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Stacking and low-temperature technique in nonaqueous capillary electrophoresis for the analysis of 3,4-methylenedioxymethamphetamine

Low-temperature and ambient-temperature nonaqueous stacking techniques in capillary electrophoresis (CE) are described for the first time. A low-temperature bath was used to control the temperature from ambient to subzero degrees, by which a novel hyphenated method, low-temperature bath-nonaqueous capillary electrophoresis stacking (LTB-NACE stacking) is demonstrated. 3,4-Methylenedioxymethamphetamine (3,4-MDMA) was determined at a concentration of 4.7×10^{-6} M (at a 92.1% confidence level) by normal nonaqueous capillary zone electrophoresis (NACZE) and this was improved to 2.6×10^{-8} M and 5.0×10^{-9} M, respectively, when the NACZE stacking and LTB-NACZE stacking techniques were applied. The content of 3,4-MDMA in an illicit drug and a suspect urine sample was readily detected. Upon application of the LTB to the separation of isomers the resolution (*R*) for the separation of 2,3-/3,4-MDMA was improved from 0.6 (LTB, 22°C) to 1.6 (LTB, −55°C) and for (+)3,4-MDMA/(−)3,4-MDMA, from 0.4 (LTB, 25°C) to 1.0 (LTB, −10°C).

Keywords: Low-temperature bath / 3,4-Methylenedioxymethamphetamine / Nonaqueous capillary electrophoresis / Stacking DOI 10.1002/elps.200305884

1 Introduction

Several reports on sample concentration techniques in capillary electrophoresis (CE) have recently appeared, describing the so-called "stacking" and "sweeping" techniques [1–10]. Such techniques have opened new perspectives in investigations of compounds that exist in low levels, even in the parts per billion (ppb) range. 3,4-Methylenedioxymethamphetamine (3,4-MDMA), a widespread illicit drug, has been discussed in many papers. We previously reported on the utility of a nonaqueous CE buffer for the detection of 3,4-MDMA in urine samples [11]. A field-amplified sample stacking procedure for the analysis of abused drugs, including 3,4-MDMA, has been reported [12], but, to the best of our knowledge, a nonaqueous stacking technique has not been attempted.

Nonaqueous capillary electrophoresis (NACE) has rapidly grown in popularity over the past few years because of its high separation efficiency and short analysis time due to the fact that, at a lower temperature, Ohm's law is linear at higher voltages and thus peak efficiency can be increased, thereby decreasing separation times. Furthermore, untoward effects of Joule heating can be avoided even when a higher electric field strength is used [13–21]; improved detection limits can be achieved due to the fact that many compounds have a higher fluorescence quantum yield in an organic solvent. In addition to the above advantages, NACE because of its possible use at lower temperatures then can be tolerated by CE, and separations can be readily performed at subzero temperatures. In general, when a CE separation is carried out at low temperature, plate efficiency is enhanced, but the analysis time is prolonged because of the increased viscosity of the CE buffer. However, since at a fixed electric field the current decreases with temperature, the capillary can be shortened, thus speeding up the analysis. As a matter of fact, already in the 1970s, gel electrophoresis in glass tubes was performed at −10°C [22]. Low-temperature (25°C to −20°C) CE, in which the applied voltage could be increased without any loss of resolution, has also been reported [23]. More recently, subzero separations have been described [24, 25]. A modified Beckman P/ACE unit can be used at temperatures as low as −20°C [26, 27]. We have also demonstrated that NACE can be applied as an ultra low-temperature technique to separate structurally similar molecules at ~ −70°C [21].

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Abbreviations: BGS, background solution; **LTB**, low-temperature bath; **NACZE**, nonaqueous capillary zone electrophoresis; **3,4-MDMA**, 3,4-methylenedioxymethamphetamine

In this study, we report on the feasibility of NACE stacking, low-temperature bath-NACE stacking (LTB-NACE stacking) and LTB-NACE separation for the separation of structural (2,3- and 3,4-MDMA), and chiral isomers ((±)3,4-MDMA). Several electrophoretic parameters, such as temperature and the injection length required for the separation, were optimized.

2 Materials and methods

2.1 Apparatus

The CE setup (Fig. 1) was fabricated in-house and is similar to a previously described unit [21]. Briefly, a high-voltage power supply (Model RR30–2R, 0–30 kV, 0–2 mA, reversible, Gamma, Ormond Beach, FL, USA) was used to drive the electrophoresis and a 75 μm ID fused-silica capillary (J&W Scientific, Folsom, CA, USA) was used for separation. The sample was hydrodynamiclly injected by raising the sample reservoir to provide the height difference for the injection length (depending on the specific situation). The excitation source was selected by a monochromator (Model SP-150; ARC, Acton, MA, USA) connected to an Xe lamp (SVX/LAX 1450; Müller GmbH Elektronik Optik, Moosinning, Germany). The excitation wavelength was 285 nm; emission was measured at 320 nm. Fluorescence data were collected at a right angle to the light source and dispersed by a second monochromator, followed by detection by means of a photomultiplier tube (ARC Model P2-R928). The analog signal was converted to a digital signal by an A/D converter (ADAM-4012 module; Advantech Taipei, Taiwan). Electropherograms were collected with a data acquisition system connected to a personal computer. The LTB was an insulated container, which could be used as a temperature controller *via* mixtures of ice/rock salt (0°C to −20°C) or dry ice/organic solvents (−15°C to −100°C) [28].

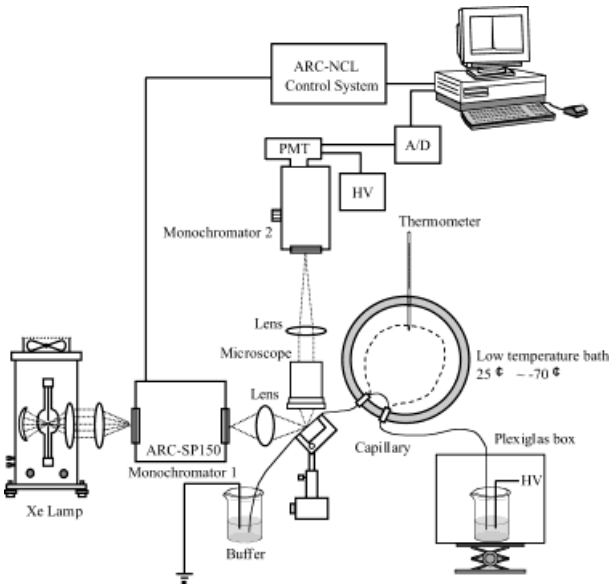

Figure 1. Schematic of the LTB-CE system.

2.2 Methodology

Figure 2 shows schematic diagrams of (A) NACE stacking, (B) LTB-NACE stacking, and (C) LTB-NACE separation, respectively.

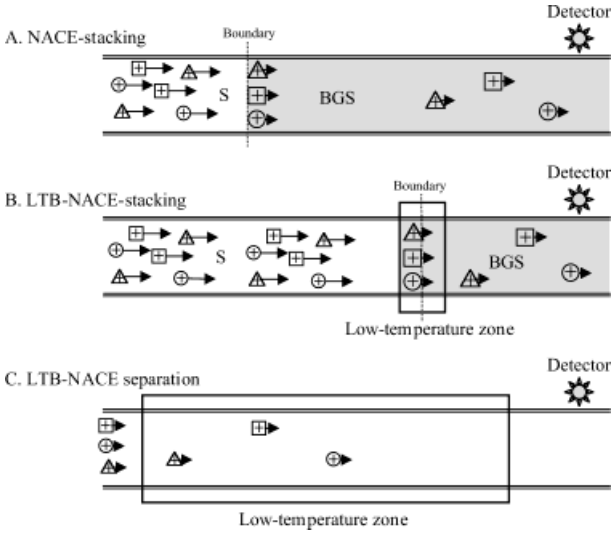

Figure 2. Schematic diagram of (A) NACE stacking, (B) LTB-NACE stacking, and (C) LTB-NACE separation. BGS, background solution; S, sample solution.

2.2.1 NACE stacking

In NACE stacking, the background solution (BGS) consisted of 50 mM ammonium acetate in a mixed methanol-water solution (90:10 v/v), pH* 7.6 (pH* is frequently used in dealing with nonaqueous solutions due to the uncertainties in the liquid-junction potential of the glass electrode) and 2.1 mS/cm conductivity. The sample was dissolved in a 1/100 diluted BGS in the same mixed methanol-water solution (90:10 v/v) resulting in a low-conductivity buffer (pH* 7.7; conductivity, 17 μS/cm). Hydrodynamic injection was achieved by raising the sample reservoir to a height of 30 cm relative to the exit reservoir, thus generating a flow rate of 0.0575 cm/s. When the injection was completed, +25 kV was applied to power the CE separation. This procedure permits the 3,4-MDMA cation, which is moving rapidly, in the sample zone (low conductivity), to then slow down at the junction between the sample solution and the BGS (high conductivity). As a result, the sample became concentrated at the boundary.

2.2.2 LTB-NACE stacking

In LTB-NACE stacking, the buffer solutions and experimental conditions were exactly the same as those used in NACE stacking. A portion of the capillary (near the junction between the sample solution and the BGS) was immersed in the LTB, which served as a low-temperature zone. When stacking was applied, the sample stacked along the capillary axis, becoming almost immobilized near the junction because of the sudden decrease in temperature. Thus, the sample was further focused at the junction. This method permits more sample to accumulate, compared to a normal stacking method.

2.2.3 LTB-NACE separation

In this mode, a longer length of the capillary was immersed in the LTB (2/3–3/4 to the total length). Thus, untoward effects of Joule heating by a higher electric field strength could be eliminated.

2.3 Liquid-liquid extraction procedures

Tablet: Tablet powder (1 mg) was dissolved in 10 mL methanol. After 2 min of sonication and a 2 min centrifugation at 5000 rpm at room temperature, the upper layer was collected and used directly. Urine: Two mL of a urine sample was made alkaline by the addition of excess K_2CO_3. The free bases were then extracted into 4 mL of a hexane/CH_2Cl_2 (3:1 v/v) solution by stirring the suspension for 1 min. After centrifugation, the upper layer was collected and this organic phase was then evaporated to dryness. The residue was dissolved in 20 μL of methanol for the subsequent CE separation.

2.4 Reagents

MDMA was acquired from Radian International (Catalog No. M-013, 99%; 1 mg/1 mL methanol). Sodium cholate ($C_{24}H_{39}O_5Na$) and methyl alcohol (99.8%) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Ammonium acetate (CH_3COONH_4) was obtained from Riedel-de Haën (RdH Laborchemikalien, Seelze, Germany). 2,3-MDMA was synthesized and generously donated by the Command of the Army Force of Military Police, Forensic Science Center, Taiwan.

3 Results and discussion

3.1 NACZE and NACZE stacking

Figure 3 shows typical CE electropherograms of a 3,4-MDMA standard separated by NACZE (frame A) and NACZE stacking (frame B) (the inset in frame A shows the molecular structure of 3,4-MDMA). The total length of the capillary was 100 cm (94 cm to the detector); the applied voltage was +25 kV and the currents were ~ 32 (NACZE) and ~ 2 μA (NACZE stacking), respectively. The complete, optimal separation of 3,4-MDMA was achieved using an ammonium acetate buffer (50 mM) in a methanol-water solution (90:10 v/v). Herein, in the NACZE mode, the sample injection length was ~ 0.3 cm, whereas in the NACZE stacking mode the corresponding length was ~ 48 cm. The sample concentrations were 6.5×10^{-5} M and 4.0×10^{-7} M (signal intensity, both 1.0 V; applied voltage of photomultiplayer tube (PMT), −900 V) in frames A and B, respectively. In comparison with the two electropherograms in frames A and B, a ~ 160-fold improvement in detection sensitivity could be obtained. In the NACZE mode, the effective length of the capillary was 94 cm and this provided a satisfactory separation efficiency ($N = 3 \times 10^5$) but entailed a longer migration time (19.5 min). In contrast, in the NACZE stacking mode, the effective length of capillary was shorter (46 cm). This provided a shorter migration time (9.5 min) and poorer separation efficiency ($N = 8 \times 10^3$).

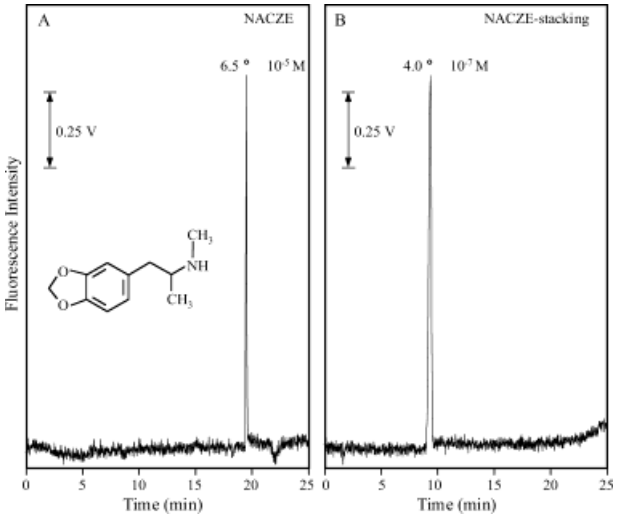

Figure 3. CE electropherograms of 3,4-MDMA obtained by (A) the normal NACZE mode (6.5×10^{-5} M) and (B) by the NACZE stacking mode (4.0×10^{-7} M), respectively. CE conditions: (A) sample injection length, ~ 0.3 cm; ammonium acetate buffer (50 mM) in a methanol-water solution (90:10 v/v). (B) BGS, ammonium acetate buffer (50 mM) in a methanol-water solution (90:10 v/v); sample was solved 1/100 in BGS; injection length, 48 cm. Capillary length, 100 cm (94 cm to the detector).

Under exactly the same experimental conditions used for NACZE stacking, when a low temperature bath (−15°C in this case) was used, a longer (69 cm) sample injection could be used. As a result, a further ~ 4-fold improvement in detection sensitivity was obtained. It is obvious that, by

the use of LTB, the sample could be injected for a longer period of time than a regular stacking method and this also provided a nonlinear improvement in sensitivity. To investigate the appropriate length of the capillary in LTB, different column lengths (8, 24, and 32 cm) were selected for the following experiment and the findings show that a longer low-temperature zone did not improve the sensitivity further. Unless a low-temperature separation is necessary (as described in Section 3.2), a length of 8 cm is sufficient and this length was used in the following experiments.

In order to investigate the effects of sample injection length and the corresponding signal intensity when the NACE stacking mode and LTB-NACE stacking method were applied, respectively, under exactly the same experimental conditions, two series of column lengths were selected: lengths of 41, 48, 55, and 62 cm for NACE stacking and of 55, 62, 69, and 76 cm for LTB-NACE stacking. As a result, the 48 cm and 69 cm column lengths for NACE stacking and LTB-NACE stacking provided the most satisfactory results. Table 1 summarizes part of these results as well as the equation for the calibration curves, coefficient of correlation, and limit of detection for the above experiments.

In Fig. 4A, electropherogram (a) shows a typical CE electropherogram of the extract from a suspect urine sample using the NACZE mode. In order to examine this peak, we spiked 1.3×10^{-5} M (2.5 ppm) 3,4-MDMA standard and found that this peak indeed increased, as shown in the electropherogram (b). We assigned this peak as 3,4-MDMA and its concentration was determined to be 2.1×10^{-6} M (0.4 ppm). In Fig. 4B, electropherograms (c) and (d) show typical CE electropherograms of the extract from a tablet seized from the Taiwan illicit market during 2001 and the 3,4-MDMA standard (1.6×10^{-6} M), respectively, using the NACZE stacking mode. By comparison with the standard, the content of 3,4-MDMA in the seized tablet was 91.1%.

Table 1. Sample injected length, calibration curve, coefficient of correlation and LOD values (at a 92.1% confidence level) for 3,4-MDMA for NACZE, NACZE stacking, and LTB-NACZE stacking

A. NACZE	
Sample injected length	0.3 cm
Equation of the line	$y = 9.19 \times 10^4 x + 0.4821$
Coefficient of correlation	$R^2 = 0.9990$
Detection range	$2.6 \times 10^{-4} - 1.3 \times 10^{-5}$ M
LOD	4.7×10^{-6} M
B. NACZE stacking	
Sample injected length	48 cm
Equation of the line	$y = 3.56 \times 10^7 x + 0.0457$
Coefficient of correlation	$R^2 = 0.9986$
Detection range	$1.6 \times 10^{-6} - 1.0 \times 10^{-7}$ M
LOD	2.6×10^{-8} M
C. LTB-NACZE stacking	
Sample injected length	69 cm
Equation of the line	$y = 3.17 \times 10^7 x - 0.1278$
Coefficient of correlation	$R^2 = 0.9999$
Detection range	$8.1 \times 10^{-7} - 8.1 \times 10^{-8}$ M
LOD	5.0×10^{-9} M

Light source: Xe lamp (total ~ 6 W), λ_{ex} = 285 nm, λ_{em} = 320 nm; capillary, total length/effective length = 100/94 cm

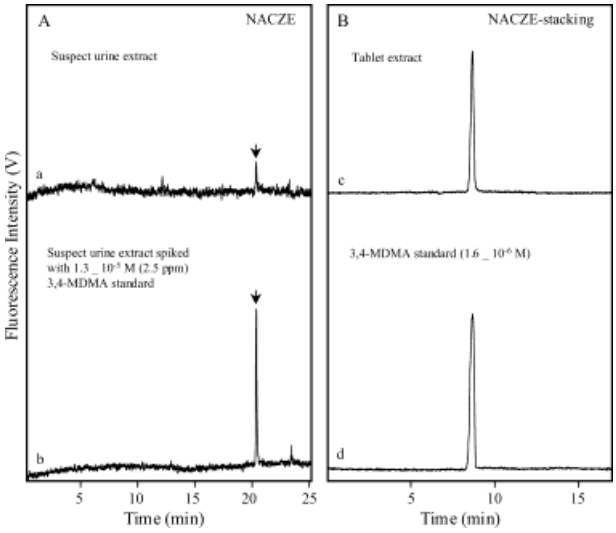

Figure 4. Electropherograms (a) and (b) show typical CE electropherograms of the extract from a suspect urine sample and the extract from the spiked urine sample, respectively, by applying the NACZE mode. (B) Electropherograms (c) and (d) show typical CE electropherograms of the extract from a seized tablet and 3,4-MDMA standard (1.6×10^{-6} M), respectively, by applying the NACZE stacking mode.

3.2 LTB-NAMEKC separation

In our previous research, 3,4- and 2,3-MDMA isomers were separated and identified on-line by NACZE separation [29]. Their resolution could be improved by LTB-NAMEKC. Figure 5A shows typical fluorescence CE chromatograms for the 3,4- and 2,3-MDMA isomers. The total length of the capillary used in this experiment was 35 cm (30 cm to the detector) and only the middle part (15 cm) was passed through the LTB, which was maintained at a low temperature. The sample concentrations were 2.6×10^{-4} M and 5.2×10^{-4} M for 3,4- and 2,3-MDMA, respectively. The NACE buffer consisted of a methanol-hexane solution (7:3 v/v) containing 100 mM sodium cholate and 20 mM ammonium acetate. By spiking with the standards,

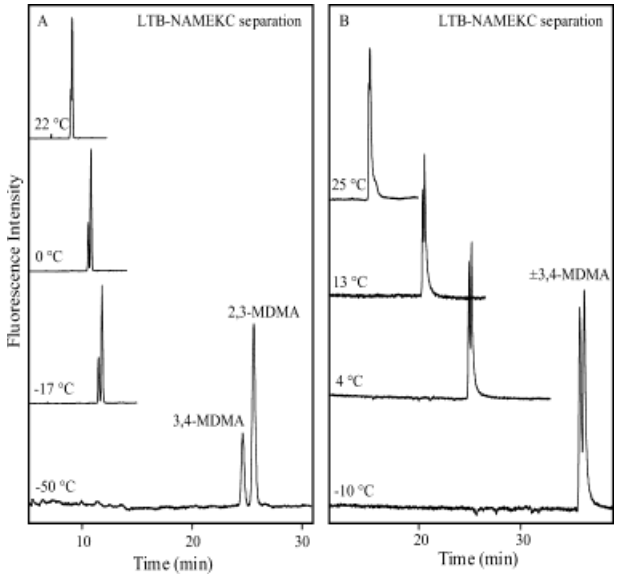

Figure 5. (A) CE fluorescence chromatograms of 3,4- (left) and 2,3-MDMA (right) at different temperatures. Conditions: capillary, 35 cm (30 cm to the detector); nonaqueous buffer, 100 mM sodium cholate, 20 mM ammonium acetate in methanol-hexane solution (7:3 v/v); applied voltage, +20 kV. (B) CE fluorescence chromatograms of (±)3,4-MDMA at different temperatures. Conditions: capillary, 50 cm (45 cm to the detector); buffer, 100 mM sodium cholate, 150 mM β-CD, and 30 mM ammonium acetate in methanol-formamide solution (7:3 v/v); applied voltage, +20 kV.

the earlier peak (corresponding to 3,4-MDMA) emerged, followed by a later peak (corresponding to 2,3-MDMA). The resolution (*R*) of the two peaks was improved from 0.6 to 1.6. Figure 5B shows the separation of (±)3,4-MDMA using the same system and a longer (50 cm/45 cm to the detector/30 cm in LTB) capillary. The NACE buffer consisted of a mixture of methanol-formamide (7:3 v/v) containing 150 mM β-CD, 100 mM sodium cholate, and 30 mM ammonium acetate. A +20 kV voltage provided sufficient separation (currents: 18.5, 13.2, 11, and 7 μA at 25, 13, 4, and −10°C, respectively). The resolution of the two peaks was improved from 0.4 to 1.0.

4 Concluding remarks

This work describes NACZE stacking and LTB-NACZE stacking, respectively. The complete, optimal nonaqueous buffer for the separation of 3,4-MDMA consisted of an ammonium acetate buffer (50 mM) in a methanol-water solution (90:10 v/v). When the stacking mode was applied, a 160-fold improvement in detection sensitivity was obtained compared with the normal NACZE method. This can be further improved by 4-fold when an LTB is used because a longer injection length can be achieved. The LTB was also useful for isomer separation when the LTB-NAMEKC mode was applied.

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