Non-aqueous capillary electrophoresis with electrochemical detection[1]

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Abstract

The application of electrochemical detection (ED) in conjunction with non-aqueous capillary electrophoresis (CE) is described. Using an acetonitrile-based buffer system only minor effects of the high voltage on the electrochemical detection circuit occur during CE experiments. Thus, for the first time CE–ED measurements are performed employing 75-μm I.D. capillaries without a special capillary modification or interruption. Capillaries from various sources are evaluated regarding their suitability for non-aqueous CE–ED. All fused-silica capillaries tested allow reproducible measurements. However, there are differences in separation efficiency depending on the fused-silica material used. The analytical characteristics of non-aqueous CE–ED are explored using ferrocene and (ferrocenylmethyl)trimethylammonium perchlorate ([FcMTMA]ClO$_4$) as model analytes. The reproducibility of migration time and signal height is expressed by relative standard deviations of 0.18 and 1%, respectively ($n=10$). The limit of detection for [FcMTMA]ClO$_4$ is 6×10^{-8} M. Practical applications concerning the determination of dye compounds such as malachite green, crystal violet and rhodamine B are discussed. A remarkably good stability of electrode response is observed for extended measuring periods owing to the well-defined surface characteristics of the platinum electrode in the acetonitrile-based medium. © 1998 Elsevier Science B.V.

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1. Introduction

Capillary electrophoresis (CE) separations in non-aqueous solvents have recently received considerable attention [1–7]. The use of organic solvents in place of aqueous systems may provide a means of selectivity enhancement. The changes in selectivity are due to differences in the solvation and the protolytic and other equilibrium constants of the solutes in the respective solvent. In addition, the so-called *levelling effect* which is typical for aqueous solutions and restricts the differentiation between acids (bases) of different strength is usually less significant in organic solvents.

Another attractive feature of non-aqueous separation media is that compounds with poor solubility in water may become accessible for CE investigations.

Among a variety of organic solvents that have been studied with regard to their suitability for CE methanol [3–6], *N*-methylformamide [2,4,6,7], *N,N*-

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dimethylformamide [4–7], dimethyl sulfoxide [4,6] and acetonitrile [4,6,8] seem to be particularly useful.

Capillary electrophoresis with electrochemical detection (CE–ED) is attractive due to the inherent sensitivity of ED and the simplicity (low cost) of the experimental set-up. However, the main problem of CE–ED is the interference of the high voltage with the detection circuit. Various electrical-field decouplers have been proposed to overcome this drawback [9–11]. As an alternative, Ewing and co-workers [12,13] introduced end-column detection using capillaries of 2 and 5 μm I.D. and a 10-μm carbon fiber electrode positioned at the end of the capillary. Lu et al. [14] extended this approach of detection to capillaries of 10 and 25 μm I.D. Recently it has been demonstrated that the end-column ED principle can operate in conjunction with capillaries of I.D. up to 50 μm without using electrical-field decouplers [15–17]. In the case of non-aqueous solvents it can be expected that the higher impedance of these media will further reduce the undesirable effects of the high voltage on the detection circuit.

From the viewpoint of compatibility with ED acetonitrile is the most promising solvent. Acetonitrile systems offer a considerable extension of the useful potential range when compared to aqueous buffers where hydrogen and oxygen evolution determine the ultimate cathodic and anodic potential limits, respectively. Provided that suitable supporting electrolytes can be selected, acetonitrile allows electrochemical studies within a potential window of more than 6 V [18]. As a consequence the applicability of electrochemical detection can be greatly expanded involving compounds that are hardly oxidized or reduced [19]. An important practical advantage is associated with the use of noble metal electrodes in acetonitrile systems because their surface state can more easily be controlled than in aqueous solutions and effective in situ conditioning is possible by simple anodic pretreatment [20].

A recent paper by Salimi-Moosavi and Cassidy [5] deals partly with CE–ED in *N,N*-dimethylformamide systems. For several inorganic anions quite low limits of detection ranging between 5×10^{-8} M (NO_2^-) and 1×10^{-9} M (N_3^-) were reported. However, the authors also encountered limitations regarding reproducibility and linearity of the calibration plots.

The present study seeks to elucidate the potential of CE–ED in acetonitrile-based media. Attention is paid to the choice of suitable electrolytes and capillaries, the influence of the high voltage on ED and the reliability of the overall CE–ED system. Following the determination of the performance characteristics applications concerned with the analysis of dye compounds will be presented.

2. Experimental

2.1. Apparatus and equipment

The electrochemical cell for end-column amperometric detection has been described in detail elsewhere [15]. Briefly, the detector cell consists of a cylindrical glass body and contains all necessary electrodes including a voltammetric microdisc electrode, a platinum coil, and 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 electrode used in this study was a platinum microdisc electrode (disc diameter 25 μm) which was prepared by glass sealing of a fine platinum wire (Goodfellow, Cambridge) into a glass tube with a conical tip (tip diameter 0.4 mm). Fused-silica capillaries with comparable O.D. can easily be aligned above the microdisc electrode. The distance between the electrode tip and the capillary outlet was 75 ± 25 μm.

Fused-silica capillaries with I.D. of 50 or 75 μm and O.D. of 360 μm were obtained from Internationale Chemie-Technik (ICT) (Bad Homburg, Germany), CS Chromatographie Service (Langerwehe, Germany) and Photonetics (Kehl, Germany). If not stated otherwise, capillaries from the latter source were used for the present investigations. Before use the capillaries were washed with pure acetonitrile for 5 min and with the operating electrolyte system for 10 min. The capillaries were stored with both ends in the gas phase of a vial filled with acetonitrile.

The high-voltage supply (Model HCN 7E-35000, F.u.G. Elektronik, Rosenheim-Langenpfunzen, Germany) was capable of delivering 0–35 kV. An isolating transformer was used to avoid ground loops with the electronics of the detection circuit. 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 in the three-electrode mode using a voltammetric analyzer Model Autolab (Eco Chemie, Utrecht, The Netherlands) equipped with a low-current amplifier module ECD system. The current signal was filtered through a third-order Sallen-Key filter with a time constant of 10 ms.

Between successive CE runs, a potential of 2.75 V was applied for 5 s in order to ensure high stability of the detector response.

2.2. Chemicals

Acetonitrile (99.9% HPLC grade, water <0.02%), acetic acid (99.99%) were obtained from Sigma–Aldrich (Steinheim, Germany) and were used as received. Ferrocene was from Riedel-de Haën (Seelze-Hannover, Germany) and was purified by sublimation. (Ferrocenylmethyl)trimethylammonium perchlorate, [FcMTMA]ClO_4, was prepared by metathesis of the corresponding iodide salt, which was obtained from Lancaster Synthesis (Mühlheim am Main, Germany). The [FcMTMA]ClO_4 was recrystallized from ethanol.

The dye compounds were obtained from the following sources: malachite green (hydrogen oxalate salt) and crystal violet (hexamethylpararosaniline chloride) from Feinchemie K.-H. Kallies (Sebnitz, Germany) and rhodamine B from T. Schuchardt (München, Germany).

All other chemicals used for buffer and supporting electrolyte preparation were of analytical-reagent grade.

3. Results and discussion

3.1. Choice of electrolytes and capillaries

The selection of suitable electrolytes is very important for both the CE and the ED characteristics. Initial experiments were conducted using acetonitrile solutions containing 10 mM tetrabutylammonium perchlorate (TBAP). This electrolyte is frequently used for non-aqueous electrochemical studies due to its electrochemical inertness. However, when performing CE measurements using the AN/TBAP system the electroosmotic flow (EOF) was not stable for a longer time and baseline irregularities occurred. In addition, the solubility of many inorganic substances is limited in this protophobic medium owing to poor solvation both of cations and anions. By adding some hydrogen-bond donor, e.g. acetic acid (HAc), the solubility of salts can be considerably enhanced based on phenomena of homo- or heteroconjugation [21]. In a recent paper by Hansen et al. [6] ammonium acetate was considered to be the most suitable electrolyte for non-aqueous CE. The proper operation of this electrolyte system was also confirmed in the present study. However, in the context of CE–ED, sodium acetate (NaAc) was found to be a preferable electrolyte because the anodic breakdown potential is about 350 mV more positive than in the case of ammonium acetate. In conclusion, among the electrolyte systems studied, acetonitrile containing 1 M HAc and 10 mM NaAc was the most favourable medium for CE–ED, taking into consideration its solubilizing power, the reliability of CE performance and the voltammetric characteristics. All subsequent experiments were done using this electrolyte system.

An essential point during the initial stage of non-aqueous CE–ED experiments was the determination of the influence of the separation HV on the detection circuit. As a general rule, the effect of the HV on the detection potential was found to be smaller than in common aqueous buffers. Using a 50-μm capillary of 1.03 m length the application of 20 kV (electrophoretic current, 1.0 μA) resulted in a shift of the working electrode potential (E) of less than 100 mV which compares to about 500 mV in case of an aqueous phosphate buffer [15]. Even 75-μm capillaries could be employed without serious complications. Fig. 1 illustrates the comparison of cyclic voltammograms of the background electrolyte and a ferrocene-containing solution recorded in the detector cell with and without 20 kV. The application of the HV (electrophoretic current, 2.5 μA) results in a negative shift of the working electrode potential of
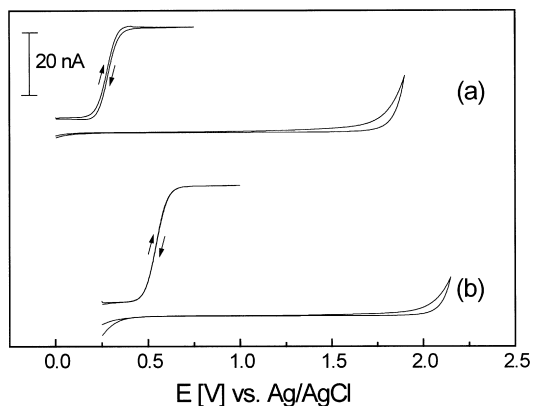
Fig. 1. Cyclic votammograms of acetonitrile background electrolyte (1 *M* acetic acid and 10 m*M* sodium acetate) and 2 m*M* ferrocene in the detector cell recorded with 50 mV/s, (a) without and (b) with a high voltage of 20 kV. Capillary dimensions, 0.95 m×75 μm I.D.

Fig. 2. Electropherograms of [FcMTMA]ClO$_4$ and ferrocene (both $9 \cdot 10^{-6}$ *M*) were obtained using capillaries from the following sources: (a) ICT (1.03 m×50 μm I.D.), (b) CS Chromatographie Service (1.20 m×75 μm I.D.), (c) Photonetics (0.95 m×75 μm I.D.) Experimental conditions: running electrolyte, acetonitrile containing 1 *M* HAc and 10 m*M* NaAc; electrokinetic injection, 20 s at 5 kV; separation voltage, 20 kV; detection potential (virtual), 1 V.

250 mV, i.e. the voltammetric events proceed at more positive potentials with reference to the virtual potential scale. Consequently, a potential setting via the potentiostat of $E+250$ mV is necessary to adjust the desired working electrode potential correctly as compared with voltammetry in absence of a HV.

Another interesting aspect that can be derived from Fig. 1 is the hydrodynamic effect of the electroosmotically driven flow on the voltammetric characteristics. In the batch experiment in quiescent solution (Fig. 1a) a quasi-steady-state voltammogram exhibiting some hysteresis between the forward and backward scan is obtained for ferrocene oxidation under the given conditions. In contrast, with the help of hydrodynamic mass transport the voltammetric response becomes truly steady state, as illustrated in Fig. 1b, with a limiting current exceeding that in quiescent solution by 30%. The flow-rate corresponding to a HV of 20 kV was determined to be 7.1 cm/min. According to the common classification of hydrodynamic electrochemical detection systems the present configuration can be termed as a wall-tube design because the capillary I.D. is larger than the electrode disc diameter [22].

Capillaries from different sources (see Fig. 2 for specifications) were examined regarding their suitability for non-aqueous CE–ED. Fig. 2 shows electropherograms obtained for the separation of neutral (ferrocene) and cationic ([FcMTMA]ClO$_4$) model compounds using three different capillaries. In
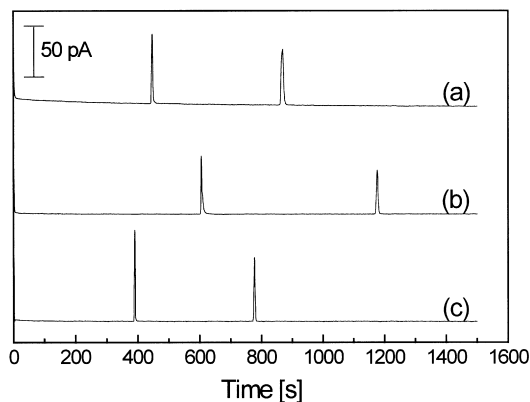
all cases the reproducibility of electropherograms and the stability of the EOF were quite good, as will be quantified in Section 3.2. The magnitude of EOF depends only slightly on the fused-silica material used. However, there are significant differences regarding efficiency obtained for the three types of capillaries. The most important contribution to zone broadening is probably due to solute interactions with the capillary walls. This is particularly indicated by the tailing of the [FcMTMA]$^+$ peak in Fig. 2b. An additional band broadening that can potentially be introduced by the detector is probably very small in the case of the present wall-tube detection cell because there is no chance for analyte dispersion to occur in the centre of the eluting zone where detection is performed. This is confirmed by the impressive numbers of theoretical plates obtained for ferrocene, which are typically larger than 2×10^5 under optimum conditions (capillary as in Fig. 2c, injection plug length smaller than 3 mm).

3.2. Performance characteristics of non-aqueous CE–ED

For the characterization of the non-aqueous CE–ED system capillaries with I.D. of 75 μm were employed owing to the convenience of measurements with larger size capillaries (reduced clogging

problems, ease of optimum electrode–capillary alignment).

The reproducibility of the migration time was determined using ferrocene as marker compound for the EOF. Evaluating 10 successive CE runs, the relative standard deviation (R.S.D.) for the migration time of ferrocene was 0.18%. After extended measuring periods of several hours the EOF becomes slightly slower probably due to changes in composition of the buffer solution in the 1-ml reservoirs. For example, after 4 h of continuous CE–ED measurements (electrophoretic current, 2.5 μA) the migration time for ferrocene was 1.4% longer than at the beginning.

The reproducibility of signal height was evaluated on the basis of repetitive CE–ED measurements of 2×10^{-6} M [FcMTMA]ClO$_4$. Under conditions of manual injection management (electrokinetically, 20 s, 5 kV), a R.S.D. of 1.0% (n=10) was obtained for the signal height.

The linearity of the detector response was studied using [FcMTMA]ClO$_4$ as test analyte. In the concentration range between 1×10^{-7} and 1×10^{-5} M the peak height depends linearly on the concentration. The sensitivity (defined as the slope of the calibration plot) is 7.22 pA/μM and the limit of detection (calculated for the signal that is twice as much as the peak-to-peak noise) amounts to 6×10^{-8} M.

The coulometric efficiency, i.e. the percentage of the eluting analyte that is actually converted at the sensing electrode is 0.8% in the case of ferrocene derivatives. This rather small value is not surprising for a wall-tube detector arrangement where the diameter of the tube outlet is several times larger than that of the disk electrode. On the other hand the small electrode surface area is a prerequisite to achieve a low baseline noise level of about 350 fA, and to ensure a fast stabilization of the baseline within less than 1 min after switching on the HV. Therefore, despite the low coulometric efficiency, an appreciable signal-to-noise ratio and low limit of detection can be attained.

3.3. Analysis of dye substances

Various dye compounds, including malachite green, crystal violet and rhodamine B, were studied by CE–ED. Prior to CE measurements, the analytes were characterized regarding their electrochemical behaviour in acetonitrile solution with the help of cyclic voltammetry. It was found that the dye substances undergo electrochemical oxidation leading to mass-transport-controlled current signals in the potential range between 1.3 and 1.4 V vs. Ag/AgCl. Taking the potential shift in the presence of a separating voltage of 20 kV into account, a virtual potential setting of 1.55 V is suitable for CE–ED measurements. Fig. 3 shows an example for the separation of a mixture of dye substances and ferrocene (internal standard) by CE–ED. All three dye compounds which form cationic species are clearly resolved and the reproducibility of the analysis is very good as illustrated by repetitive electropherograms shown in Fig. 3. In particular, it is worth noting that no problems of electrode deactivation were encountered over measuring periods of several hours. This practically important aspect is further manifested by the experiment illustrated in Fig. 4 where CE–ED measurements were performed on aliquots of the same sample with a time interval of 9 h between the individual CE runs. During this quite long time period a reasonable number of related CE–ED experiments were done with an effective measuring time of about 6 h. As can be seen from Fig. 4, the electrode response is main-
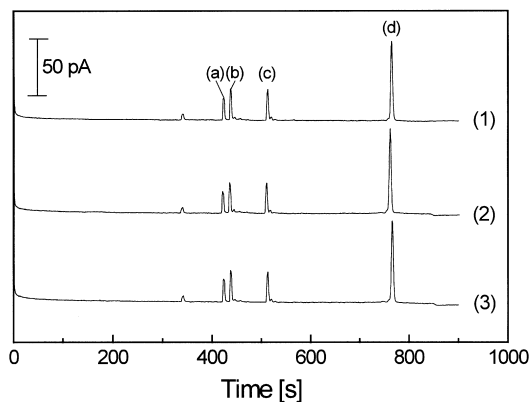

Fig. 3. Non-aqueous capillary electrophoresis with electrochemical detection of a dye mixture containing (a) 1.7 μg/ml malachite green, (b) 0.70 μg/ml crystal violet, (c) 4.3 μg/ml rhodamine B and (d) 9.1×10^{-6} M ferrocene. Experimental conditions were as in Fig. 2c, except that a detection potential (virtual) of 1.55 V was applied. Electropherograms (1), (2) and (3) are successive recordings.
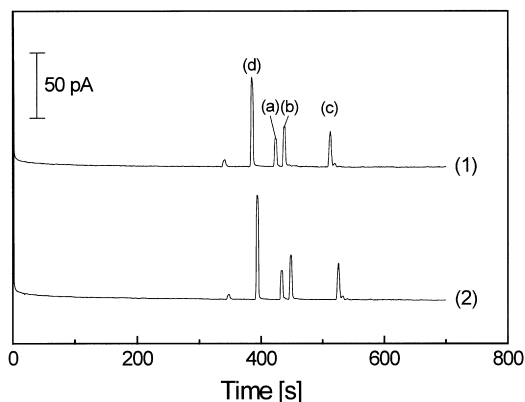
Fig. 4. Electropherograms for the separation of (a) 1.9 μg/ml malachite green, (b) 0.77 μg/ml crystal violet, (c) 4.35 μg/ml rhodamine B and (d) 7.0×10^{-6} M [FcMTMA]ClO$_4$. Experimental conditions were as in Fig. 4. Electropherogram (2) was recorded 9 h after (1) with a discontinuous operation of the CE–ED system characterized by an effective measuring period of 6 h between the shown recordings.

tained constant over the whole time. The only difference that occurs is the slight shift of the electropherogram (2) (Fig. 4) towards longer migration times as a result of partial depletion of the acetonitrile buffer which was not renewed.

4. Conclusions

The compatibility of ED with non-aqueous acetonitrile-based CE has been demonstrated in this work.

In comparison to measurements in aqueous systems the application of ED to non-aqueous CE is particularly attractive due to the following reasons:

(1) The effect of the HV on ED is considerably reduced. As a consequence, capillaries with 75 μm I.D. can be used for CE–ED without any electrical-field decoupler.

(2) The accessible potential range for detection is widened, which extends the spectrum of compounds that can be detected electrochemically.

(3) The reliability of non-aqueous CE–ED with an acetonitrile-based buffer is very good. The electrode characteristics of a noble metal electrode are maintained stable without the necessity of frequent mechanical treatments of the electrode surface.

Because of these features, electrochemical detection methods coupled with non-aqueous CE lead to enhanced practicality and will certainly receive growing attention in the future.

Acknowledgements

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