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Improved end-column amperometric detection for capillary electrophoresis

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Abstract

An amperometric detector for end-column detection in capillary electrophoresis is described. The influence of the high voltage on the detection performance is studied. As a consequence of these studies it is shown that the end-column detection strategy operates up to capillary dimensions of 50 μm I.D. without using any electrical-field decoupler design. The shift of the working electrode potential resulting from the presence of the high voltage electrical field is compensated by selecting a more positive potential setting via the potentiostat.

The detector performance is characterized in terms of attainable separation efficiencies (300 000 theoretical plates for homovanillic acid), limits of detection ($5 \cdot 10^{-8}$ mol/l for serotonin) and coulometric efficiencies (8.9% for homovanillic acid). Practical aspects associated with the use of microdisk electrodes instead of carbon fiber electrodes are addressed.

Keywords: Detection, electrophoresis; Amperometric detection; Electrochemical detection; Serotonin; Tryptophan; DOPA; Homovanillic acid

1. Introduction

Capillary electrophoresis (CE) has become a very important technique in the field of liquid-phase separations [1]. In recent years essential progress regarding the instrumentation for CE has been achieved. Particularly so in the developments of detectors for CE. Detectors based on UV–Vis absorbance, fluorescence measurements, mass-spectrometry or conductivity have found widespread acceptance.

Electrochemical detectors (ED) have also been used for CE [2] and are attractive for a number of reasons. (i) Very low limits of detection can be attained because the miniaturization of electrode systems does not necessarily sacrifice the signal-to-noise ratio. (ii) Systems based on amperometric detection permit low dead volume arrangements, which is important to utilize the unique capability of CE with respect to separation efficiency. (iii) The proper choice of the working electrode potential can help to improve selectivity in case of co-eluting species. (iv) Electrochemical detectors can be constructed using low-cost components.

Although amperometric and voltammetric methods offer such challenging characteristics for detection in CE, they are not routinely used. Several problems associated with the combination of electrochemical measurements and CE have hindered the widespread use of CE–ED. A major problem is the interference of the high voltage (typically 10–30 kV) with the detection circuit. In order to diminish this effect various electrical-field decouplers consisting of porous material [3,4], Nafion tubing [5,6], cellulose

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acetate [7] or palladium [8,9] have been developed. Although impressive performance characteristics have been reported, the implementation of these post-column detection schemes is difficult, at best. Ewing and co-workers [10,11] introduced end-column detection using capillaries of 2 and 5 μm I.D. and a 10-μm carbon fiber positioned at the end of the capillary. However, capillaries with such small internal diameters are not practical for routine use. In this respect it was an important step forward that Lu et al. [12] succeeded in performing end-column detection using capillaries of 10 and 25 μm I.D.

Another reason for the limited acceptance of CE–ED in practice is that most electrochemical detectors described in the past have utilized carbon fibers as working electrodes. These fiber micro-electrodes have the disadvantage of being fragile and mechanical treatment is not possible if the electrode function fails. However, the potential user of CE–ED wishes to have similar possibilities to polish and re-use the working electrodes as in conventional ED coupled with high-performance liquid chromatography. This demand can be fulfilled by employing microdisk working electrodes.

The present paper presents an electrochemical detector arrangement that contains all necessary electrodes including a carbon microdisk working electrode. It will be shown that the end-column detection strategy operates for capillaries up to 50 μm I.D. without the need of any electrical-field decoupler design. The performance characteristics will be discussed in detail.

2. Experimental

2.1. Apparatus and equipment

The electrochemical detector cell developed for end-column detection in CE is shown in Fig. 1. The detection principle has been reported elsewhere [13]. In comparison to the previously described cell, the quasi-reference electrode was replaced by a silver/silver chloride reference system connected with the cell electrolyte via a ceramic frit that was molten through the glass wall of the cell. The working electrodes used in this study were carbon microdisk electrodes made from carbon fiber material (diameter

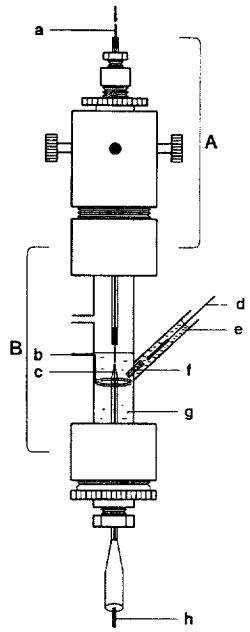

Fig. 1. Construction of the electrochemical detector. (A) x–y–z Positioning device, (B) electrochemical cell. (a) Fused-silica capillary, (b) external contact of the ground electrode, (c) microdisk electrode, (d) Ag/AgCl electrode, (e) 3 *M* KCl, (f) porous frit, (g) internal cell electrolyte and (h) external micro-electrode contact.

30 μm, AVCO, Lowell, MA, USA). The electrode preparation was done as follows. The carbon fiber was sealed in a glass tube which had a conical shaped tip. While heating with a microtorch a vacuum was applied at the other end of the glass tube. The application of a vacuum is necessary for two reasons. First it removes the oxygen which can react with the carbon surface and secondly the melting glass is pressed strongly onto the electrode material which is important to avoid leakage problems. Next the electro-active surface was exposed using fine abrasive paper and polished with alumina suspensions of different particle size down to 0.3 μm. Electrical contact with a copper lead was made via conductive paint (Doduco, Germany). The tip diameters of these electrodes were about 0.4 mm, which is close to the O.D. of the fused-silica capillaries and therefore simplifies very much the electrode alignment.

Fused-silica capillaries with an I.D. of 25 or 50 μm and an O.D. of 360 μm were obtained from CS

Chromatographie Service (Langerwehe, Germany). The high-voltage supply (Model HCN 7E-35 000, F.u.G. Elektronik, Rosenheim-Langenpfunzen, Germany) was capable of delivering 0–35 kV. The high-voltage input was housed in a Plexiglas box fitted with an interlock to protect the operator. According to the construction of the interlock and the insulation properties of the materials involved, the high voltage must not be higher than 20 kV to ensure proper functioning of the system. The detector cell was placed in a Faraday cage to minimize interference from external noise. Care was taken to ensure that the hydrostatic levels of the input and output reservoirs were the same.

All electrochemical measurements were performed using a voltammetric analyzer Model Autolab (Eco Chemie, Utrecht, Netherlands) equipped with a low-current amplifier module ED system. The current signal was filtered through a third order Sallen-Key filter with a time constant of 10 ms.

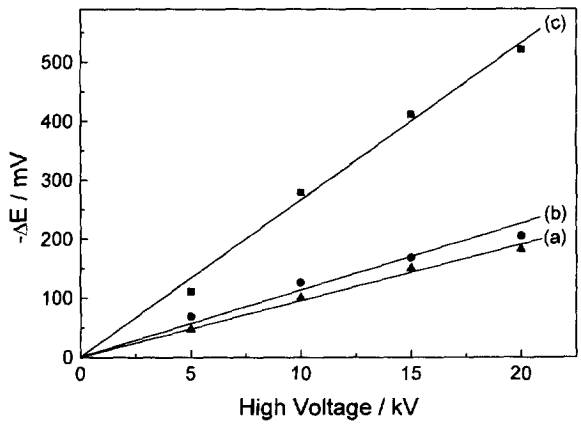

Fig. 2. Working electrode potential shift (ΔE) as a function of the applied high voltage. Experimental conditions: 0.01 *M* phosphate buffer (pH 8.00); capillary length, 1 m; inner diameter of the capillaries, (a) and (b), 25 μm, (c) 50 μm; distance between the capillary outlet and the working electrode tip, (a) 1 mm, (b) and (c) 50 μm.

2.2. Chemicals

Serotonin hydrochloride, L-tryptophan, 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) and homovanillic acid (HVA) were obtained from Fluka (Neu-Ulm, Germany), ferrocenemonocarboxylic acid was from Aldrich (Steinheim, Germany). These substances were used as received. All other chemicals including buffer substances and supporting electrolytes were of analytical-reagent grade.

Solutions were prepared from double-distilled water. Before use all buffer solutions and samples were filtered through a 0.2-μm acrodisc syringe filter (Gelman, Rossdorf, Germany).

3. Results and discussion

3.1. Influence of the high voltage on the working electrode potential

In order to quantify the effect of the high voltage on the detection performance measurements of the shift of the working electrode potential in dependence on the applied separation voltage were performed. For this ferrocenemonocarboxylic acid ($5 \cdot 10^{-4}$ *M*) was added to the running buffer and the shift of the half-wave potential (ΔE) was evaluated as a function of the applied high voltage. In Fig. 2 results for capillaries with 25 and 50 μm I.D. are shown. It can be seen that the observed potential shift depends significantly on the capillary dimension. This can easily be understood because a smaller capillary diameter corresponds to a higher resistance. Consequently, the percentage of the high voltage which drops across the detection arrangement is smaller compared with that in the larger diameter capillaries. However, even for the 50 μm I.D. capillary the shift of the working electrode potential resulting from a 20 kV high voltage is still in a range in which this effect can be compensated by selecting a more positive potential setting via the potentiostat.

The influence of the electrode alignment on the working electrode potential shift has also been studied. The comparison of curves (a) and (b) in Fig. 2 shows that for a distance of 1 mm between the electrode tip and the capillary outlet the working electrode potential shift is somewhat less than in the case of the closer positioning. However, the observed effect of electrode alignment on the shift of the working electrode potential is rather small. Thus, electrode-capillary distances of 50 ± 10 μm have been used in further studies in order to minimize the dead volume of the detector.

3.2. End-column amperometric detection in conjunction with 50 μm I.D. capillaries

From the practical point of view it is highly desirable to extend the amperometric end-column detection scheme to capillaries which are commonly used in practice. The following part describes the performance characteristics of CE–ED with 50 μm I.D. capillaries. Fig. 3 shows an electropherogram for the separation of some neurotransmitter substances and their corresponding biological precursors or metabolites. From cyclic voltammograms of the individual compounds it was derived that 0.8 V vs. Ag/AgCl (3 *M* KCl) is a suitable working electrode potential at which all these substances are oxidized under transport controlled conditions. On the basis of the results illustrated in Fig. 2 a virtual working electrode potential of 1.35 V was selected to be applied, which corresponds to an actual electrode potential of 0.85 V if the high voltage is switched on. Baseline characteristics (regarding noise and slope) such as shown in Fig. 3 were established within less than 1 min.

There was no negative effect of keeping the working electrode potential at 1.35 V between the CE–ED measurements. However, applying electrode potentials higher than 1.5 V may lead to a modification of the electrode surface resulting in an alteration of the electrode properties. In case of necessity of higher "real" detection potentials this effect can be circumvented by performing the "virtual" potential setting after starting the CE run.

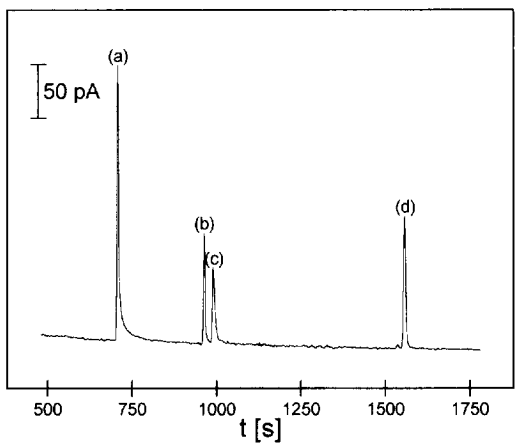

Fig. 3. Electropherogram for the separation of (a) $4.2 \cdot 10^{-6}$ *M* serotonin, (b) $5.1 \cdot 10^{-6}$ *M* L-tryptophan, (c) $9.6 \cdot 10^{-6}$ *M* L-DOPA and (d) $9.4 \cdot 10^{-6}$ *M* HVA. Experimental conditions: 0.01 *M* phosphate buffer (pH 8.00); fused-silica capillary of 1 m×50 μm I.D.; high voltage, 19 kV; injection, 20 s at 5 kV; applied working electrode potential, 1.35 V.

The reproducibility of the CE–ED system including the manual injection procedure was evaluated from five consecutive electropherograms corresponding to injections of a solution containing 10^{-6} *M* serotonin and 10^{-5} *M* L-tryptophan. The relative standard deviation of the peak heights obtained for serotonin and L-tryptophan were 6 and 4%, respectively. However, the variations in peak currents of measurements that involve a replacement of the microdisk electrode are about four times higher than in a fixed configuration. This can be attributed to both the changes in electrode activity due to the polishing step and the precision of electrode alignment.

The attainable separation efficiency of the CE–ED system can be characterized by evaluating the nearly Gaussian-shaped signal obtained for HVA. Under conditions given in Fig. 3 the number of theoretical plates (calculated from peak width at half peak height) was approximately 241 000. By reducing the injection volume to 5 nl (5 kV, 10 s) an even higher efficiency of 300 000 theoretical plates could be obtained. This excellent efficiency illustrates that no significant additional band broadening is introduced by the detector. The theoretical plate number obtained for HVA rivals favorably with that reported for comparable substances on the basis of other end-column ED arrangements (74 000 for ribose [14], 140 000 for catechol [11]). For a recently introduced CE–ED system based on an end-column electrical decoupler which is close to the end-column detection strategy a comparable high theoretical plate number of 230 000 (isoproterenol) has also been achieved [15].

Another criterion of the performance characteristics is the detector response at low analyte concentrations. Fig. 4 illustrates the signal-to-noise structure for a CE–ED measurement of a serotonin sample at a concentration close to the limit of detection. In the concentration range between 10^{-7} *M* and 10^{-6} *M* the peak height of the serotonin signal depends linearly on the concentration. The sensitivity (calculated as the slope of the calibration

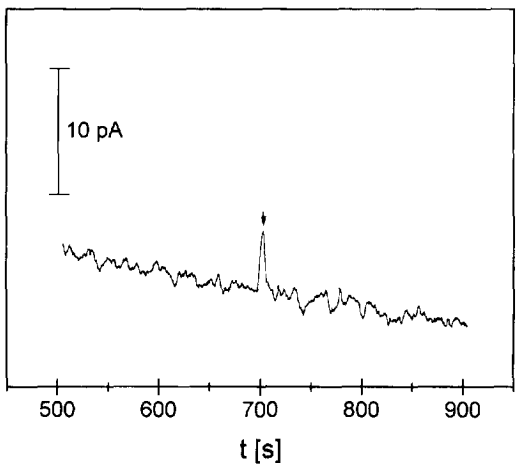

Fig. 4. Capillary electrophoresis with electrochemical detection of $1.1 \cdot 10^{-7}$ M serotonin. Experimental conditions as in Fig. 3.

plot) is 36.1 pA/μM and the limit of detection (calculated for the signal that is twice as much as the peak-to-peak noise) amounts to $5 \cdot 10^{-8}$ M.

The detector arrangement was further characterized regarding the coulometric efficiency. The coulometric efficiency expresses the ratio between that part of an electro-active substance which contributes to the current signal and the total amount of this substance injected into the capillary. The calculation is based on Faraday's law:

$$\int idt = nFz \tag{1}$$

where $\int idt$ represents the charge corresponding to a current i registered during a time t, n is the quantity of the electro-active compound in moles, F is Faraday's constant and z is the number of electrons transferred. The quantity injected by electromigration can be calculated using the following equation:

$$n = \frac{\mu_{\text{eff}} E A c t_{\text{inj}}}{l} \tag{2}$$

where μ_{eff} is the effective electrophoretic mobility of the analyte species in presence of electro-osmotic flow (determined on the basis of the migration time), E is the injection voltage, A is the cross-sectional area of the capillary, c is the concentration of the analyte, t_{inj} is the injection time and l is the capillary length.

In CE–ED the peak area represents a charge. The charge that would correspond to the complete electrochemical reaction of the total amount of the injected analyte can be calculated using Eqs. (1,2). The ratio of both charges gives the coulometric efficiency. The average coulometric efficiency determined for HVA is 8.9% ($n = 3$). In case of carbon fiber based end-column detector arrangements [12], it is probable that higher coulometric efficiencies can be obtained. However, only a part of the electrode surface is in effective contact with the eluting analyte. This is disadvantageous because the larger surface area, i.e. the higher capacitance of carbon fiber electrodes may result in higher noise level. In practice, however, the potentially lower noise associated with microdisk electrodes can only be utilized with perfectly sealed electrode material. Furthermore, the use of a microdisk instead of a carbon fiber electrode can minimize the additional band broadening introduced by the detector because the detection zone is restricted to a smaller space region.

4. Conclusion

It has been shown that the end-column detection strategy of CE–ED operates for capillaries up to 50 μm I.D. without the need of an electrical-field decoupler. That means the simple end-column ED can now be performed in conjunction with capillaries which are used in routine applications. Further advantages of the CE–ED system are the ease of alignment of the microdisk electrode at the capillary outlet due to the conical shaped electrode tip and the possibility to polish and re-use the working electrode.

Accordingly, the presented detector should facilitate acceptance of ED as a practical detection method in CE.

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