Forensic Applications of Capillary Electrophoresis

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Introduction

During the last decade, capillary electrophoresis (CE) has developed into a widely applied method for the analysis of pharmaceuticals (both for the evaluation of pharmaceutical formulations and metabolites). These applications established the basis for introducing CE into the forensic field also. Today, capillary electrophoresis can be applied to a number of analytical problems in forensic science, including the analysis of gunshot residues, explosives, inks, dusts, soils, and, of course, illicit drugs, diverse toxicants, DNA fingerprinting, protein analysis, and so forth (for reviews, see Refs. 1 and 2).

Several features of capillary electrophoresis are particularly interesting for forensic scientists, namely high separation efficiency, sensitivity, and small amount of samples (nanoliters) and solvents (a few milliliters per day). Regarding different operational modes, all of them are applied, although to a different extent, depending on the type of compounds to be assayed.

Forensic Toxicology

Capillary electrophoresis has a particularly wide potential for the analysis of illicit drugs [3]. For this category of applications, mainly capillary zone electrophoresis (CZE) and micellar electrokinetic chromatography (MEKC) are used; however, other modifications of this approach, such as separation in buffers containing a high proportion (up to 20%) of an organic modifier in the background electrolyte, can be used also. More recently, some hints appeared indicating the possibility of applying electrochromatographic techniques for this purpose as well, although, admittedly, this latter approach has not reached the stage of maturity of other techniques. It is to be foreseen that particularly electrokinetic separations exploiting the properties of reversed-phase packings, will be used for these purposes in the near future.

Regarding tissues and body fluids to be analyzed, blood and urine serve most frequently as source material, although the interest of forensic analytical chemists can be easily extended to other specimens (e.g., saliva, bile, or vitreous humor). A tissue that has attracted a lot of interest in the course of recent years is hair [4]; at the root end, the tissue is penetrated by a number of drugs during hair matrix formation. Because the hair stalk is basically devoid of metabolic processes, the drugs, once sorbed, remain in the tissue (depository effects). If one considers the average hair growth of about 1 cm/month the analysis of hair sections may yield information about past exposure of the individual to the toxicants; it has to be kept in mind, however, that external contamination may contribute also to the amount of the toxicant recovered.

As the analytes to be assayed are nearly always present in minute amounts, preconcentration steps are almost always necessary. The techniques used for this purpose have been mostly adopted from analytical procedures using gas or liquid chromatography as the separation step [5]. This fact is emphasized here because the appropriate adjustment of the sample preparation is necessary because the original procedures do not respect the fact that the sample volume injected into a CE system represents a few nanoliters only and requires a relatively high concentration of analytes assayed. However, in selected types of analysis, direct sample application is also possible (for a review, see Ref. 3).

There are three points emphasized in the quoted review which limit the use of direct injection: (a) protein-bound drugs display a different mobility in CZE in comparison to unbound species, (b) high conductivity of the untreated biological samples may cause undesirable peak broadening, and (c) selectivity of the analysis may be negatively influenced by using a nonselective wavelength for detection (usually 200 nm), which is needed to reveal low concentrations of the analytes of interest. Consequently, desalting and deproteinization (at last partial) is frequently done by adding different proportions of organic solvents to the sample ($\sim 1{:}1 \rightarrow 1{:}4$). If MEKC is to be used, it is recommended to remove the organic solvent prior to analysis (for the first application of MEKC for forensic purposes, see Ref. 6; for additional information, see Ref. 7). Standard preconcentration conditions, such as solid-phase and liquid–solid extractions, are widely used.

Some idea about actual conditions applicable for the separation of drugs of forensic interest can be obtained from Table 1. Extensive information about the MEKC of drugs is offered in a review of Nishi and Terabe [8].

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Table 1 Examples of the Capillary Electrophoretic Separations of Misused Drugs, Their Enantiomers, and Metabolites

Analytes (remarks)	Detection	Separation
17 Basic drugs (amphetamine, lidocaine, codeine, diazepam, methaqualone, etc.; extracted by chloroform–2-propanol, 9:1 v/v)	UV at 214 nm	CZE, 50 m*M* phosphate buffer pH 2.35
Abused drugs (includes heroin, heroin impurities, *cis*- and *trans*-cinnamoyl cocaine)	UV at 210 nm	MEKC, 85 m*M* SDS, 8.5 m*M* borate, pH 8.5; containing 15% acetonitrile
Abused drugs and metabolites (includes benzoylecgonine, morphine, heroin, methamphetamine, codeine, amphetamine, cocaine, methadone, benzodiazepines)	Fast scanning UV	MEKC, borate–phosphate buffer pH 9.1, 75 m*M* SDS
Amphetamines (enantiomers; LLE[a] or SPE[b])	UV at 200 nm	CZE, 20 m*M* (2-hydroxy)-propyl-β-cyclodextrin in 200 m*M* phosphate pH 2.5
Amphetamines and ephedrine (enantiomers; LLE)	UV at 200 nm	CZE, 20 m*M* β-cyclodextrin in 150 m*M* phosphate pH 2.5
Barbiturates (after LLE)	UV at 214 nm	MEKC, 100 m*M* SDS in 10 m*M* borate, 10 m*M* phosphate, pH 8.5, 15% acetonitrile added (by volume)
Barbiturates (phenobarbital can be assayed without sample pretreatment)	Multiwavelength detection (195 and 320 nm)	MEKC, 50 m*M* SDS, phosphate–borate buffer, pH 7.8
Caffeine metabolites (direct injection, LLE)	UV at 254 nm	MEKC, 70 m*M* SDS, phosphate–borate, pH 8.43
Caffeine metabolites (LLE)	Scanning UV 195–320 nm	MEKC, 70 m*M* SDS, 16.2 m*M* phosphate, pH 8.6
Cannabis constituents (alkaline hydrolysis of urine followed by SPE[b])	Fast scanning UV	MEKC, 75 m*M* SDS in phosphate–borate buffer pH 9.1)
Dextromethorphan and dextrophan (direct injection)	UV at 200 nm	CZE, 175 m*M* borate pH 9.3
Dihydrocodeine metabolites and O-demethylation (hydrolysis, direct injection, SPE)	UV at 213 nm, scanning UV 195–320 nm	MEKC, 75 m*M* SDS, 6 m*M* borate, 10 m*M* phosphate, pH 9.2
Flurazepam metabolites, sulfonamides (hydrolysis, LLE)	UV at 254 nm; MS	CZE, 15 or 0.2 m*M* ammonium acetate pH 2.5 or 1.3 adjusted with TFA, 15% methanol
Haloperidol metabolites (SPE)	UV at 214 nm, scanning UV 195–320 nm, MS	CZE, 50 m*M* ammonium acetate, 10% methanol, 1% acetic acid, pH 4
Mephenytoin and dextromethorphan metabolites (hydrolysis)	Scanning UV 195–320 nm	MEKC, 75 m*M* SDS, 6 m*M* borate, 10 m*M* phosphate pH 9.2–9.3 or CZE, 140 m*M* borate pH 9.4
Morphine and cocaine in hair (hydrolysis by 0.25 *M* HCl at 45°C followed by LLE)	UV at 200 nm (230 nm for cocaine, 214 nm for morphine)	CZE, 50 m*M* borate pH 9.2
Nitrazepam and metabolites (SPE)	UV at 220 nm	MEKC, 60 m*M* SDS in 6 m*M* phosphate–borate buffer pH 8.5, 15% methanol (by volume)
Opiates (heroin, morphine, and metabolites; SPE)	Spectral UV analysis	CZE, 12 m*M* borate, 20 m*M* phosphate, pH 9.8 or MEKC, 75 m*M* SDS in phosphate borate buffer, pH 9.2
Opiates (morphine, heroin, codeine, etc., SPE)	UV at 200 nm	CZE, 100 m*M* phosphate pH 6
Opiates (morphine, heroin, codeine, amphetamine, caffeine)	UV at 200 nm	MEKC, 50 m*M* SDS, 50 m*M* glycine, pH 10.5
Purines, substituted (direct injection, LLE, SPE)	Scanning UV 195–320 nm	MEKC, 75 m*M* SDS, 6 m*M* borate, 10 m*M* phosphate pH 9
Racemethorphan, racemorphan (optical isomers)	UV at 200 nm	MEKC, 60 m*M* β-cyclodextrin in 50 m*M* borate pH 9.05, 50 m*M* SDS, 20% 1-propanol
Theophylline metabolites (SPE)	Scanning UV 195–320 nm	MEKC, 200 m*M* SDS in 100 m*M* borate, 100 m*M* phosphate, pH 8.5 (ration 12:7, final pH 6.5)

Note: For detailed specifications of individual procedures, see Ref. 2.
[a]Liquid–liquid extraction.
[b]Solid-phase extraction.

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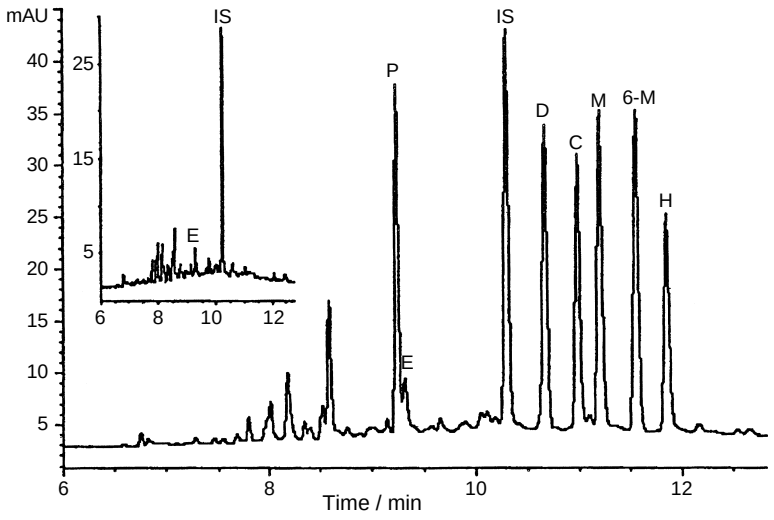

Fig. 1 Capillary electrophoretic separation of opiates. Running conditions: 100 m*M* phosphate buffer pH 6.0. Electrokinetic injection with field-amplified sample stacking after solid-phase extraction of spiked urine using "double mechanism" cartridges. Precision of migration times 1.2% R.S.D. (relative standard deviation), resolution >2 with all peaks shown. Within the day and day-to-day repeatability 1–4% R.S.D., respectively; detection by UV at 200 nm. Peak identification: pholcodine (P), MAM (6-M), heroin (H), codeine (C), morphine (M), dihydrocodeine (D), and levallorphan (I.S.); E represents an unidentified endogenous compound present in urine (see inset). (From Ref. 9 with permission.)

As indicated in Table 1, ultraviolet (UV) light at short wavelengths (190–220 nm) is routinely used for detection. In these cases, laser-induced fluorescence appears to be the method of choice. Unfortunately, the commercially available laser units emit at wavelengths not suitable for direct drug analysis, which limits the practical applicability of this approach. Nevertheless, where applicable, laser-induced fluorescence can easily improve the detection limit by a factor of 1000, in comparison with UV detection; Ar-ion lasers (emitting at 488 nm wavelength) or He–Cd lasers (emitting at 325 nm wavelength) are commonly used for this purpose. Typically, with enzymatically hydrolyzed urine, the detection limit can be about 2 ng/mL of the assayed compound (zolpidem).

If the investigated drug does not possess a suitable fluorophore, derivatization may be required [fluorescein isothiocyanate for compounds possessing a free amino group (e.g., amphetamines) may serve as a typical example; an Ar-ion laser emitting at 488 nm was used for this purpose]. Generally, detection limits achieved with laser-induced fluorescence after derivatization can be around 3 m*M* in concentration terms (or 3 μmol in terms of absolute mass detection). This is about three orders of magnitude less than what can be achieved with gas chromatography–mass spectrometry.

Another possibility is to use xenon-arc lamp irradiation for the same purpose, which extends the possibilities of excitation wavelengths to the 272–382-nm range. Exploiting competitive binding of trace amounts of (misused) drugs with fluorescence-labeled immunotracers can be spotted in the literature; however, this is not a widely used approach at the moment.

Amperometric detection, which generally offers quite high sensitivity, has not been used so far for forensic (toxicological) applications. The reason probably reflects some problems with commercially available coupling of the high-performance amperometric cell with the capillary electrophoresis device.

Surprisingly, not very many methods using the capillary electrophoresis–mass spectrometry (CE–MS) combination (mainly electrospray ionization) are in use in the area of forensic drug analysis. The first application of CE–MS for forensic purposes was described by Johansson et al. in 1991 [10] for the analysis of sulfonamides and benzodiazepines in urine. The main problem faced in this case is the need of improving the concentration sensitivity of the CE–MS combination. In order to improve the concentration of ana-

lytes, a special method of in-capillary coupling of isotachophoresis with CZE–MS has been proposed. CE–MS instrumentation and its application was reviewed by Cai and Henion [11].

Forensic Biology

One of the major areas of forensic science is DNA fingerprinting, which is used for personal identification and paternity testing. Most of these analyses are based on the polymerase chain reaction (PCR) of individual loci, followed by analysis of differences in length or sequence. Capillary electrophoresis, with laser-induced fluorescence detection, is becoming an alternative method to polyacrylamide gel electrophoresis (PAGE) and agarose slab gel electrophoresis. Fully automated CE-based instruments are now available for DNA sizing, quantitation, screening, and sequencing. Detailed information about the separation of nucleic acids is a matter of a specialized entry; for detailed information, specialized reviews are available (see, e.g., Refs. 12 and 13).

Using the separation of DNA fragments in media with cross-linked polyacrylamide or agarose gels makes it possible to achieve high efficiency (tens of millions of theoretical plates); however, from the practical point of view, replaceable, entangled polymers (e.g., derivatives of cellulose, linear polyacrylamide) are preferred. The main advantage of replaceable media is the ease of renewing the gel media in the capillary with every single run.

Ultraviolet light is routinely used for detection; however, laser-induced fluorescence of labeled DNA offers better results. For example, a PCR-amplified DNA fragment comprised of 120–400 base pairs can be separated with a resolution up to four base pairs using 1% hydroxyethylcellulose and DB-17 capillary (60 cm effective length $\times$ 0.1 mm inner diameter with 0.1 μm phase thickness). Laser-induced fluorescence detection can yield a sensitivity of about 500 pg/mL of DNA (after staining with fluorescent intercalating dye YO-PRO-1) [14].

Capillary electrophoresis can be also applied for DNA sequencing. For this purpose, multicapillary (array) instruments with laser-induced fluorescence detection are being developed. Detailed descriptions of these methods is beyond the scope of this entry.

Proteins and enzymes are also of interest in forensic science. In this context, it is possible to mention acetaldehyde–protein adducts, which can be used as potential markers of alcoholism. Another application is the determination of globins, saliva, and semen proteins. Capillary electrophoresis of proteins is a broad and complex area of analytical chemistry which, like the separation of nucleic acids, is beyond the scope of this entry (for a review, see, e.g., Ref. 15 and many others). In principle, it is possible to use different operational modes such as CZE in acid or alkaline media, capillary isotachophoresis, capillary isoelectric focusing, capillary gel electrophoresis, and, recently, MEKC. A considerable problem in protein–enzyme separations in untreated capillaries is sticking of these analytes to the inner capillary wall, which can be eliminated (at least in part) by running the separation at very high or very low pH values, by adding some modifiers (as salts, etc.) to the buffer, or by appropriate modification of the capillary surface.

Conclusion

In conclusion, CE is a valuable analytical tool that offers a number of possibilities for the analysis of a wide spectrum of forensically interesting compounds. Practically all compounds which have been traditionally analyzed by GC, high-performance liquid chromatography, thin-layer chromatography, or slab–gel electrophoresis, can be assayed by capillary electrophoretic procedures. All methods of capillary electrophoresis can be validated and can meet the demands of good laboratory practice.

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