution containing 10⁻⁵ M alkali and alkaline-earth metals. Capillary I.D. 25 μm; length 0.99 m; buffer 20 mM magnesium acetate; pH 7.5; injection electrokinetically, 5000 V for 2 s; potential 15 kV; 15 s membrane as described previously; detection post-column.
magnesium acetate as buffer electrolyte, the mobile phase showed an extremely low potential and the eluting sample zones of the different cations (all from Fluka Chemie AG, Buchs, Switzerland) could be detected with the sensitivity according to their relative selectivity ratio to magnesium (Fig. 2).

The internal filling electrolyte consisted of MgCl₂. The concentration of magnesium was chosen to be the same as in the background electrolyte. In this way, diffusion processes across the membrane phase could be eliminated and a constant baseline was obtained.

3.3. Preparation of the Ion-Selective Microelectrode

The preparation, pulling procedure and sterilization of the glass microcapillaries has been described in [16][17]. The microcapillary was filled with a 20 mmol/l soln. of MgCl₂. A slight pressure onto the back-end of the glass body was applied to fill the tip with soln. Then, the front of the microcapillary was dipped into the membrane cocktail. By applying a short vacuum pulse onto the back-end of the glass body, the membrane phase was sucked into the tip (front filling technique). The length of the filled zone was between 100 and 300 μm.

4. Applications

With the previously described system and the ISE sensor, we studied some aqueous solutions containing alkali and alkaline earth metals by free zone electrophoresis. Separations were carried out in fused silica capillaries of 25 μm I.D. with magnesium acetate as background electrolyte. Sample injections were performed electrokinetically simply by applying a certain voltage over a short time period onto the vessel containing the sample solution. The signals were measured in a differential mode against a Pt wire in the detector cell. We observed that under the high-voltage conditions in CE the Pt reference is in terms of noise and signal stability (drift) superior to the calomel reference. Fig. 7 shows an electropherogram of a solution containing 10⁻⁵ mol/l alkali and alkaline earth metals. The integrals of the ion-peaks behave in accordance with their individual selectivity coefficients relative to Mg (note that the range was switched after 13 min). The half-width of the Ba signal corresponds to 893,000 theoretical plates. It is important to understand that the ISE signal follows a logarithmic function and, therefore, the peaks are displayed broader than they would appear to be in linear scale [12].

The electropherograms shown in Figs. 8–10 are some studies on the quality of deionized and doubly distilled H₂O and illustrate very clearly the analytical power of this system. Solutions were injected by electromigration. The separations were performed at 15 and 20 kV. Although we expected a drop in the sensitivity with decreasing pH, we still obtained considerably high signals; up to 30 mV for CE runs at pH 5.14 (magnesium acetate/HCl). An interesting result is the appearance of a Li peak in doubly distilled deionized H₂O, but not in the original deionized H₂O (Fig. 9). Furthermore, we observed that the doubly distilled H₂O from washbottles was more contaminated. This contamination comes not from the plastic material, but from the absorption of aerosols, as the surrounding air gets re-aspirated through the tip of the washbottle.

A problem was the determination of the detection limit. Since all the H₂O samples we analyzed contained traces of Na⁺, K⁺, NH₄⁺, and Ca²⁺, we monitored the K⁺ signal from a 10⁻⁵ M solution by diluting it to 10⁻¹⁰ M. It has to be pointed out that the detection limit depends strongly on the selectivity ratio of the individual cation relative to Mg.
5. Conclusion

In this contribution, we have presented some of the first successful results of the combination CZE-ISE. With its low detection limits for various cations, the ISE is a powerful sensor for charged species. Due to its high internal resistance, this sensor is almost perfectly suitable as a detector in zone electrophoresis. To fully exploit the analytical possibilities of this method, efforts are in progress to extend this technique to anions and organic cations.

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Fig. 10. Free zone electropherogram of doubly distilled water stored in a washbottle. Capillary I.D. 25 μm; length 0.99 m; buffer 20 mM magnesium acetate (HCl); injection electrokinetically, 5000 V for 5 s; potential 20 kV; ISE membrane as described previously; detection post-column.

(S)-Trolox™ Methyl Ether: a Powerful Derivatizing Reagent for the GC Determination of the Enantiomers of Aliphatic Alcohols

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Abstract. (S)-Trolox™ methyl ether 5a prepared from the racemic commercial antioxidant Trolox™ is presented as a new chiral reagent for the GC analysis of stereoisomeric primary and secondary alcohols. The superiority to the hitherto known reagents is demonstrated by some examples.

1. Introduction

New methods of stereoselective organic synthesis, particularly some involving homogeneous asymmetric catalysis [1], yield products with high enantiomeric excess. For the development and the improvement of such techniques, reliable analytical procedures for ee determinations are essential.

In the course of our work aimed at the total synthesis of naturally occurring toco-pherols 1 and other isoprenoids, we realized that general methods for the exact determination of the (high) optical purity of intermediates comprising Me-branched C–C chains were lacking. Thus, we developed a practical GC method for the stereochemical analysis of acyclic terpenoid compounds using acetics derived from (+)-l-tartaric esters [2].

In the case of Me-branched primary alcohols, we could obtain no satisfactory result by the application of commercially available chiral reagents. Recently, reports on the direct separation of a few such alcohols on GC columns coated with (chiral) cyclo-dextrine derivatives have appeared [3]. In this communication, we would like to offer a promising new derivatizing reagent for the analysis of such alcohols using conventional (achiral) GC columns.

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