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Determination of cannabinoids in hair using high-pH* non-aqueous electrolytes and electrochemical detection Some aspects of sensitivity and selectivity

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Abstract

Non-aqueous capillary electrophoresis with electrochemical detection (NACE–ED) was applied to the determination of cannabinoids in hair. The effect of different electrolyte compositions on the selectivity of the separation of tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) and tetrahydrocannabinol carboxylic acid (THCA) was studied. Complete electrophoretic resolution was obtained using a strongly basic background electrolyte consisting of 5 m*M* sodium hydroxide dissolved in acetonitrile–methanol (1:1). Electrochemical detection yielded well defined signals in the oxidation mode. In order to obtain low limits of detection experimental parameters, which determine the sensitivity and the noise level, were optimized. A crucial parameter for sensitive measurements using a wall-tube flow cell as end-column detector is the distance between the capillary outlet and the working electrode. The highest signal-to-noise ratio using a 50 μm I.D. capillary was obtained at a distance of 25 μm. When the capillary outlet was moved away from the working electrode, thus reducing the strength of the separation field present at the working electrode, a large low frequency noise developed. This rise was attributed to disturbances of the hydrodynamic pattern in the flow cell. Analytical aspects such as sensitivity, reproducibility and selectivity were addressed in this work. The precision of NACE–ED regarding migration time and peak height for a sample containing 1 μg/ml THC was 0.4% and 1.1% (RSD), respectively ($n=5$). The calibration curve was linear for concentrations ranging between 0.1 and 10 μg/ml ($r=0.998$). The limit of detection for THC was 37 ng/ml, which is almost two orders of magnitude lower when compared with on-column UV detection. The method was evaluated using hair samples containing cannabinoids as sample material.

Keywords: Sensitivity; Selectivity; Non-aqueous capillary electrophoresis; Forensic analysis; Cannabinoids

1. Introduction

Capillary electrophoresis (CE) has matured over the last 2 decades into an indispensable method for liquid phase separations. The principles underlying the separation have been investigated in detail and the method has been applied to a broad variety of analytes. Occasionally, organic solvents have been added to the separation buffer to improve the solubility of hydrophobic compounds or to alter the selectivity, particularly in micellar capillary electro-

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chromatography (MECC). In the recent past many reports have appeared in the literature based on work using purely organic solvents which offer unique means to manipulate selectivity [1–10]. Much of this research was focused on the separation of basic drugs [1,2]. Typically ammonium acetate or sodium acetate and acetic acid have been added to solvents such as methanol, acetonitrile, *N*-methylformamide, *N*,*N*-dimethylformamide or mixtures of these. These studies have confirmed improvement of selectivity for the separation of basic drugs by non-aqueous capillary electrophoresis (NACE). The separation of acidic analytes is, however, less favorable since capillary electrophoresis at high pH relies on a rapid and stable electroosmotic flow (EOF). Only solvents with a large dielectric constant to viscosity ratio such as acetonitrile give sufficiently short migration times.

The addition of organic solvents shifts the dissociation constants for both acidic and basic compounds to various degrees depending on the physicochemical properties of the solvents. Generally, pK_a values for acids become greater. Hence, strong acids which are leveled in water can be differentiated in non-aqueous solvents. Organic solvents may also alter the selectivity by replacing water molecules in the solvation sphere, thereby changing the effective radius of the solvated analyte ions. Furthermore, non-aqueous solvents can promote the formation of homo- and heteroconjugates, which does not occur in protic solvents. This is due to hydrogen bonding in these solvents, which prevents the association of the dissolved species with one another [3,4]. The use of ion-pair reagents or other additives, such as cyclodextrins has been exploited in NACE as well.

A number of articles describe the analysis of acidic species using high pH* (pH* – apparent pH which is measured in the non-aqueous media using a glass electrode calibrated with standard non-aqueous buffer solutions) non-aqueous solvents, although such analysis has received only little attention compared to NACE of basic species. Chiari and Kenndler improved the selectivity for the separation of aromatic and aliphatic acids by using a tris(hydroxymethyl)aminomethane–acetate buffer system with methanol as the solvent [5]. A wide range of acidic compounds of different pK_a were quantitatively assessed by Altria and Bryant [6]. Belder et al. studied the influence of the pH on the electroosmotic flow in hydrophilic coated capillaries [7]. Tjørnelund et al. used hexadimethrine bromide as an EOF modifier and obtained very fast separations of anionic compounds in the coelectroosmotic mode [8]. Morales and Cela were able to demonstrate a 400fold improvement in sensitivity when field amplified sample stacking was applied to the analysis of priority pollutant phenols [9].

The use of organic solvents is also beneficial for detection. The low electrophoretic currents allow the use of larger capillaries with a longer path length for optical on-column detection schemes [10]. The coupling of mass spectrometry is facilitated by the high volatility of organic solvents. Electrochemical detection (ED), which is attractive due to its very low detection limits [11,12] benefits from the wide accessible potential window [13,14]. For instance, in dry acetonitrile potentials between -3 and $+3$ V can be applied to the working electrode. Compounds which cannot be oxidized (reduced) in water may become detectable with high sensitivity. Also, the absence of oxide layers at the electrode reduces the background current, hence improving the limit of detection. Two typical problems which hamper the broader use of ED can be solved by the use of non-aqueous media. Namely the low conductivity of electrolytes dissolved in organic solvents reduces interference between the high voltage separation field and the detection circuit and simple detector configurations without a decoupler can be employed [15–17]. The eluting analyte molecules are detected at an easy-to-maintain microdisk electrode. Disk shaped electrodes also provide a very high signal-to-noise ratio [18–20]. The problem of decreasing sensitivity over extended periods of measurement with amperometric electrodes is also addressed by organic solvents. The high solvation power of these media significantly reduces the risk of unwanted deposits of redox products at the surface of the electrode, thus improving the reproducibility of the surface state of the electrode [21].

Cannabinoids were the target analyte for NACE–ED in this study. They represent a major class of illicit drugs, owing to its easy accessibility and perhaps low addictive potential. Additionally, some cannabinoids exhibit a therapeutic potential [22].

Cannabinoids are routinely determined by means of gas chromatography–mass spectrometry (GC–MS), thereby enabling the identification of the active compounds [23,24]. This approach however, requires complex instrumentation and all samples must be derivatized prior to injection [25]. Therefore, there is an interest in an economical method with a low detection limit for the analysis of cannabinoids in biological matrices. The first attempt to determine illicit drugs by capillary electrophoresis, which does not necessitate the derivatization of heat instable compounds, was published by Weinberger and Lurie [26]. Subsequently many reports concerning the analysis of biosamples by CE appeared [27,28]. Capillary electrochromatography allowed the simultaneous determination of acidic, basic and neutral organic compounds [29]. However, capillary-based separation techniques are often not fully exploited for the analysis of low concentrations in micro samples due to the limitations of standard on-column UV detection. More adequate detection limits for cannabinoids in the context of miniaturized analytical techniques can by obtained by electrochemical detection [30]. In high-performance liquid chromatography (HPLC), there has been a long tradition of electrochemical detection of cannabinoids in the oxidation mode [31–33]. Studies by Isenschmid and Caplan [34], Thompson and Cone [35], Zweipfenning et al. [36], Fisher et al. [37] and Kraemer and Kovar [38] have demonstrated the potential and reliability of this approach. Detection limits in the low ng/ml range were reported for tetrahydrocannabinol (THC), cannabinol (CBN), cannabidiol (CBD) and tetrahydrocannabinol carboxylic acid (THCA). However, an experimental difficulty encountered by several research teams is the high detection potential required for the oxidation of cannabinoids. Anodic potentials of up to 1.2 V were applied to the working electrode. This voltage is very close to the decomposition potential of water, thus increasing the background current and consequently, the noise.

In this work, non-aqueous electrolyte systems of high pH* were evaluated regarding the sensitive and selective determination of cannabinoids. In addition, the analytical system was systematically characterized and optimized with respect to the signal-to-noise ratio. The method was applied to the analysis of cannabinoids in hair.

2. Experimental

2.1. Apparatus and equipment

The detector used for end column detection in this work was similar to a detector design which has been reported elsewhere [15]. Briefly, the detector cell consisted of a cylindrical glass body with an electrolyte volume of about 1 ml (Fig. 1). The separation capillary and the microdisk working electrode were vertically mounted in the cell, thereby forming a wall-tube configuration. An annular platinum electrode incorporated in the glass body served as a counter electrode for the detection circuit as well as for the separation circuit. The reference electrode was connected to the cell via a ceramic frit. The capillary outlet and the working electrode were precisely aligned by means of a simple x–y–z micropositioner. For improved mechanical stability the thin capillary was guided in a glass tube with an appropriate inner diameter at the end. The advantage of this design is that the capillary can be removed from the cell between measurements without the need to realign it. Platinum and gold microdisk electrodes (disk diameter 25 μm) were prepared by sealing fine wires (Goodfellow, Cambridge, UK) into soft glass capillaries with an O.D. of 0.4 mm at the tip. A coil made of silver wire (0.5 mm in diameter) served as a quasi reference electrode. The

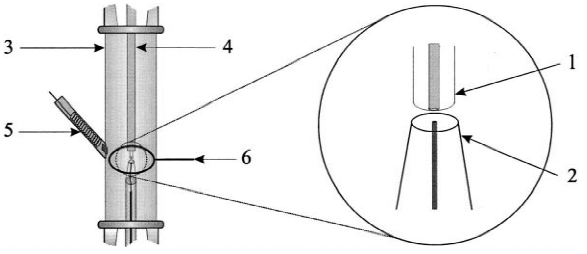

Fig. 1. Schematic diagram of the detector cell. 1, Separation capillary; 2, microdisk working electrode; 3, cylindrical cell body; 4, guide capillary; 5, reference electrode; 6, counter electrode.

detector cell was placed in a Faraday cage to reduce interference from external noise.

Fused-silica capillaries with an I.D. of 50 μm and an O.D. of 360 μm were obtained from Polymicro Technologies (Phoenix, AZ, USA). Before initial use, new capillaries were flushed with 0.1 *M* NaOH for 10 min, with distilled water for 2 min and finally with the buffer for 10 min. After use, the capillaries were washed with pure acetonitrile and stored with the open ends in a vial containing acetonitrile. Since it was found that acetonitrile can lead to a considerable swelling of the polyimide coating, which can eventually block the capillary, 2 to 3 mm of the coating were removed from each end of the capillary using a flame.

The separation voltage was provided by a high-voltage power supply (Model SL40 PN10, Spellman, Plainview, NY, USA). Both the high voltage and the electrophoretic current were monitored using digital voltmeters for better resolution of small readings. The buffer reservoir connected to the high-voltage source was housed in a Plexiglas box fitted with an interlock to protect the operator from electric shock. The high-voltage source was connected to the main power supply using an isolating transformer to prevent ground loops.

All electrochemical measurements were performed in the three-electrode mode using a BAS LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN, USA). In order to reduce noise introduced by the cables connecting the working electrode with the detector, a battery operated, laboratory-made current amplifier was used and placed inside the Faraday cage. For optical detection a Thermo Separation Products UV detector Model UV 1000 was used. Electropherograms were collected using a Keithley DAS-1600 analog-to-digital converter board. The sample rate was set to 10 samples/s for all experiments. For cyclic voltammetric experiments, a BAS voltammograph was connected to the BAS LC-4C amperometric detector providing a triangular voltage ramp. The apparent pH of non-aqueous buffers was measured by means of an OAKTON pH/mV-meter series 35617 and a combination pH glass/Ag/AgCl-reference electrode. UV spectra were recorded using a Cary 3 Bio UV–visible spectrometer (Varian). The scan rate was set at 600 nm/min. The measurements were performed in the double beam mode with baseline correction.

2.2. Chemicals

Acetonitrile (99.9% HPLC grade, water <0.002%), methanol (assay, 99.9%; suitable for use in spectrophotometry, water, 0.01%), sodium acetate (>99.0%), sodium hydroxide (98.5%) and acetic acid (glacial) were obtained from Fisher Scientific. Tetraethylammonium hydroxide solution (1.5 *M* in methanol) was purchased from Fluka (Buchs, Switzerland). Drug standards (methanolic solutions), crystal violet and ferrocene were obtained from Sigma (St. Louis, MO, USA). All substances were used as received except ferrocene which was purified by sublimation before use. Solid-phase extraction cartridges (LC-8, WAX) were purchased from Supelco. Worldwide monitoring CSTHC101 solid-phase extraction cartridges were purchased from United Chemical Technologies (Bristol, PA, USA).

2.3. Sample preparation

2.3.1. Liquid–liquid extraction

In order to destroy the protein matrix of the hair and to liberate the cannabinoids a sample preparation procedure described by Cirimele et al. [39] was employed. A 100-mg amount of hair was washed with phosphate buffer (pH 7) followed by distilled water and cut in very small pieces using a pair of scissors. The sample was then incubated in 1 ml 1 *M* sodium hydroxide for 10 min at 95°C. After cooling, the homogenate was extracted with 5 ml hexane–ethyl acetate (9:1, v/v). The organic phase was evaporated to dryness, and the extract was reconstituted in 50 μl of run buffer. An aliquot was hydrodynamically injected (Δ*h*, 5 cm; *t*, 20 s).

2.3.2. Solid-phase extraction

Sample preparation following the procedure of Wernly and Thormann [40] was conducted as follows: the CSTHC101 solid-phase extraction cartridges were activated immediately prior to use by passing through 3 ml methanol followed by 3 ml distilled water. The cannabinoids were liberated from the hair matrix as described above. The pH of the

digest was adjusted to 3.5 by adding 0.3 ml glacial acetic acid and an appropriate amount of hydrochloric acid (18%). The sample was applied to the cartridge and drawn through at a flow-rate of 1 ml/min. The cartridges were then sequentially rinsed with 2 ml of distilled water and 2 ml of 0.1 *M* HCl–acetonitrile (70:30). Prior to elution the cartridges were rinsed with 0.2 ml hexane. Thereafter the cannabinoids were eluted with 2 ml of hexane–ethyl acetate (50:50) at a flow-rate of 1 ml/min. The organic phase was evaporated using a desiccator connected to a water-jet aspirator and the residue was reconstituted in 100 μl of acetonitrile.

3. Results and discussion

3.1. Optimization of the selectivity for the separation of cannabinoids

In initial experiments moderately basic background electrolytes of various compositions containing sodium acetate or ammonium acetate dissolved in mixtures of methanol and acetonitrile were tested for the separation of cannabinoids. These electrolyte systems are stable over time and have been reported to be well-suited for the separation of medium and strong acids [6]. However, no satisfactory resolution of cannabinoids could be accomplished by means of these electrolyte systems. Organic bases such as benzoate, pyridine, imidazole and 2,6-lutidine were also tested, but not considered for further investigation either because of significant absorption in the UV range or because of deposition of redox products onto the surface of the working electrode.

Strong bases which do not interfere with electrochemical detection or UV detection and which are capable of deprotonizing cannabinoids are tetraalkylammonium hydroxides. The electropherogram of CBN, THC and CBD shown in Fig. 2a was obtained using 3.75 m*M* tetraethylammonium hydroxide (TEA-OH) dissolved in MeOH–acetonitrile (1:1) as background electrolyte. However, despite the high pH* of 24, an incomplete separation was obtained. A possible explanation might be the formation of ion pairs between the TEA cation and the deprotonized cannabinoids, which affects the hydrodynamic radius of the molecules and thus their mobility. Another very basic electrolyte system which has been applied to the separation of weak bases [6] and which does not form ion pairs consists of sodium hydroxide dissolved in methanol–acetonitrile. Although a solution consisting of 2.5 m*M* NaOH dissolved in MeOH–acetonitrile (1:1) is less basic than the electrolyte containing TEA-OH, all cannabinoids including THCA were baseline resolved (Fig. 2b). As expected, THCA had the longest migration time followed by CBN, THC and CBD. It is interesting to note that CBD, which has two phenol moieties, migrates faster than CBN. Moreover, the migration order of CBD and CBN is reversed when compared with the separation performed in the presence of TEA-OH (Fig. 1a). In a further set of experiments the effect of an increased salt concentration in the background electrolyte was investigated. A high salt concentration is potentially useful to increase the conductivity for electrochemical detection and to improve the sample capacity. As can be seen from Fig. 2c the addition of 2.5 m*M* sodium acetate to the background electrolyte leads to longer migration times but not a significantly improved resolution. A high salt concentration generally reduces the rate of the electroosmotic flow thereby increasing the time before the analyte molecules reach the detector. For sensitive electrochemical detection at a very high detection potential it was important to minimize the concentration of methanol in the separation electrolyte since methanol can be oxidized and thereby increase the background current. Unfortunately, an increase of the acetonitrile content resulted in longer migration times and slightly broader peaks (Fig. 2d). A background electrolyte based on pure acetonitrile was not considered because of the poor solubility of sodium hydroxide in this medium.

3.2. Robustness of the separation

During the optimization of the buffer composition it was observed that with a freshly prepared buffer the noise level of the baseline and the background absorption at 220 nm was lower than after the use of the buffer for several days. This rise of absorption

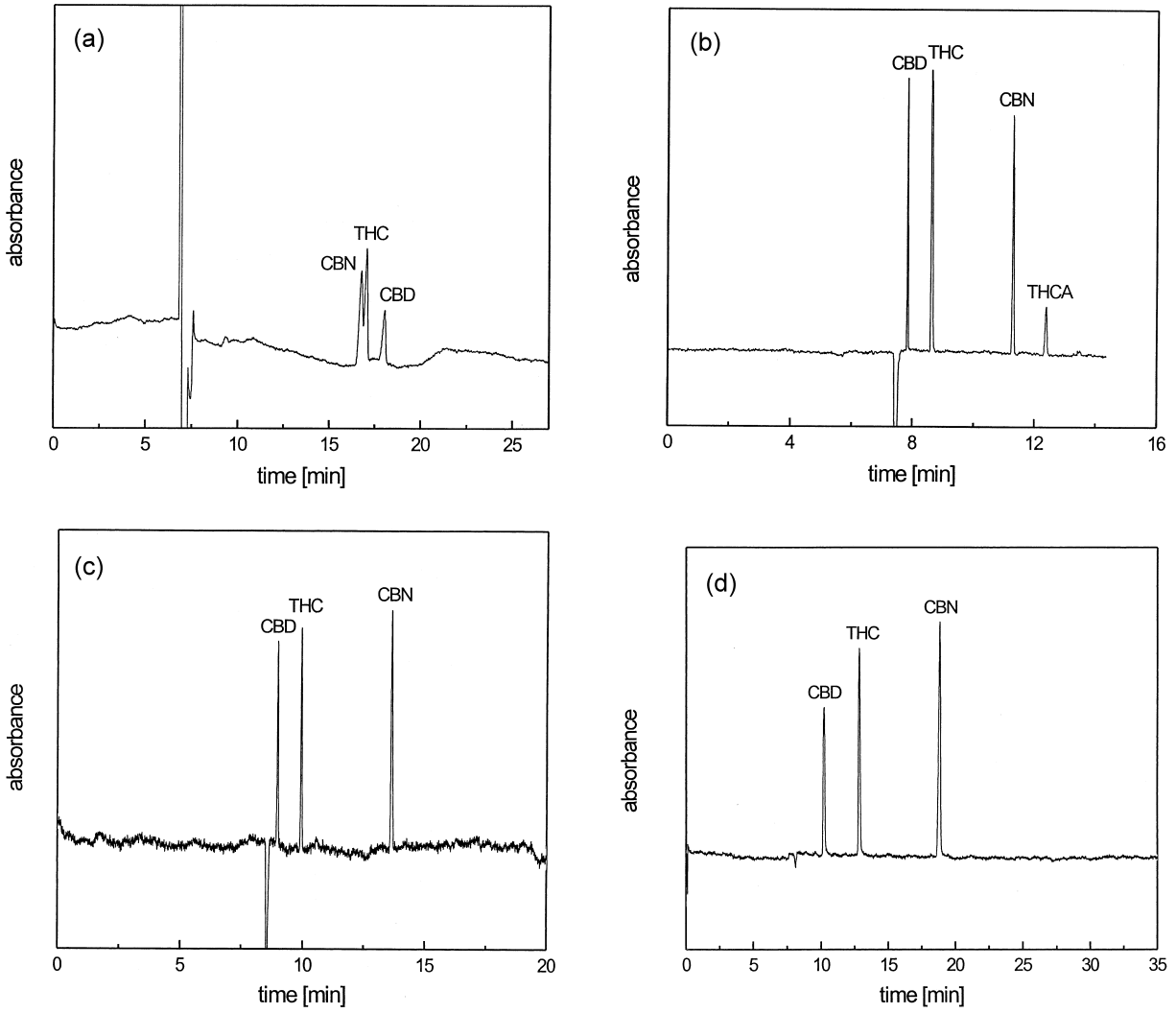

Fig. 2. Electropherograms for mixtures of cannabinol (CBN), cannabidiol (CBD) tetrahydrocannabinol (THC) and tetrahydrocannabinol carboxylic acid (THCA) were obtained using run buffers consisting of (a) 3.75 m*M* tetraethylammonium hydroxide in acetonitrile, (b) 2.5 m*M* NaOH in acetonitrile–MeOH (1:1), (c) 2.5 m*M* sodium acetate–2.5 m*M* NaOH in acetonitrile–MeOH (1:1) and (d) 2.5 m*M* sodium acetate–2.5 m*M* NaOH dissolved in acetonitrile–MeOH (3:1). Experimental conditions: capillary 95 cm (effective length 80 cm)×50 μm I.D.; hydrodynamic injection at h_{inj}, 12 cm for t_{inj}, 20 s; sample concentration, (a), (c) and (d) 10 μg/ml each, (b) CBD, THC and CBN 20 mg/ml each, THCA, 5 μg/ml; separation voltage, 20 kV; detection wavelength, 220 nm.

was attributed to the formation of carbonate. Although the UV spectrum of the buffer showed no defined signals for carbonate a significant rise in absorbance was found after bubbling air containing carbon dioxide through the buffer solution. Since the formation of carbonate in the separation electrolyte also alters the pH* and subsequently the separation performance it was found to be important to prepare a fresh buffer twice a week. Furthermore, the volumetric flask containing the buffer was stored in a desiccator filled with sodium hydroxide pellets. When the separation capillary was flushed after every run with the run buffer for 1 min (p, 10 p.s.i.; 1 p.s.i.=6894.76 Pa) an excellent reproducibility of the migration time for THC (0.7%), CBN (2.0%) and CBD (0.4%; n=5) could be obtained. All

injections were performed hydrodynamically from the same sample vial (Δh, 5 cm; t, 20 s) and the separation electrolyte was renewed after 5 runs. The vials used in the laboratory-made CE system were capped with well fitting (but not perfectly air tight) PTFE stoppers.

3.3. Optimization of the signal-to-noise ratio in ED

Special attention was paid to the optimization of the S/N ratio in order to match the low detection limits required for the analysis of illicit drugs in biological matrices. The amperometric signals in Fig. 3 show that the highest sensitivity using a 50 μm capillary is obtained at a distance of 25 μm between the capillary outlet and the surface of the working electrode. For larger distances the peak height decays rather rapidly until at a distance of 500 μm no signal could be measured. This behavior is qualitatively similar for the neutral ferrocene (t_{mig}, 14.8 min) and the cationic crystal violet (t_{mig}, 6.2 min; data not shown) and therefore independent of the migration rate. Interestingly, significant band broadening, which generally reduces sensitivity and limits electrophoretic resolution, was not observed even for larger distances.

Another crucial parameter for the analysis of very low concentrations besides the sensitivity is the background noise level including the peak-to-peak noise. The graph in Fig. 4 illustrates the background current (baseline, no electroactive compound) as well as its fluctuations for a set of different distances between the capillary outlet and the working electrode. The stepwise increase of the level of the background current is due to a shift of the actual potential present at the working electrode towards a higher potential when the capillary outlet is being moved away from the working electrode thereby reducing the affect of the separation voltage. The lowest peak-to-peak noise (300 fA) was obtained at a distance of 25 μm. A preamplifier with a gain of 1000 placed inside of the Faraday cage was used to reduce electronic noise introduced by the cable to the amperometric detector. Note that the peak-to-peak noise increases as the strength of the high voltage field at the working electrode is reduced. This somewhat unexpected behavior might be explained by hydrodynamic disturbances of the wall-tube flow

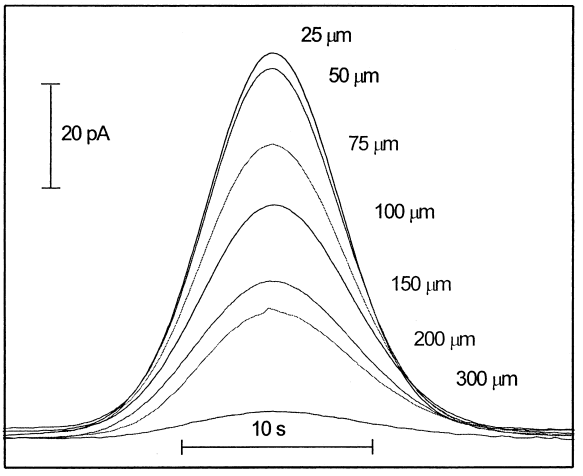

Fig. 3. Sensitivity in end-column NACE–ED: effect of the distance between the capillary outlet and the surface of the working electrode on peak height and peak width. Experimental conditions: capillary I.D., 50 μm; working electrode, 25 μm Pt-microdisk electrode set to a ''virtual'' potential of 800 mV for the detection of ferrocene (50 μ*M*); electrokinetic injection (5 kV; 20 s), background electrolyte, acetonitrile containing 1 *M* acetic acid and 10 m*M* sodium acetate; high voltage field, 219 V/cm.

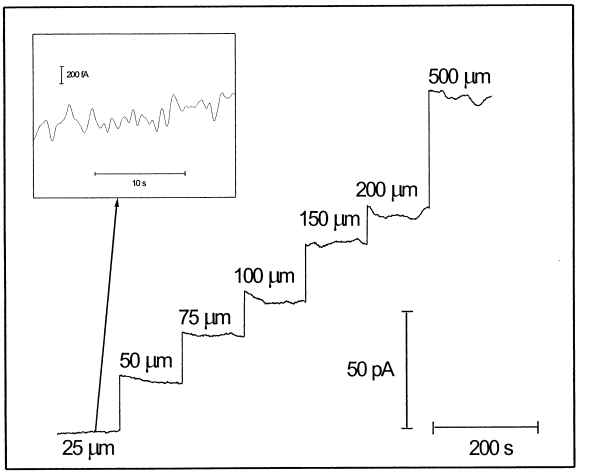

Fig. 4. Background current and noise level in NACE–ED for different distances between the capillary outlet and the working electrode. Experimental conditions: capillary I.D., 50 μm, 25 μm Pt-microdisk electrode, detection potential, 1100 mV, high voltage field, 219 V/cm, electrophoretic current, 1.3 μA; background electrolyte, 1 *M* acetic acid, 10 m*M* sodium acetate dissolved in acetonitrile.

characteristics. Furthermore, the higher potential present at the working electrode at longer distances might also give rise to additional noise. The noise component with a low frequency in the mHz range and an amplitude of up to 5 pA (at 500 μm) at distances longer than 150 μm was attributed to uncontrolled convective effects in the flow cell. The experiment illustrated in Fig. 4 was also conducted in the presence of 1 m*M* ferrocene as an electroactive compound and similar noise characteristics were observed although for larger distances a lower Faradaic current was observed. The lowest peak-to-peak noise with an amplitude of 18 pA also occurred at distance of 25 μm.

3.4. Voltammetric behavior of cannabinoids

The voltammetric behavior of cannabinoids was studied in non-aqueous solution by cyclic voltammetry. Cyclic voltammograms of THC were recorded using platinum and gold microdisk electrodes. The use of an non-aqueous electrolyte system consisting of 2.5 m*M* NaOH dissolved in MeOH–acetonitrile (1:1) allowed for a wide accessible potential window ranging from −250 to +1400 mV for gold electrodes and from −200 to +1200 mV for platinum electrodes. THC exhibited a well defined voltammetric wave on a gold microdisk electrode at 1020 mV (data not shown). Five successive scans indicated only negligible electrode fouling. However, for amperometric detection of cannabinoids platinum electrodes were chosen because of their lower background current in the given solvent and therefore potentially lower detection limits. The cyclic voltammograms of THC, CBN, CBD and the background electrolyte recorded on a Pt microdisk electrode are depicted in Fig. 5. The cyclic voltammogram curves reflect the individual voltammetric characteristics of each compound. Although the cannabinoids exhibit no clear mass-transport-controlled current plateau, potentials within the region between 800 and 1000 V appear to be suitable for amperometric detection. For the proper selection of a suitable detection potential in NACE–ED experiments the effect of the separation field on the detection circuit must be taken into account. In the present configuration the application of a separation voltage of 20 kV resulted in a 250 mV shift of the detection potential. Consequently, an offset potential of +250 mV has to be added via the potentiostat to compensate for this shift.

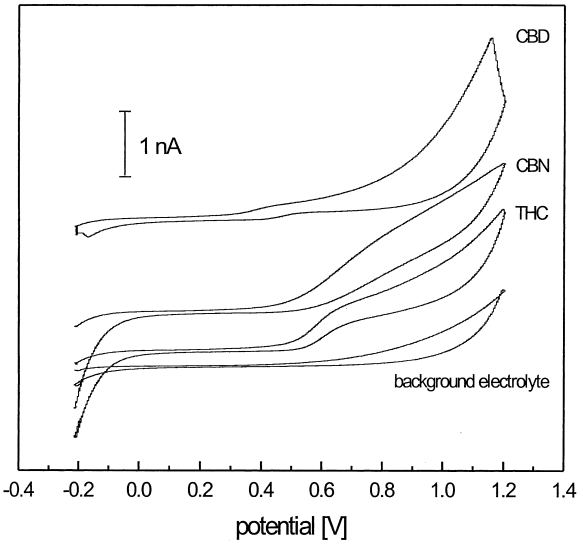

Fig. 5. Cyclic voltammograms of THC, CBN, CBD and background electrolyte [2.5 m*M* NaOH in MeOH–acetonitrile (1:1)]. Experimental conditions: working electrode, 25 μm Pt-microdisk electrode set at scan rate of 25 mV/s. Potentials measured vs. a silver quasi reference electrode. The concentration of the cannabinoids was 160 μ*M* each.

3.5. Analytical characterization of NACE–ED

Electrochemical detection was performed in an electrolyte consisting of 5 m*M* NaOH dissolved in acetonitrile–MeOH because it allowed complete separation of THC, CBN and CBD within less than 14 min and allowed for reliable detector performance (Fig. 6). Magnification of the broad peak at 9.3 min shows that Δ^8- and Δ^9-THC migrate at different rates although the compounds could not be baseline resolved. Table 1 summarizes the separation performance.

Five consecutive samples containing THC, CBN and CBD (1.0 μg/ml each) were injected for the determination of the reproducibility of migration times as well as of peak heights. The experimental conditions are specified in Fig. 6. The concentration

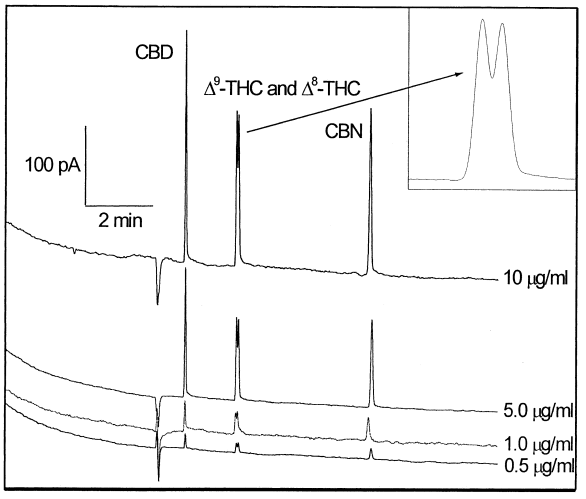

Fig. 6. NACE–ED separation of cannabinoids. Experimental conditions: capillary I.D., 50 μm; running electrolyte 5 m*M* sodium hydroxide dissolved in methanol–acetonitrile (1:1); separation field 219 V/cm; sensing electrode, 25 μm Pt-microdisk electrode set at a detection potential of 1.0 V.

dependence of the peak current i_P was studied for concentrations ranging from 0.1 to 10 μg/ml. The results of linear regression were as follows THC, $i_P=36$ (pA ml/μg)·$c+13$ pA, $r=0.998$, $n=5$; CBN, $i_P=46$ (pA ml/μg)·$c+0.6$ pA, $r=0.998$, $n=5$ and CBD, $i_P=55$ (pA ml/μg)·$c+17$ pA; $r=0.997$, $n=5$. The limit of detection (LOD) based upon the peak-to-peak noise and a signal-to-noise ratio of 2 was calculated according to an injection of 0.1 μg/ml of THC, CBN and CBD, respectively, and the following data were obtained: THC, 37 ng/ml; CBN, 30 ng/ml; CBD, 20 ng/ml. These detection limits are about one to two orders of magnitude lower when compared with on-column UV detection [30] and compare favorably with limits of detection reported for GC–MS [23].

3.6. Determination of cannabinoids in a hair sample

The developed NACE–ED methodology for the analysis of cannabinoids was applied to the determination of THC, CBN and CBD in hair. The analysis of hair samples can indicate exposure to cannabis and can give insight as to whether someone uses illegal drugs regularly and at what doses.

Fig. 7 shows an electropherogram of a hair sample after sample preparation by liquid–liquid extraction. The lower trace (gray) shows an electropherogram of a standard solution containing CBD, THC and CBN (1 μg/ml each) for comparison. Only for CBD a signal completely resolved from the hair matrix could be obtained. This was proved by comparison with a blank hair sample. THC as well as CBN co-migrated with compounds extracted from the hair sample and could therefore not be quantified. The investigated sample, originated from a drug abuser, contained 0.61 ng/mg CBD. This finding is consistent with analytical data previously obtained by a police laboratory for the same sample and indicates a considerable intake of cannabinoids.

An alternative route for sample clean up commonly employed for hair samples is solid-phase extraction. After preliminary tests using standard non-polar (LC-8) and weak anion-exchange columns we concentrated on the use of special cartridges designed for the analysis of THC. These cartridges usually contain a mixed bed consisting of above mentioned stationary phases. Good recoveries of about 80% were obtained using Clean Screen THC columns when the extraction protocol of Wernly and

Table 1
Parameters for the separation of cannabinoids by NACE–ED

Compound	t_{mig} (min)	*N*	*r*	Reproducibility (t_{mig}) (%)	Reproducibility (peak height) (%)
CBD	7.6	204 000		0.4	0.9
THC	9.3	200 000	9.5	0.7	1.1
CBN	13.5	184 000	19	2.0	1.7

Experimental conditions are specified in Fig. 6.

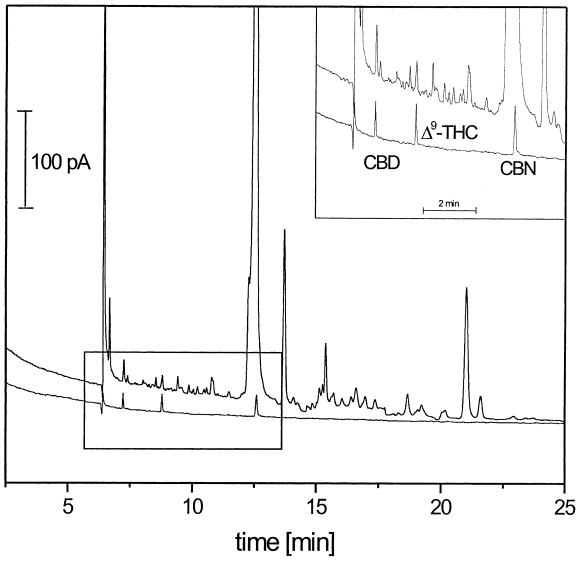

Fig. 7. Determination of cannabinoids in a hair sample originated from a drug abuser after sample preparation by liquid–liquid extraction. The lower trace shows the electropherogram of a standard containing CBD, THC and CBN (1 μg/ml each) injected for comparison. Experimental conditions as in Fig. 6.

Thormann [40] was employed. A procedure according to the manufacturer of the column yielded slightly lower recoveries. In both cases the recovery was highest for THC followed by CBN and CBD.

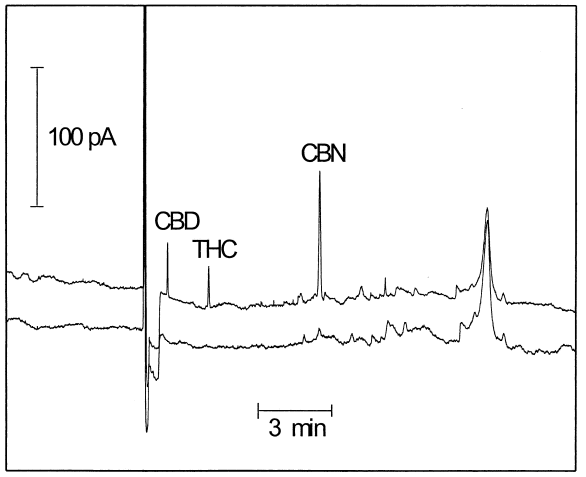

Fig. 8. Electropherogram of a blank hair sample after sample clean up by solid-phase extraction. The upper trace shows the electropherogram of the same sample after adding CBD, THC and CBN (1 μg/ml each). Experimental conditions as in Fig. 6.

An electropherogram obtained from a blank hair sample after sample digestion according to Ref. [39] followed by solid-phase extraction according to Ref. [40] is depicted in Fig. 8 (lower trace). As can be seen all potentially interfering compounds are effectively removed from the injected sample. The upper trace in Fig. 8 shows an electropherogram of the same sample after the addition of 1 μg/ml of THC, CBN and CBD, respectively.

4. Conclusion

This work demonstrates the feasibility of carrying out high-pH* NACE–ED determinations of cannabinoids and application to the analysis of human hair samples. Although rigorous toxicological method validation was not conducted the results show that the method provides reproducible results with respect to peak height and migration time.

The optimum separation electrolyte was selected according to a systematic study of different electrolyte systems. Since the analysis of cannabinoids in a biological matrix requires low detection limits the analytical system was carefully optimized with respect to the signal-to-noise ratio. It was found that a high strength of the separation field present at the sensing electrode does not result in a high electronic noise level in the detection circuit. This result is promising for the future use of electrochemical detection in CE since it indicates the potentially high-performance of easy-to-manufacture detector arrangements.

For the analysis of cannabinoids in hair proper sample clean up proved to be crucial. It was found that solid-phase extraction using a proprietary sorbent removed matrix interference much more effectively than liquid–liquid extraction thereby enabling the interference-free quantification of THC, CBN and CBD in hair samples. In order to use the developed methodology routinely, additional work should focus on the use of an internal standard, similar to the compound suggested in Ref. [37].

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