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Separation of related opiate compounds using capillary electrochromatography

Capillary electrophoretic separations have been investigated for six controlled narcotic analgesic compounds having related structures. Owing to the similar charge-to-mass ratios of these compounds, capillary zone electrophoresis failed to provide a satisfactory separation, whereas a baseline-resolved separation was achieved in 10 min using micellar electrokinetic chromatography. Column efficiencies of 40 000–150 000 plates/m were obtained with a 50 cm long, 50 μm inner diameter (ID) capillary using 50 mM sodium dodecyl sulfate (SDS) in a 50 mM borate solution containing 12% isopropanol. In contrast, separation of this mixture by capillary electrochromatography proved to be significantly superior. The capillary was 15 cm long, with an ID of 75 μm, and was packed with 1.5 μm nonporous octadecyl silica (ODS) particles. The mobile phase consisted of 80% 10 mM tris(hydroxymethyl)aminomethane (Tris) and 20% acetonitrile, and contained 5 mM SDS. A complete separation was obtained in 2.5 min with an efficiency of 250 000–500 000 plates/m.

Keywords: Capillary electrochromatography / Opiate drugs / Micellar electrokinetic chromatography
EL 3824

1 Introduction

The analysis of opiate drugs such as heroin and morphine is necessary for legal and intelligence gathering purposes [1]. Therefore, screening and confirmation of forensic drugs is important for the investigation of potential users of drugs and the control of drug addicts following withdrawal therapy. These drugs are usually screened by gas chromatography-mass spectrometry (GC-MS) [2, 3], high performance liquid chromatography (HPLC) [4, 5], thin-layer chromatography (TLC) [6, 7], and, to a lesser extent, by supercritical fluid chromatography (SFC) [8]. On-line coupled LC-GC has been applied in the determination of heroin and its metabolites in urine [9]. An advantage of GC-MS is its sensitivity and the possibility of identifying both the native compounds and their metabolites on the basis of their mass spectra. Many drug substances are polar, thermally degradable, or nonvolatile, however, which makes their analysis by GC-MS impractical. Although the chemical nature of compounds is not a problem in HPLC, the separation efficiency is often poorer than in GC. TLC is used mainly for screening of drugs, but the sensitivity of TLC is not sufficient for confirmation of substances present in low concentrations.

During the past several years, capillary electrophoretic methods such as capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC), capillary gel electrophoresis (CGE), and capillary electrochromatography (CEC) have received considerable attention from analytical chemists. In particular, interest in CEC for analytical applications has intensified because of its high efficiency and relatively high separation speeds [10–12]. CE is a useful technique for the simultaneous screening of different types of drugs, *e.g.*, beta-blockers [13, 14], diuretics [15], and narcotics [16–20]. Both CZE and MEKC have been applied to the same class of compounds. The main advantage of MEKC over CZE is that both neutral and charged compounds can be separated in a single run.

CEC is a separation technique in which the selectivity of LC is combined with electrophoretic migration. In CEC the mobile phase is driven through the column by electroosmosis, resulting in a near plug-shaped flow [21, 22], which avoids dispersion effects that are present in hydrodynamically driven chromatographies. The first electrochromatographic separations were done in 1974 by Pretorius *et al.* [23], who applied an electrical field across a chromatographic column. This work was followed by a classic study in 1981 by Jorgenson and Lukacs [24], who helped to develop CZE into a powerful and widely used technique in analytical chemistry. In 1987, Knox and Grant [25] published a paper on experiments with electroosmotically driven LC in packed capillaries that indicated that this method had the potential for high-efficiency separations if columns could be packed with small, uniform particles. Indeed, this promise has been realized in a number of instances. Recent applications of CEC include separation of polycyclic aromatic hydrocarbons (PAHs)

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Abbreviation: ODS, octadecyl silica

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[12, 26]; various nitrotoluenes, bisphenyls, and thiourea [27]; alkylbenzoates [28]; chiral separations of chlorthalidones and mianserin enantiomers [29]; benzoic and mandelic acid [30]; and aminoacetophenone as well as several substituted phenols [31], pharmaceutical compounds [32], and *N*-, *O*-, and *S*-containing PAHs [33]. The separation of forensic drugs by CEC has not been studied much, although Lurie, Meyers, and Conver [34] reported the CEC separation of cannabinoids. In contrast, many papers have reported the separation of controlled drugs by CE or MEKC. This paper describes the analysis and the effects of varying several chromatographic parameters on the CEC separation of related opiate drugs, an important class of compounds not previously analyzed using this method.

2 Materials and methods

2.1 Column packing

CEC columns were produced using an electrokinetic method described previously [35]. Briefly, a temporary packing frit is made at one end of 50 cm section of a polyimide-coated, fused silica capillary, 360 μm OD by 75 μm ID (Polymicro Technologies, Phoenix, AZ) by plugging it with a paste of 5 μm silica particles suspended in methanol. This structure was gently sintered in place using a butane micro torch. A suspension of 1.5 μm nonporous octadecyl silica (ODS) packing material (Micra Scientific, Northbrook, IL) in methanol was electrokinetically packed into the column. New inlet and outlet frits were made in the packed section under pressure using a resistively heated wire stripper (Teledyne Kinetics, Solana Beach, CA) and the temporary frit was removed. A 2 mm detection window was burned in the polyimide coating approximately 2 mm downstream of the outlet frit. The total column length used in this study was 28 cm, of which 15 cm was packed with the stationary phase.

2.2 Chemicals

Six opiate drug samples (1 mg/L in methanol) were obtained from Altech (State College, PA). Figure 1 shows the structures of the six opiate drugs for which CEC was performed. Acetonitrile (HPLC grade), 2-(*N*-mopholino)ethanesulfonic acid monohydrate (MES>98%), and Tris (>98%) were purchased from Sigma-Aldrich (Milwaukee, WI) and were used without additional purification. Electrophoresis grade (>99.5%) sodium dodecyl sulfate (SDS) was obtained from Life Technologies (Gaithersburg, MD). Water was purified using a Labconco Water Pro PS (Kansas City, MO) purification system.

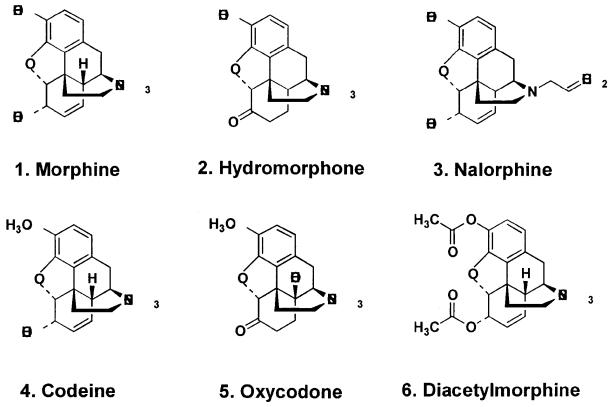

Figure 1. Structure of six opiate drugs studied.

2.3 CEC system

Packed capillaries were inserted into an on-column flow cell (Model 9550-0155; Thermo Separation Products, San Jose, CA) installed with a UV/Vis detector. The flow cell contained a spherical lens that illuminated only a 100 μm section of the capillary detection window. All absorption measurements in this study were made at 215 nm. Samples (50 mg/L each component) were injected electrokinetically. Columns were conditioned with a mobile phase using a manual syringe pump. After conditioning, each end of the capillary was inserted into a vial containing mobile phase that had been degassed by sonication under vacuum for at least 15 min. Electroosmotic flow was established by applying a potential across the column using a 0–30 kV power supply. The stability of the electroosmotic flow was checked by measuring the current, which was determined by monitoring the voltage drop across a 10 kΩ resistor in series with the capillary. Analog output from the detector was digitized and recorded with a PC-compatible computer using a program written in LabView (National Instruments, Austin, TX).

3 Results and discussion

Figure 2 presents a chromatogram of the CEC separation of six opiates. The column was 28 cm long (15 cm packed with 1.5 μm nonporous ODS particles), 75 μm ID, and was operated at an applied voltage of 20 kV. The mobile phase was 80% 10 mM Tris, and 20% acetonitrile with 5 mM SDS at pH 8.3. Baseline separation of the six compounds was achieved in 2.5 min.

The number of theoretical plates/m for each compound, shown in Table 1, is between 250 000 and 500 000/m for the six components. In order to determine the optimal separation condition, both inorganic and organic buffers were used with various concentrations and various volume percentages of methanol, isopropanol, and acetoni-

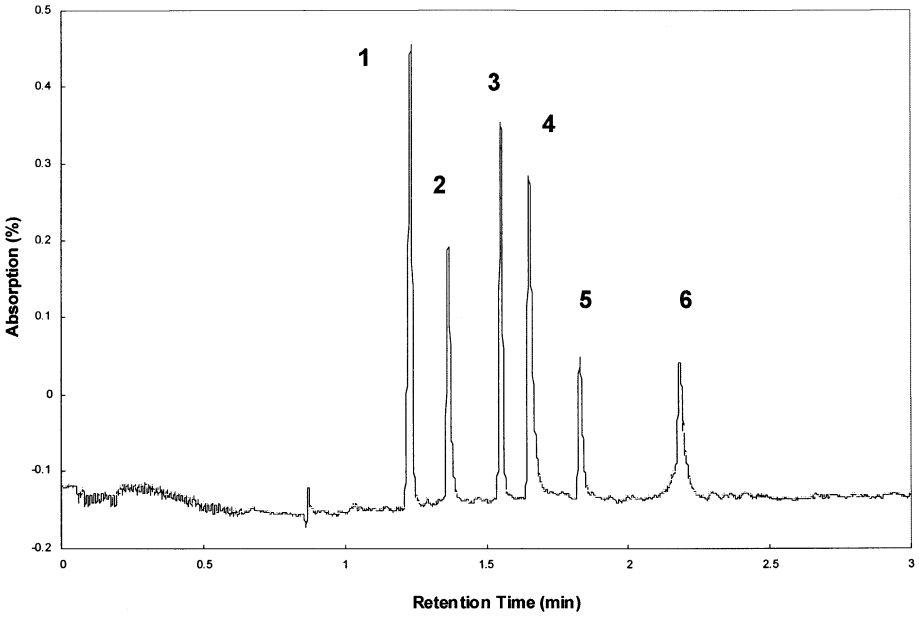

Figure 2. CEC separation of six opiate drugs. Conditions: 10 mM Tris/5 mM SDS/20% CH_3CN buffer, pH 8.3; column, 75 μm ID, 15 cm long, packed with 1.5 μm nonporous ODS particles; voltage, 20 kV; injection, 0.5 kV for 2 s; detection, UV absorption at 215 nm. The numbers refer to the opiates identified in Fig. 1.

trile as organic modifiers. Phosphate and borate were used as the inorganic buffer. At more than 5 mM of inorganic buffer, the probability of bubble formation is very high because of the high conductivity of phosphate and borate buffer even though a high volume percentage of organic modifiers is present. At less than 5 mM of inorganic buffer, the bubble problem is decreased, but separation efficiencies are not satisfactory. Under these conditions runs took more than 30 min and showed peak broadening. The organic buffer is better in CEC separations because the conductivity of organic buffer is lower than that of inorganic buffer. Both MES and Tris were used as an organic buffer. While the conductivity of the MES buffer is less that of Tris, it was found that most rapid and most efficient separations could be obtained using a 10 mM Tris buffer.

To prevent the generation of too much Joule heating, a number of approaches have been tried. One has been the use of a high percent of organic modifier in the mobile phase, but this method does not give good separation efficiency. Other research groups have tried pressurized CEC separations to reduce or eliminate bubble formation [36, 37], but this approach is somewhat complicated. Another approach is to add a surfactant such as SDS to the mobile phase. This method is easy and very effective for reducing bubble formation in CEC separations, as described previously by several research groups [38, 39]. For the prevention of bubble formation in the CEC separation of opiates, therefore, we added 5 mM SDS, whose concentration is lower than the CMC of 8.1 mM [40]. We found that the addition of 5 mM SDS is effective for preventing bubbles as well as for improving the separation efficiency. The way in which SDS acts to improve the CEC separation is not known presently, although it is believed that SDS plays an important role in preventing bubble formation by decreasing the surface tension at the solid-liquid interface.

Table 1. Column efficiency (number of plates/m) of CEC (Fig. 2) and MEKC (Fig. 5) of the six opiate drugs under study

Opiate	CEC	MEKC
1. Morphine	248 000	147 000
2. Hydromorphone	305 000	90 000
3. Nalorphine	499 999	115 000
4. Codeine	362 000	119 000
5. Oxycodone	444 000	86 000
6. Diacetylmorphine	322 000	36 000

To investigate the effect of varying the pH in our system, the concentration of the SDS was changed from 0 to 5 mM as the pH was changed from 2 to 10. In the absence of SDS and with the pH at 2, 4, or 6, the six opiates appeared before the EOF marker and showed a longer retention time with increasing pH. On the other hand, at pH 8 and at pH 10, the six opiates came out after the EOF marker and had a shorter retention time than those at lower pH (Fig. 3a). We interpret this behavior to mean that the opiates under study have a positive charge at low pH because they have pK_a values between 7.0 and 7.8. Therefore, all opiates under study are ionized and have a positive charge less than pH 7; therefore opiates came out before the EOF marker. The retention time is longer than those at higher pH because total mobility, which is

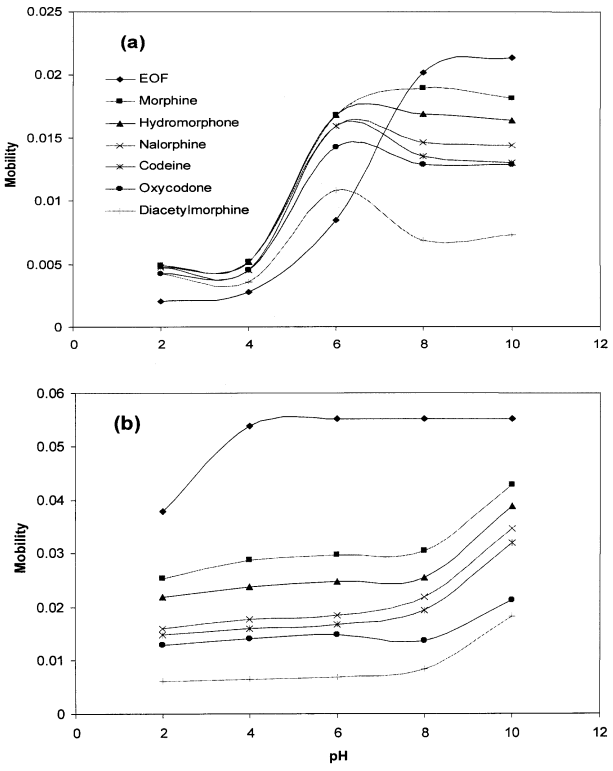

Figure 3. The mobility of the EOF marker and six opiates as a function of pH and concentration of SDS. (a) Without SDS, (b) 5 mM SDS.

the sum of the electroosmotic mobility and the electrophoretic mobility, is smaller at lower pH. The electroosmotic mobility increased with increasing pH value. However, in 5 mM SDS, all samples appeared after the EOF marker, and the retention time of the EOF marker was almost the same at different pH values (Fig. 3b). From these results, we conclude that at low concentrations of SDS and at low pH values, the opiates came out before the EOF marker, which implies that all samples have a positive charge under these separation conditions. However, at higher concentrations of SDS (below the critical micelle concentration) even though the pH is low, the opiates came out after the EOF marker. Under these conditions, all the opiates are neutral or ion-paired owing to the presence of the SDS. From these results, the separation mechanism at high concentrations of SDS at all pH values is expected to be based on the interaction with the hydrophobic moiety of the C_{12} chain on the ion-paired SDS and the C_{18} chain of the stationary phase.

As is expected in reversed-phase chromatography, compounds with more polar and less bulky groups have shorter retention times. Consequently, morphine, which has two hydroxy groups and fewer bulky groups, was the first to appear. Diacetylmorphine, which is less polar and which is more conjugated and more bulky, was the last to appear. This behavior supports the idea that the partitioning between the mobile phase and stationary phase plays an important role in the separation mechanism.

Figure 4 illustrates the effects of acetonitrile concentration on the separation of the six opiate drugs. The retention time decreased with an increase of the acetonitrile concentration for all samples. At the low volume percentage of acetonitrile, near the optimal separation condition, the electroosmotic mobility increased with an increase of the acetonitrile concentration. For CZE in aqueous systems, the variation of the electroosmotic mobility with the content of the organic solvent has been studied previously [41]. The overall trend is the same for all protic and aprotic solvents, a steady decrease in the electroosmotic mobility with an increasing volume percent of the organic solvent; that is, retention time increases with an increasing volume percent of the organic solvent. These results can be attributed to a decrease in viscosity and dielectric constant with an increase of volume percent of acetonitrile. Also, the same trend was shown to occur with inorganic buffers [42].

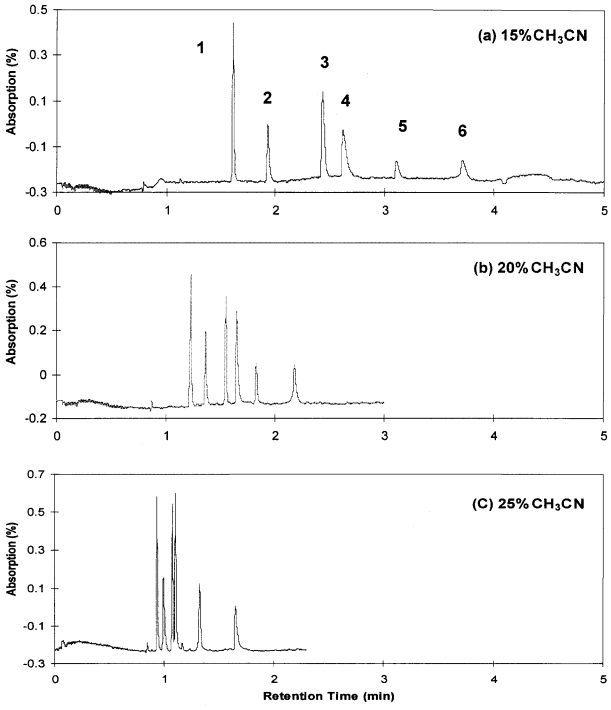

Figure 4. Effect of percent acetonitrile on the CEC separation of six opiate drugs. Conditions: buffer, 10 mM Tris/5 mM SDS/(a) 20%, (b) 25%, and (c) 30% CH_3CN; column, 75 μm ID, 15 cm long, packed with 1.5 μm nonporous ODS particles; voltage, 20 kV; injection, 0.5 kV for 2 s; detection, UV absorption at 215 nm.

For CEC, the effect of the organic modifier is rather different than for CZE. Dittmann and Rozing [43] observed an increase in mobility with increasing acetonitrile content in MES and Tris buffers. Also, Lelievre *et al.* [29] observed an increase in mobility with increasing acetonitrile content in MES and Tris buffers. It is expected that the polarity of the buffer influenced the retention behavior of the opiates in reversed-phase CEC. The polarity of the buffer is increased with an increase in the volume percentage of acetonitrile. The highly polar buffer interacted more strongly with opiates and the retention times are decreased. The relative electroosmotic mobilities are comparable to EOF. For all opiates, the relative electroosmotic mobilities increased with an increase in the volume percentage of acetonitrile. We conclude that the mobility of each opiate is influenced by the ratio dielectric constant and viscosity of the buffer in CZE and by the polarity of the buffer in CEC. Also, it is expected that the partition interaction with the stationary phase is more important than the electroosmotic mobility as a separation mechanism for CEC.

The separation of the opiates achieved by using MEKC is presented in Fig. 5. The capillary column was 63 cm long (50 cm to detector), 50 μm ID. The mobile phase was 88% of a solution of 50 mM borate and 50 mM SDS with 12% isopropanol at pH 8.3. In order to determine the optimal condition of MEKC for the six opiates, the phosphate, borate, and Tris buffer were used with various volume percentages of methanol, isopropanol, and acetonitrile. In 50 mM borate buffer with 15% methanol organic modifier, the six opiates are separated with baseline resolution, but the separation required 30 min and showed peak tailing. In a high volume percentage of isopropanol and acetonitrile, the retention time is so short that the separation resolution is unsatisfactory. The number of theoretical plates for the six opiates in MEKC was between 36 000 and 147 000. These values are somewhat lower than those of other samples separated by MEKC but this behavior appears to be reasonable when compared with data that others have reported. For example, Tagliaro *et al.* [44] separated forensic drugs using a capillary column (50 μm ID, 35 cm to detector) with 25 mM borate (pH 9.24), 20% methanol, and 100 mM SDS as a buffer and an applied voltage of 20 kV. They found that the number of theoretical plates was between 80 000 and 90 000. Krogh *et al.* [45] could separate illicit drugs such as heroin and amphetamine with a theoretical plate number between 120 000 and 290 000. by MEKC using a capillary column (50 μm ID, 50 cm to detector) with 25 mM SDS, 10 mM NaH_2PO_4, 10 mM $Na_2B_4O_7$, pH 9.0, with 5% acetonitrile and an applied voltage of 20 kV. For most components these values are lower than those obtained from CEC (Table 1).

4 Concluding remarks

The separation of six controlled drug compounds by CEC has been successfully demonstrated. The separation efficiencies are remarkably high, the theoretical plate number approached 500 000 plates/m, and all components appeared within 2.5 min with good resolution and good reproducibility. The same compounds were also separated by MEKC, but the separation efficiencies were not as good as with CEC.

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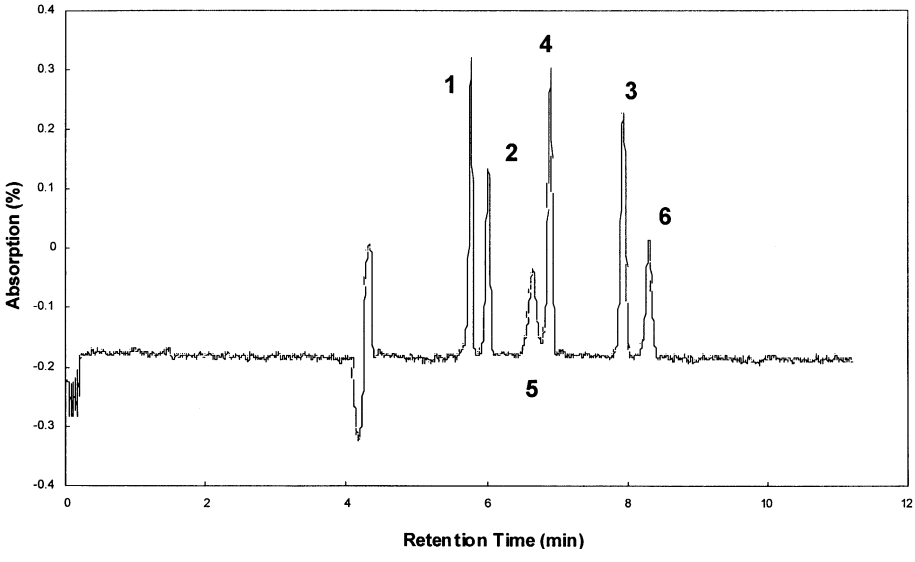

Figure 5. MEKC separation of the six opiate drugs under study. Conditions: buffer, 88% (50 mM borate, 50 mM SDS), 12% isopropanol, pH 8.3; column, 50 μm ID, 50 cm to detector; voltage, 30 kV; injection, 2 kV for 2 s; detection, UV absorption at 215 nm. The numbers refer to the opiates identified in Fig. 1.

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