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Application of non-aqueous capillary electrophoresis with electrochemical detection to the determination of nicotine in tobacco

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Abstract

Non-aqueous capillary electrophoresis with electrochemical detection (NACE–ED) was applied to the determination of nicotine. The measurements were performed using an acetonitrile-based buffer. Nicotine was shown to yield well defined voltammetric signals suitable for oxidative detection. The precision of NACE–ED regarding migration time and peak height for samples containing 8 μg/ml nicotine is expressed by relative standard deviations of 0.1% and 1.6%, respectively ($n=8$). The limit of detection for nicotine was 13 ng/ml (286 fg). For nicotine determination in tobacco samples various solutions were studied regarding the extraction efficiency in an ultrasonic bath. The highest extraction efficiency was obtained using a solvent mixture consisting of acetonitrile–acetic acid–water (20:5:75, v/v). The results for nicotine determination in tobacco were evaluated using tobacco reference material with certified nicotine content. Analytical aspects such as accuracy, reproducibility and selectivity were addressed in this work. The measurements were based on the use of a newly developed electrochemical detector cell which was found to enable user-friendly operation of NACE–ED measurements.

Keywords: Detection, electrophoresis; Electrochemical detection; Instrumentation; Non-aqueous capillary electrophoresis; Tobacco; Alkaloids; Nicotine

1. Introduction

In recent years a number of reports on non-aqueous capillary electrophoresis (NACE) have appeared [1–11] and it has been demonstrated that the substitution of aqueous buffer solutions by non-aqueous systems offers new possibilities for liquid-phase separations. In particular, the manipulation of selectivity by varying the organic solvent choices and ratios can help to solve complicated separation problems and to verify peak purity [2]. Further advantages include improved solubility of hydrophobic compounds [12] and reduced electrophoretic currents [13].

Several organic solvents such as methanol [2,3,5], *N*-methylformamide [2,5], *N*,*N*-dimethylformamide [2,3,5], dimethyl sulfoxide [2,5] and acetonitrile [2,5] have been studied with regard to their suitability for NACE. Among these solvents acetonitrile exhibits the largest ratio of dielectric constant to viscosity which leads to high electroosmotic flow velocity and fast separations [14]. Acetonitrile is also an ideal solvent for performing electrochemical measurements [15] owing to the extended accessible potential range and the enhanced stability of electrochemical responses associated with this medium. In recent work it has been shown that the combination

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of acetonitrile-based NACE with electrochemical detection (ED) provides reliable performance and offers new applications that cannot be studied by conventional aqueous CE–ED systems [16–19]. For example, various alkaloids become accessible for oxidative detection in acetonitrile-based media. For the present study nicotine was chosen as the target analyte. Nicotine is of obvious interest to the tobacco industry and there are several pharmaceutical products containing nicotine. Over the last few years a number of methodical approaches for the determination of nicotine were reported. These include methods such as gas chromatography with nitrogen-specific detection [20], high-performance liquid chromatography with UV detection [21], supercritical fluid chromatography–ion mobility detection [22] and aqueous capillary electrophoresis with UV detection [23,24]. The tobacco matrix is rather complex which is one of the main challenges addressed in previous work. Because ED is known to be a very sensitive detection method it could be expected to reduce matrix effects by applying sufficiently diluted extracts.

In this work, NACE–ED was evaluated for its potential to enable selective and sensitive determinations of nicotine in tobacco extracts. In addition, a new electrochemical detector cell, which was particularly developed for NACE–ED applications is introduced to enhance practicality.

2. Experimental

2.1. Apparatus and equipment

Special electrodes were fabricated to be used in conjunction with the electrochemical detector. For the preparation of the electrodes glass capillaries (Hilgenberg, Malsfeld, Germany) with an I.D. of 0.10 mm and an O.D. of 0.35 mm were cut to a length of 24 mm and a 50-μm platinum wire (Goodfellow, Cambridge, UK) was inserted into the capillary and sealed at one end by a short exposure to a microtorch. The other capillary end was glued into a piece of copper tube (1 mm O.D.×0.35 mm I.D.) using epoxy resin. Then electrical contact was made by soldering the protruding platinum wire to the copper tube. The electrode assembly was connected to a PTFE adapter by heat shrinking. The microdisk electrode surface was exposed and polished with the help of polishing foils (Ehret Datentechnik, Emmendingen, Germany) with particle sizes ranging from 60 μm to 0.3 μm.

The electrochemical detector cell developed for NACE measurements is illustrated in Fig. 1. It operates without the implementation of an electrical-field decoupler. The cylindrical cell body was made from PTFE. Two pieces of stainless steel tubes (Sigma–Aldrich, Deisenhofen, Germany) with an O.D. of 1.6 mm were mounted into precisely drilled holes of the detector wall applying a heat shrinking procedure. The stainless steel tubes guide the separation capillary and the working electrode into the detector cell. At the ends of the stainless steel tubes the I.D. meets exactly the dimensions of the electrode and separation capillary, respectively. There is no need for an additional axial capillary-to-electrode alignment. The capillary and working electrode are fixed in place by PTFE adapters fitted to the stainless steel tubes. One of the stainless steel tubes served as counter electrode and high voltage ground. A capillary-to-electrode distance of 75±5 μm was adjusted under a stereomicroscope by carefully pushing the separation capillary towards the working electrode tip. The cell was ready for operation after filling it with 2.5 ml of the separation buffer and placing a PTFE cap with an attached silver/silver chloride reference electrode on top. The internal solution of the reference electrode was the non-aqueous acetonitrile-based buffer used for CE separations. All potentials given in this work were measured with respect to this reference system. The electrolyte solutions in the detector can easily be changed or renewed with the help of a syringe. After use, the cell was cleaned with acetonitrile and stored dry without disassembling the capillary-to-electrode arrangement.

Fused silica capillaries with an I.D. of 75 μm and an O.D. of 360 μm were obtained from Polymicro Technologies (Phoenix, AZ, USA). Before use, the capillaries were washed with pure acetonitrile for 5 min and with the buffer for 10 min. After use, the capillaries were again washed with pure acetonitrile, after which the capillaries were blown dry for storage.

The high-voltage supply (Model HCN 7E-35000, Elektronik, Rosenheim-Langenpfunzen, Germany) was capable of delivering separation voltages up to

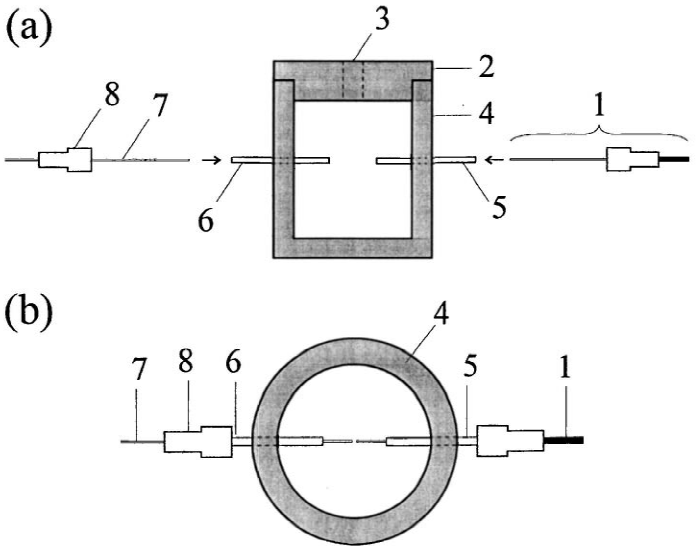

Fig. 1. Schematic of the electrochemical detector (a) side view, (b) top view. 1=Working electrode; 2=PTFE cap; 3=hole for inserting the reference electrode; 4=PTFE cell body; 5=guiding stainless steel tube for the working electrode; 6=guiding stainless steel tube for the separation capillary; 7=separation capillary; 8=PTFE adapter holding the separation capillary.

35 kV. The electrophoretic currents measured under the conditions of the present study were 3.3 ± 0.1 μA and 5.2 ± 0.1 μA corresponding to separation voltages of 20 kV and 30 kV, respectively.

An isolating transformer was used to ensure galvanic separation between the high voltage supply and the potentiostat. 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 identical. Sample injection was done by elevating the sample vial to a height difference of 10 cm above the cell electrolyte level for a duration of 10 s. According to capillary flow injection measurements [25] carried out with the same system an injection volume of 22 nl was determined by calibration.

All electrochemical measurements were performed in the three-electrode mode using a voltammetric analyzer Model Autolab PGSTAT10 (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 100 ms. The interval time of current measurements for amperometric recordings of electropherograms was 0.3 s throughout. Before running a new electropherogram a working electrode potential of 3.0 V was applied for 5 s followed by −0.5 V for 5 s to ensure long-term stability of the detector response.

2.2. Chemicals

Acetonitrile (99.9% HPLC grade, water <0.02%) and 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 precipitated [FcMTMA]ClO_4 was recrystallized from ethanol–water. (−)-Nicotine and (±)-nornicotine were purchased from Fluka (Buchs, Switzerland). DL-Anabasine and (−)-cotinine were obtained from Lancaster Synthesis. Standard solutions of the above alkaloids were prepared in the acetonitrile-based separation buffer prior to the capillary electrophoresis experiments.

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

Tobacco samples were obtained from SAL (Sig-

maringen, Germany). A reference material had a certified nicotine content of 3.66±0.04% (w/w) with respect to the dry tobacco sample. The water content was 8.85% (w/w).

The extraction procedure for nicotine determinations in tobacco was as follows: a ground tobacco sample (50 mg) was placed in a 25-ml glass vial. Then 10 ml of the extracting solution (see Table 1) was added and the capped vial was sonicated for 10 min in an ultrasonic bath (Sonorex TK 52, Brandelin). A portion of the extract was filtered through a 0.45 μm syringe filter (Gelman Sciences) and diluted with the separation buffer at a ratio 1:10. This solution was used for injection into the NACE–ED system.

3. Results and discussion

3.1. Voltammetric behavior of nicotine in non-aqueous solution

The voltammetric characteristics of nicotine were studied by cyclic voltammetry (CV). Fig. 2 shows CV results for repetitive measurements of nicotine performed in the acetonitrile buffer which was the same as used for NACE–ED determinations. The extended potential window in the non-aqueous system allows to record well-resolved voltammetric signals corresponding to the oxidation of nicotine. However, there is no clear mass-transport-controlled current plateau, but potentials within the region from 1.45 V to 1.75 V appear to be suitable for amperometric detection. For the selection of an appropriate detection potential for NACE–ED measurements the shift of the working electrode potential in presence of a high voltage electrical field has to be considered [16]. For the present experimental configuration the application of high voltages of 20 kV and 30 kV result in negative shifts of the working electrode potential of 250 mV and 375 mV, respectively. Consequently, a more positive ''virtual'' potential setting via the potentiostat is necessary to adjust the desired working electrode potential correctly as compared with voltammetric experiments in the absence of a high voltage electrical field. In addition, hydrodynamic voltammetry experiments under NACE–ED conditions, applying different detection potentials, led to results which were in agreement with the above conclusions derived from CV measurements.

The decreasing voltammetric signals for succes-

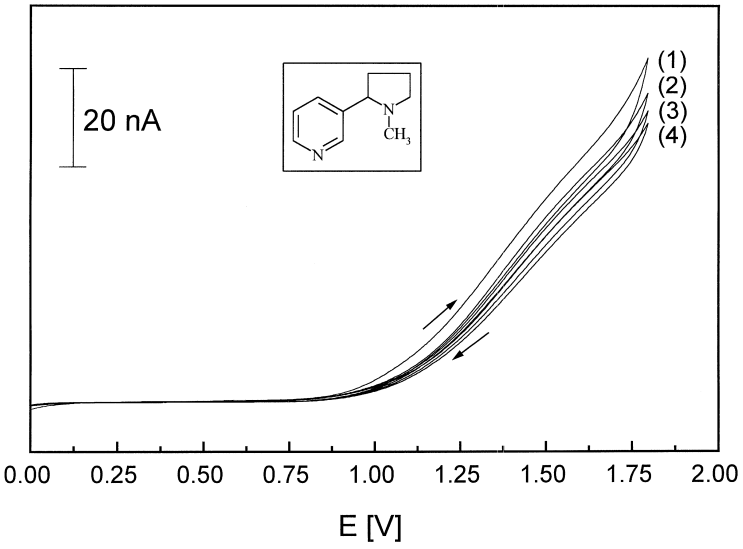

Fig. 2. Cyclic voltammograms of 5 m*M* nicotine in acetonitrile containing 1 *M* acetic acid and 10 m*M* sodium acetate. Scans 1–4 represent the first, second, third and fourth voltammogram, respectively. A platinum microdisk electrode (d_{Pt}=25 μm) was used and the scan rate was 25 mV/s.

sive CV scans as shown in Fig. 2 indicate some effect of electrode fouling. Usually, the extent of electrode deactivation is much lower under real conditions of NACE–ED because the concentration of analyte is magnitudes of order lower than in the CV experiments and the nicotine zone is only for a short time in contact with the electrode. Nevertheless, it is desirable to find an appropriate pretreatment procedure to restore the electrode response. In the case of nicotine oxidation a combination of an anodic and a cathodic potential pulse (3.0 V, 5 s/−0.5 V, 5 s), applied to the working electrode which was placed in the background electrolyte solution, resulted in complete restoration of the electrode response characteristics. This pretreatment procedure was therefore applied before starting a new NACE–ED experiment.

3.2. Analytical characteristics of NACE–ED determination of nicotine

The electrolyte system used for NACE–ED experiments was an acetonitrile solution containing 1 *M* acetic acid and 10 m*M* sodium acetate which was previously shown to yield very reliable NACE–ED results [16]. Fig. 3 illustrates the NACE–ED behavior of nicotine in comparison to those of $[FcMTMA]^+$ and ferrocene. The relative migration time obtained for nicotine suggests that its predominant form in the non-aqueous solution is a monoprotonated species.

The reproducibility of migration times and peak heights were determined on the basis of eight consecutive NACE–ED measurements of samples containing 8 μg/ml nicotine applying experimental conditions as specified in Fig. 3. The relative standard deviations (RSDs) for migration times and peak heights were 0.1% and 1.6%, respectively. The concentration dependence of the peak current i_p was studied for concentrations ranging from 0.1 μg/ml to 10 μg/ml. The results of linear regression were as follows: $i_p = 89.3 \pm 1.3$ (pA ml/μg)·$c + 10.9 \pm 5.4$ pA ($r = 0.9997$, $n = 6$).

The limit of detection (LOD), calculated on the basis of the peak-to-peak noise and a signal-to-noise ratio of 2, was 13 ng/ml (286 fg). The obtained LOD compares favorably to previously reported results [21,22,26].

3.3. Investigation of tobacco samples

The high sensitivity of NACE–ED determinations

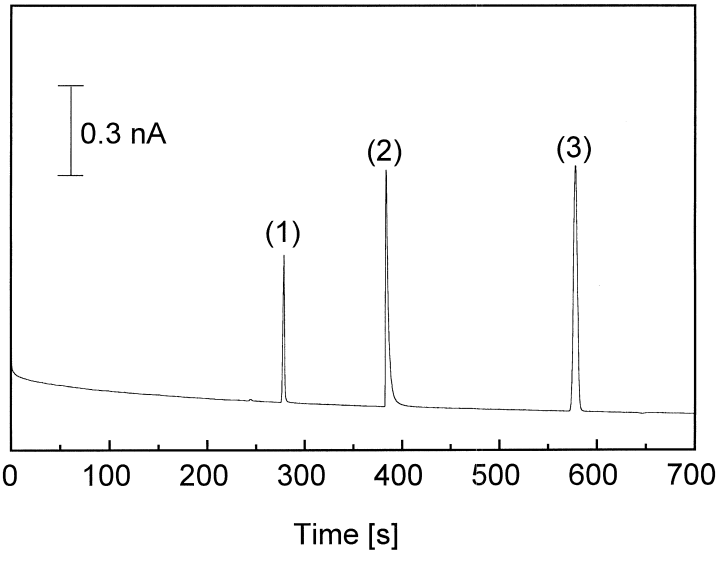

Fig. 3. Electropherogram for a mixture of $2 \cdot 10^{-5}$ *M* $[FcMTMA]ClO_4$, $5 \cdot 10^{-5}$ *M* nicotine and $2.2 \cdot 10^{-5}$ *M* ferrocene. Experimental conditions: capillary dimensions, 79.5 cm×75 μm I.D.; running electrolyte, acetonitrile containing 1 *M* acetic acid and 10 m*M* sodium acetate; injection volume, 22 nl; separation voltage, 20 kV; sensing electrode, 50 μm Pt disk electrode; detection potential, 1.75 V.

of nicotine allows one to apply a high dilution ratio for the sample preparation procedure which should be advantageous to reduce potential matrix effects. Various extracting solutions were studied regarding the extraction efficiency. The extraction was performed in an ultrasonic bath as described in the experimental section. The nicotine determination was done by NACE–ED measurements applying standard addition technique. Typical electropherograms for a tobacco sample and additions of nicotine standards are illustrated in Fig. 4. The recovery of nicotine was evaluated with respect to a tobacco reference material with a known content of nicotine. The differences in extraction efficiency obtained for the different extracting solutions are summarized in Table 1. Clearly, the highest average recovery for nicotine was obtained by using a mixture of acetonitrile–acetic acid–water (20:5:75, v/v). The relatively high acidity of this solution facilitates the extraction of nicotine via the conversion to protonated species that are readily soluble in this medium. A comparable solvent mixture was also used by Troje et al. [21] whereas in other reports alkaline solutions [20,23] or chloroform [22] were employed for the extraction of nicotine from tobacco. For subsequent measurements of nicotine in tobacco samples the acetonitrile–acetic acid–water mixture was used throughout as the extracting solution. The accuracy of these determinations was evaluated by comparison with the known nicotine content of the reference material. A nicotine content of 3.5±0.25% (n=10) was measured which agrees well with the certified value of 3.66±0.04%. The precision for repetitive determinations of nicotine in tobacco is considerably influenced by the extraction procedure. A prolongation of the extrac-

Table 1
Efficiency for the extraction of nicotine from tobacco using the following extracting solutions: (1) acetonitrile, (2) acetonitrile containing 1 *M* acetic acid and 10 m*M* sodium acetate, (3) aqueous 0.1 *M* sodium hydroxide, (4) acetonitrile–acetic acid–water mixture (20:5:75, v/v)[a]

Extracting solution	Nicotine recovery, % ±SD (n)
(1)	19.4 (2)
(2)	30.0 (2)
(3)	89.5 (2)
(4)	96.4±7.0 (10)

[a] Extraction conditions are specified in Section 2.2 and the nicotine determinations by NACE–ED were based on standard addition technique (see Fig. 4). SD=standard deviation, n= number of samples.

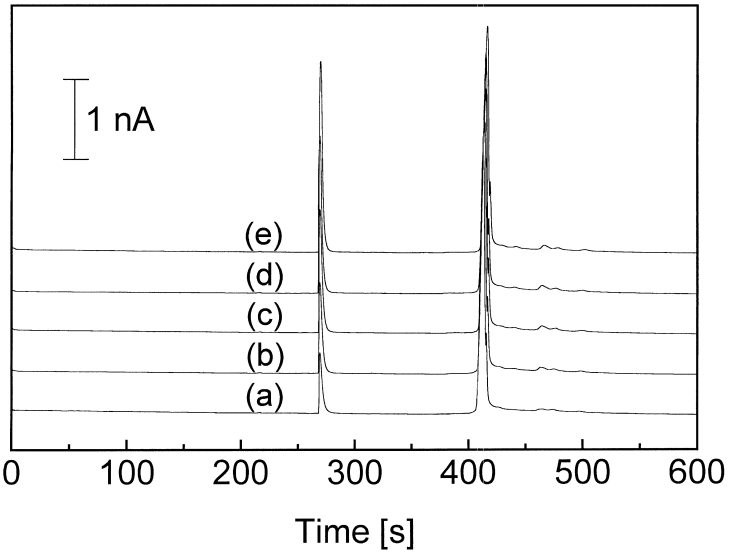

Fig. 4. NACE–ED determination of nicotine extracted from a tobacco sample. Tobacco sample (50 mg) was extracted by 10 ml of a solvent mixture (see text for details) and diluted 1:10 with the separation buffer. The electropherograms a–e correspond to injections of sample plus additions of 0, 20, 40, 60 and 80 μl of 0.8 mg/ml nicotine to 2 ml sample solution. Experimental conditions as in Fig. 3 except that a separation voltage of 30 kV was applied and the detection potential was 1.85 V.

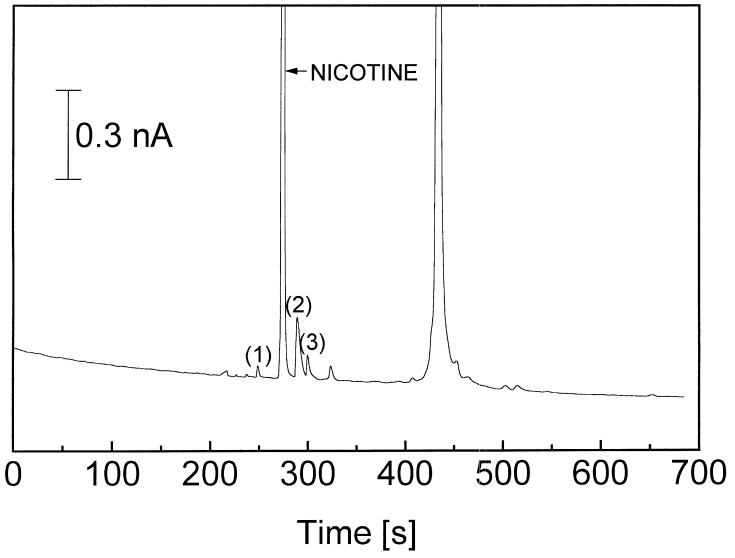

Fig. 5. NACE–ED measurement of a tobacco extract spiked with (1) 0.16 mg/ml cotinine, (2) 0.07 mg/ml nornicotine and (3) 0.07 mg/ml anabasine. Experimental conditions as in Fig. 4.

tion time might help to ensure complete extraction of nicotine and to approach the precision obtained for NACE–ED determinations of standard solutions of nicotine.

Under the conditions used for the electropherograms shown in Fig. 4 it appears that nicotine is the only detectable compound for migration times shorter than that of the electroosmotic flow. However, to ensure that other tobacco alkaloids do not interfere with the determination of nicotine an extracted tobacco sample was spiked with other common tobacco alkaloids such as nornicotine, anabasine and cotinine. The corresponding NACE–ED measurement is shown in Fig. 5. The signals obtained for the minor tobacco alkaloids are well separated from the nicotine peak. In addition, it was found that the sensitivity of nicotine determinations is much higher than that obtained for the other alkaloids. Therefore, it is not surprising that for the considerably diluted tobacco extracts only nicotine signals were registered.

4. Conclusions

The suitability of NACE–ED for the analysis of nicotine has been studied in this work. Owing to the extended accessible potential range well-defined oxidative detection of nicotine can be performed in the non-aqueous medium. It has been demonstrated that NACE–ED represents an attractive approach for highly sensitive and selective determination of nicotine in tobacco.

The detection was based on the use of a newly developed detector cell which was found to be very convenient for daily operation. Advantages of the detector include inertness of the detector components, simple capillary-to-electrode alignment, use of 75 μm I.D. capillaries without the need for electrical field decouplers and minimal effort for maintenance of the detector.

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