Forensic applications of capillary electrophoresis

David M. Northrop,[a] Bruce R. McCord,[b] and John M. Butler[c]

[a]*Washington State Patrol Crime Laboratory, Kennewick, WA, U.S.A.*
[b]*FBI Laboratory, Forensic Science Research Unit, FBI Academy, Quantico, VA, U.S.A.*
[c]*Department of Chemistry, University of Virginia, Charlottesville, VA, U.S.A.*

Correspondence: *Dr. David M. Northrop, Washington State Patrol Crime Laboratory, Rte. 7, Box 12450, Kennewick, WA 99337, U.S.A.*

Abstract

Capillary electrophoresis is an emerging technology that can be applied to problems of interest in forensic science. Rapid analysis, high efficiencies, small sample requirements, and economical costs are advantages that CE brings to forensic science. Preliminary results have demonstrated the application of CE to drug, gunshot residue, explosives, DNA, and other forensic analyses.

Forensic science is a unique branch of analytical chemistry. The primary function of the forensic scientist is to provide analysis of evidence and expert testimony to be used in legal proceedings. Evidence submitted to the laboratory may consist of a wide variety of organic, inorganic, biological, and nonbiological samples that are frequently received as heterogeneous mixtures. The goal of the forensic scientist is to resolve these complex samples into identifiable constituents, free from the interference of other substances. The methods used to achieve this goal must be reliable, rapid, economical, and provide information that is unequivocal. It is also important to preserve as much of the evidence as possible. Samples are often very limited; therefore analytical techniques need to be nondestructive or use limited amounts of sample. CE is an analytical tool that shows great promise in addressing these requirements. The excellent mass detection limits, rapid analysis time, exceptional separation and resolution of complex mixtures, economy, and small sample requirements make CE an attractive method for a wide range of forensic problems. The following discussion will focus on the preliminary work that has been done using CE for forensic analysis. Specific attention will be given to drug, gunshot residue, explosive residue, and DNA analysis.

Indexing terms

Capillary electrophoresis, forensic, DNA, drugs, explosives, spectrometry, gunshot residue

Abbreviations

CE, capillary electrophoresis; SDS, sodium dodecylsulfate; MECE, micellar electrokinetic capillary electrophoresis; LSD, lysergic acid diethylamide; Δ^9-THC, Δ^9-tetrahydrocannabinol; LAMPA, lysergic acid-*N*-(methylpropyl)amide; MDA, methylene-dioxyamphetamine; MDMA, methylene-dioxymethamphetamine; PCP, phencyclidine; GC-MS, gas chromatography-mass spectrometry; IR, infrared; GITC, 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate; CTAB, cetyltrimethylammonium bromide; GSR, gunshot residue; SEM, scanning electron microscopy; AAS, atomic absorption spectroscopy; IC, ion chromatography; HPLC, high-performance liquid chromatography; HEC, hydroxyethyl cellulose; PCR, polymerase chain reaction; LIF, laser-induced fluorescence; VNTR, variable-number tandem repeat; PAGE, polyacrylamide gel electrophoresis

Forensic drug analysis

Drug analysis requests account for a larger volume of casework than any other area in forensic science. This fact requires that the forensic laboratory dedicate significant resources both in analytical instrumentation and personnel. Analytical techniques that improve the quality and quantity of work in this area are a necessity. The following discussion will demonstrate how CE can be effectively implemented in several areas of forensic drug analysis.

CE can be used as a general drug screening tool. It was in this form that CE was first used in forensic drug analysis.[1] A separation of 18 common drugs of abuse was accomplished in less than 40 min by using a phosphate/borate buffer, with sodium dodecylsulfate (SDS) as a micellar phase and acetonitrile as an organic modifier (*Figure 1*). This method was extended to include 34 drugs and drug impurities (*Table 1*). The advantage of this method over other screening techniques is that acidic, basic, and neutral drugs can be screened using a single analytical method. Extraction procedures for class separation of unknown samples are unnecessary.

A close inspection of the compounds listed in Table 1 demonstrates the chromatographic superiority that CE has over the commonly used methods for the

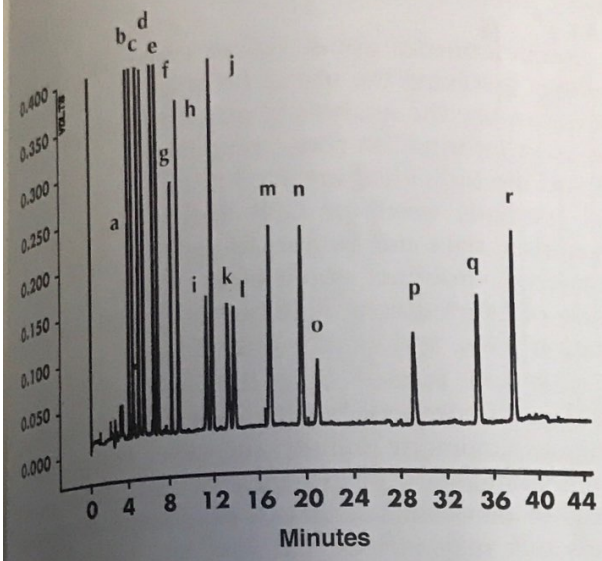

FIGURE 1 *Micellar electrokinetic capillary electrophoresis (MECE) forensic drug screen. Conditions: capillary: 50 µm i.d. x 25 cm; voltage: 20 kV; temperature: 40 °C; buffer: 85 mmol/L SDS, 8.5 mmol/L borate, 8.5 mmol/L phosphate, 15% acetonitrile, pH 8.5; detection: 210 nm; sample concentration: 250 µg mL⁻¹. a) Psilocybin, b) morphine, c) phenobarbitol, d) psilocin, e) codeine, f) methaqualone, g) lysergic acid diethylamide (LSD), h) heroin, i) amphetamine, j) librium, k) cocaine, l) methamphetamine, m) lorazepam, n) diazepam, o) fentanyl, p) phenycyclidine, q) cannabidiol, r) Δ⁹-tetrahydrocannabinol (Δ⁹-THC). (Reprinted with permission from Ref. 1.)*

Table 1
ILLICIT DRUG AND DRUG IMPURITIES SEPARATED BY CE[a]

Benzoylecgonine	Amphetamine
Psilocybin	Librium
Morphine	Cocaine
Hydromorphone (diluadid)	MDMA
Phenobarbital	Methamphetamine
Psilocin	Phentermine
O⁶-Monoacetylmorphine	Noscapine
O³-Monoacetylmorphine	*cis*-Cinnamoylcocaine
Codeine	Lorazepam
Methaqualone	Diazepam
Mescaline	*trans*-Cinnamoylcocaine
LSD	Fentanyl
LAMPA	Flurazepam
Heroin	PCP
Acetylcodeine	Cannabidiol
Papaverine	Cannabinol
MDA	Δ⁹-THC

[a]Listed in order of elution. Abbreviations: LAMPA, lysergic acid-*N*-(methylpropyl)amide; MDA, methylene-dioxyamphetamine; MDMA, methylene-dioxymethamphetamine; PCP, phencyclidine; Δ⁹-THC, Δ⁹-tetrahydrocannabinol. (Reprinted with permission from Ref. 1.)

analysis of certain drugs. Most drug analysis is conducted using either GC-MS or extraction followed by IR analysis. Separation of the benzodiazapines and ergot alkaloids by GC is difficult and differences in IR characteristics are small. The separation of these compounds by CE is readily achieved. Forensic drug chemists also find the analysis of LSD difficult because of microgram-quantity dosage units and the fact the GC-MS analysis is difficult, at best, due to column adsorption and thermal lability. This is also true for psilocybin, the psychoactive agent in illegal mushrooms. This drug contains a phosphate moiety that is very labile. Routine analysis actually identifies psilocin, the dephosphorylated form of the drug. In each of these cases, CE analysis conditions allow for the separation of each of these drugs without causing decomposition or analyte loss from complex preparative extractions.

Drugs that have many closely related isomers pose a difficult analytical problem for the forensic scientist. For this reason the phenethylamines are historically a difficult group of compounds to analyze due to the large number of isomers, including both D- and L-optical isomers. Separation of ephedrine, pseudoephedrine, norephedrine, norpseudoephedrine, amphetamine, and methamphetamine, including the optical isomers, is not easily accomplished in a single run using GC methods. The 12 optical isomers of these compounds were separated in under 30 min using CE.[2] The separation was achieved by derivatizing the analytes with 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl isothiocyanate (GITC) followed by CE analysis using 20% methanol and 80% SDS buffer (100 m*M* SDS, 10 m*M* phosphate, and 10 m*M* borate at pH 9.0). Optical isomer identification can be important in the forensic arena. For example, D-propoxyphene is a controlled substance while L-propoxyphene is not. These isomers have been separated by CE using a mixed micelle system consisting of 5 m*M* dimethyl-ß-cyclodextrin and 0.5 m*M* ß-cyclodextrin-sulfobutylether.[3]

The clandestine manufacture of illegal drugs poses additional problems for the forensic scientist. Samples submitted for analysis may contain complex mixtures of chemicals, including thermally reactive and labile compounds. Methamphetamine is frequently encountered in this form. Identification of the optical isomeric makeup of a methamphetamine sample can be used to develop evidence concerning the synthetic pathway and thus provide information as to the source of the sample. This information can be obtained using the CE methods described for the separation of the optical isomers of the phenethylamines. An additional problem with samples from clandestine laboratories is the presence of unreacted precursors and adulterants that may interfere with standard analysis. Under typical GC-MS conditions certain mixtures of chemicals with methamphetamine will derivatize the illegal drug to a compound that is not controlled. The resulting analysis is inconclusive at best.

Using the CE methods described above, the analysis can be completed without changing the composition of the original mixture.

The worldwide manufacture and distribution of heroin and cocaine have resulted in significant efforts to control these drugs. Part of the effort centers on identifying the origin and shutting down that source. Quantitation of the parent drug as well as impurities and related chemicals provides information for this purpose. The Drug Enforcement Administration is using the method developed for general drug screening to quantitate heroin samples.[1] The Australian Government Analytical Laboratories report that good results were also obtained when cetyltrimethylammonium bromide (CTAB) was used as the micellar agent instead of SDS.[4] The resulting CE analysis of illicit heroin samples provided rapid separation of complex mixtures while allowing for automated quantitation of each component. Cocaine seizures were also analyzed by CE using 7.5% acetonitrile and 92.5% buffer (50 m*M* CTAB, 10 m*M* phosphate, and 10 m*M* borate at pH 8.6).[5]

Anabolic steroids were placed on the federal list of controlled substances in 1991.[6] Work is currently under way to develop routine analytical methods for the analysis of this class of compounds. Two studies have been published demonstrating the use of CE for this purpose. The first study utilized a micellar buffer system to separate a series of testosterone esters.[7] The second study examined the development of a separation method for anabolic steroids in general.[8] The CE separation of 14 of these steroids was achieved using an acetonitrile/SDS (75 m*M* SDS, 10 m*M* phosphate, and 10 m*M* borate at pH 9.0) buffer in a ratio of 2 to 3. Analyte discrimination could be enhanced further with diode array detection.

Forensic drug analysis is not limited to bulk samples. Toxicological analysis of biological samples for the presence of illegal drugs or poisons involves complex matrices and separation of metabolites as well as the parent compound. An extensive review article[9] found more than 60 references for the CE analysis of drugs in biological samples. This included drugs of abuse in urine,[10] cocaine and morphine in hair,[11] and barbiturates in serum.[12] Other examples include the chiral separation of racemethorphan and racemorphan in urine using a cyclodextrin/SDS/propanol buffer,[13] and the micellar CE separation of nitrazepam and its metabolites in urine.[14]

The use of MS or IR will continue to be necessary because forensic drug analysis usually requires that unequivocal identification of the compound be made. CE can provide excellent complementary information in instances in which the separation is incomplete, the optical isomer needs to be identified, the analyte is thermally labile or reactive, and/or trace quantities are involved.

Forensic gunshot residue analysis

Public concern about violent crime often focuses on crimes involving the use of firearms. Forensic investigators can use the analysis of gunshot residues (GSRs) to develop information concerning the type of weapon fired and the individual involved in the crime. The analytical methods used for GSR analysis must provide characteristic data and be able to detect trace quantities of material. Routine application has been limited because of interferences, high blanks, prohibitive instrumentation costs, and lengthy analysis times.

GSRs are formed from the ammunition primer (consisting of compounds such as lead styphnate, barium nitrate, antimony sulfide, and other compounds),[15] and the ammunition propellant. The propellant usually consists of nitrocellulose plus a number of other compounds that may include nitroglycerin, dinitrotoluene, diphenylamine, ethyl or methylcentralite, and phthalate ester plasticizers.[16] The two most commonly used analytical methods for GSR analysis are scanning electron microscopy (SEM) and atomic absorption spectroscopy (AAS).[17] These techniques are used to examine evidence for the presence of the primer residues. Environmental concerns over lead and heavy metal exposure are driving ammunition manufacturers to find primer components that are free of these materials.[18] A quick survey of any shooter's catalog of ammunition will reveal that several of the major manufacturers are now offering lead-free primers and ammunition. With the advent of these ammunitions, new analytical techniques are needed.

CE has been demonstrated as an excellent separation tool for the identification of the organic GSR constituents that come from the smokeless powder used in ammunition.[19,20] Rapid separation of several GSR constituents, including nitroglycerin, was achieved using a borate buffer at pH 8.5 and SDS as a micellar modifier. Improved instrumentation, including diode array detection and improved detector cell design, has allowed the method to be modified in order to improve separation efficiency and detection capabilities. The buffer concen-

Table 2
SMOKELESS POWDER CONSTITUENTS

Constituent	Abbreviation
Dibutylphthalate	DBP
N,N'-Diethyl-N,N'-diphenylurea (ethylcentralite)	EC
N,N'-Dimethyl-N,N'-diphenylurea (methylcentralite)	MC
Diethylphthalate	DEP
2,3-Dinitrotoluene	2,3-DNT
2,4-Dinitrotoluene	2,4-DNT
2,6-Dinitrotoluene	2,6-DNT
3,4-Dinitrotoluene	3,4-DNT
Diphenylamine	DPA
Glycerol trinitrate (nitroglycerin)	NG
2-Nitrodiphenylamine	2-nDPA
4-Nitrodiphenylamine	4-nDPA
N-Nitrosodiphenylamine	N-nDPA

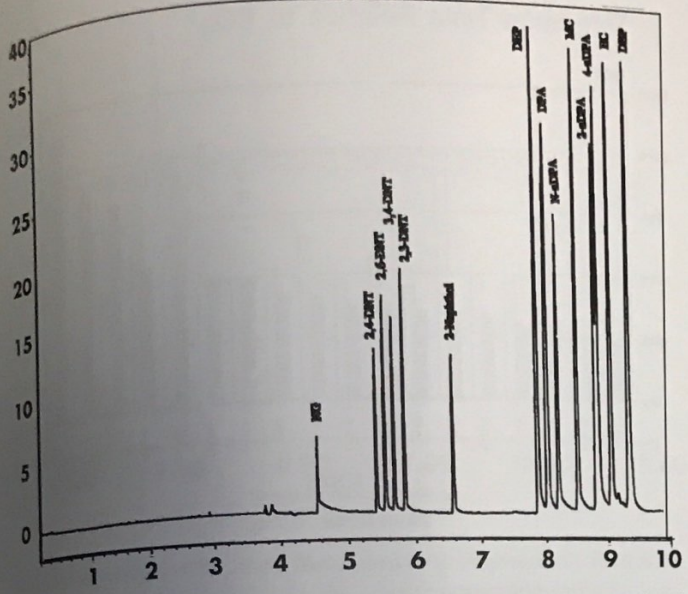

FIGURE 2 *MECE separation of gunshot residue constituents standards at 10^{-4} mol/L. Conditions: 10 mmol/L borate, 25 mmol/L SDS, pH 8.5; pressure injection: 30 mbar for 1.5 sec; voltage: 30 kV; capillary: 80 cm × 75 µm i.d. extended pathlength detection cell; detection: diode array, displayed at 200 nm.*

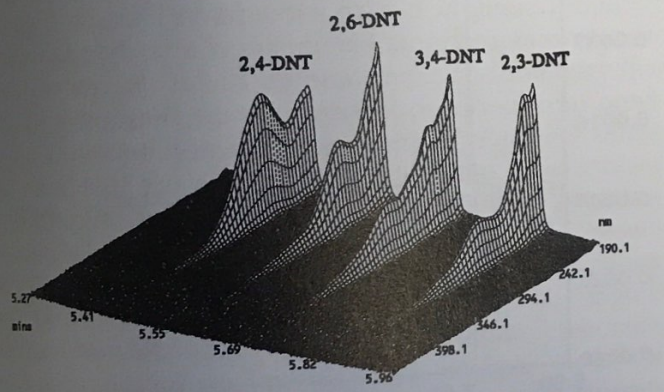

FIGURE 3 *Diode array display of four dinitrotoluene isomers separated in Figure 2. Wavelength: 190–450 nm; time: 5.3–6.0 min. Conditions: Same as in Figure 2.*

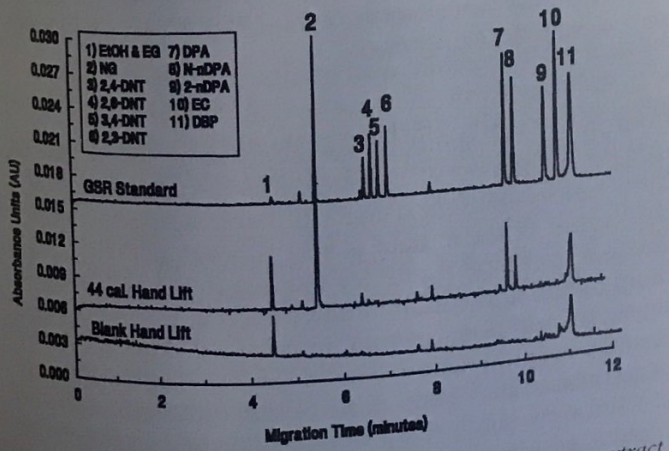

FIGURE 4 *MECE analysis of a solution of GSR standards, an extract of 2-mm²-square section of a film-lift from a hand used to fire a 44-caliber revolver, and an extract from a film-lift blank. Capillary: 100 µm i.d. × 82.5 cm; buffer: 25 mmol/L SDS, 2.5 mmol/L borate; gravity injection: 50 mm, 5 sec; voltage: 25 kV. UV detection at 200 nm.*

tration was raised from 2.5 m*M* borate and 25 m*M* SDS to 5 m*M* borate and 50 m*M* SDS using an extended-pathlength capillary with a nominal internal diameter of 75 µm and a detection window of approx 225 µm. A separation of 13 GSR standards (*Table 2*), including the isomers of dinitrotoluene and nitrodiphenylamine, is shown in *Figure 2*. Three-dimensional data can be obtained with the use of diode array UV detection (*Figure 3*). Detection limit studies found that 2.5×10^{-6} mol/L (3 pg) of nitroglycerin (the poorest UV-absorbing standard) could be detected with a signal-to-noise ratio of 3 to 1.

Firing range studies demonstrated that GSRs collected using masking tape adhesive lifts could be identified using the CE method described above.[21] Samples were collected from the hands of individuals after they had fired three rounds from a weapon. The weapons studied included a Beretta 9-mm semiautomatic pistol, a Smith and Wesson 44 special revolver, and a Colt 45 caliber semiautomatic pistol. Traces of nitroglycerin and other minor constituents were found on pieces of tape even when no residue was visible under a low-power stereo microscope (*Figure 4*). The study also found that no GSRs were found after the hands of the shooters had been washed. Preliminary experiments using the improved CE method have shown that organic GSR compounds are not found on the hands of the general population, while inorganic GSR compounds have been found at low levels in the general population,[22] making interpretation of SEM or AAS data complex.

Another possible application is the use of quantitative analysis of residue constituents to provide information to identify the type of ammunition used. Analyses using 2-naphthol as an internal standard achieve relative standard deviations in peak areas of better than 5%.[21] Determination of time of firing may also be possible by studying the quantitative changes in GSR composition with respect to time.

GSR analysis of organic residues will be the method of the future because of the trend toward the elimination of the use of heavy metal primers. CE offers a technology that can separate trace quantities of organic analytes common to GSRs. It is currently possible to determine that an individual may have fired a weapon using this method, and, with continued research, further information can be developed.

Forensic analysis of inorganic explosive residues

Capillary electrophoresis also has applications in the analysis of inorganic ions. In criminal cases, material such as black powder, flash powder, ammonium nitrate, and homemade energetic mixtures are commonly used as fillers in improvised explosive devices such as pipe bombs. The inorganic anions resulting from the explosive reaction of these materials are among the most important evidence used to determine the nature of an inorganic explosive.[23] While the extraction and

analysis of these ions is a complex and difficult operation, the results obtained can offer important clues in determining the type of material used.

For many years, the most powerful tool in these investigations has been ion chromatography (IC).[23-26] In order to analyze the wide range of sample ions produced from such explosions, special techniques have been developed using low-capacity ion exchange resins.[24,27] Unfortunately, late ... peaks and band broadening occur in these systems, reducing the overall sensitivity. While gradient techniques can alleviate this problem, they suffer from increased complexity and utilize eluants containing carbonate, an important ion in explosive analysis.[28,29]

The introduction of CE for anion analysis provides an opportunity to alleviate these problems.[30-32] This technique, developed and patented by Millipore Corp. (Bedford, MA), produces sharp peaks by achieving efficiencies 10 to 20 times greater than those found with IC.[31,32]

Separation of anions in CE occurs using uncoated capillaries and borate buffers. The ions are swept past the detector by means of the induced or electroosmotic flow. For proper analysis, this flow must be oriented in the direction of the positive electrode, where the detector is located.[33] The electroosmotic flow modifier, an amine compound, coats the capillary wall with positively charged ions, orienting the osmotic flow toward the detector and allowing the anion separation to be performed quickly and efficiently. Since many anions do not absorb well in the ultraviolet, analysis is conducted using inverse photometric detection. With this technique, an additive is placed in the buffer to make it absorbent. The loss in absorbance as the separated ions pass the detector window is monitored.

Capillary electrophoresis separations are based on differences in charge-to-mass ratios of the solvated ions, while IC separations are the result of complex interactions between the ions and the stationary phase. As a result, elution order is quite different in the two techniques and a nearly orthogonal relationship exists between the relative retention times (see *Figure 5*).[34]

These differences in elution order make peak interferences easy to detect because overlapping peaks in one technique are likely to be widely separated in the other. For example, in IC, ions such as phosphate, cyanate, and thiocyanate can cause interferences with the oxidizer ions nitrite, chlorate, and perchlorate, respectively. In CE, however, these ions are well separated from each other.

Another useful technique for determining peak identity is to perform the analysis at different wavelengths.[34] At 205 nm, bromide, nitrite, nitrate, sulfide, and thiocyanate produce peaks in a positive direction due to their UV absorbance. Anions that do not absorb at this wavelength continue to produce peaks in the negative direction, and while sensitivity is reduced, a distinctive pattern of positive and negative peaks results. *Figure 6* shows the results of an analysis of the residue

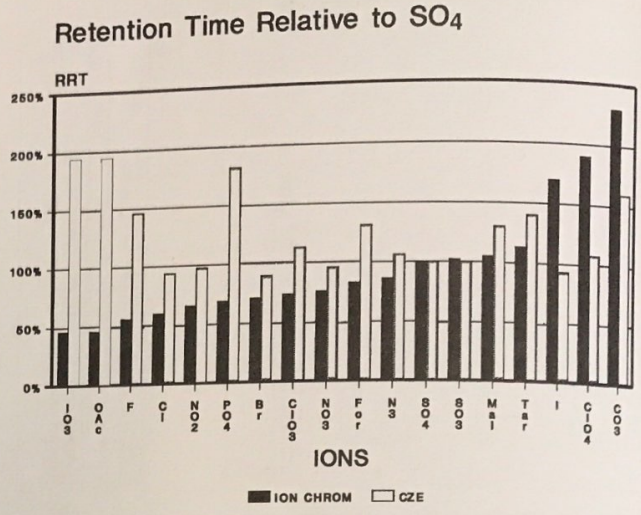

FIGURE 5 *Comparison of elution order in capillary electrophoresis and ion chromatography.*

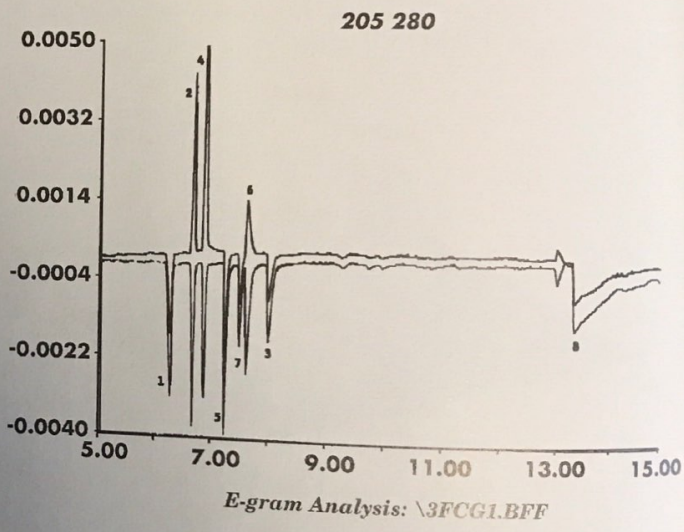

FIGURE 6 *Anion standard containing ions present in explosive residue. Analysis performed at 205 and 280 nm using capillary electrophoresis. Peak identification: 1) Cl⁻, 2) NO_2^-, 3) ClO_3^-, 4) NO_3^-, 5) SO_4^{2-}, 6) SCN^-, 7) ClO_4^-, 8) HCO_3^-.*

from an anion standard recorded at 205 and 280 nm using a scanning UV detector. It is also possible to perform cation analysis using CE,[35] and while the evidentiary value of these data is not as great as that of anion analysis, it can be useful for the determination of ammonium nitrate and certain amines used in slurry gel explosives. In addition, certain improvised explosives may contain sodium salts instead of the less-hygroscopic potassium salts.[26] Kishi et al.[36,37] have described the use of CE for the analysis of both anions and cations in the analysis of improvised explosives in Japan.

Capillary electrophoresis has become an established technique for the forensic analysis of low-explosive residues, and results from this technique have been entered as evidence in a number of cases. In a typical

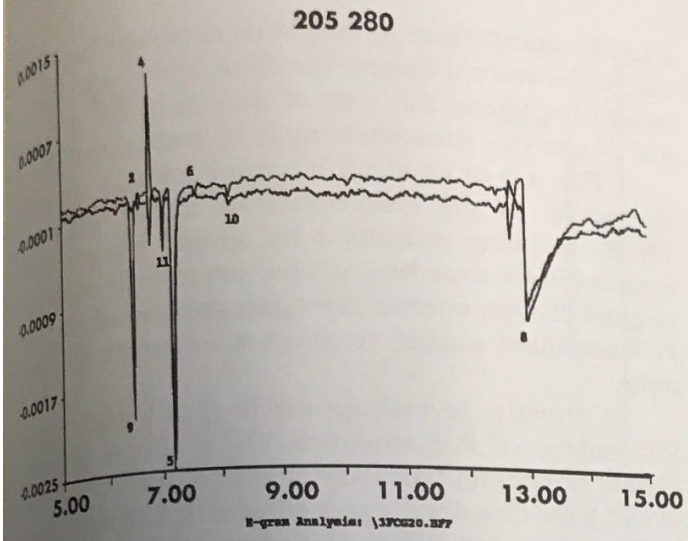

FIGURE 7 *Electropherogram of black powder pipe bomb residue. Peak identification: 1) Cl⁻, 2) NO_2^-, 4) NO_3^-, 5) SO_4^{2-}, 6) SCN^-, 8) HCO_3^-, 9) HS^-, 10) OCN^-, 11) unknown.*

analysis, evidence consisting of shrapnel and other material is taken from the crime scene and washed with distilled water. The aqueous extract is filtered, diluted, and analyzed. The resultant electropherogram consists of a pattern of ions of different type and intensity that can be compared with the results obtained from previously exploded bombs.[38] For example, *Figure 7* shows the results of the analysis of the residue from a black powder pipe bomb. Black powder consists of a mixture of potassium nitrate, sulfur, and charcoal. The results show a wide variety of ions present in the anionic residue. These ions include nitrate from the original mixture as well as the product ions, sulfide, carbonate, thiocyanate, cyanate, nitrite, and sulfate. Note the cyanate ion in Figure 7. This ion interferes with chlorate in the IC analysis, but is separated from chlorate by CE. The distinction between this peak's migration time and that of the chlorate peak allows the investigator to rule out the presence of chlorate in the original explosive mixture.

The electropherogram also illustrates the advantage of dual-wavelength detection. Peak number 9 is sulfide. At 205 nm, the sensitivity for this peak reaches a minimum due to a certain amount of inherent absorptivity. Thus, only a peak at 280 nm appears. Nitrite, nitrate, and thiocyanate also appear as positive peaks in this analysis. The ability to overlay the results of analysis at different wavelengths improves confidence in peak assignments.

CE offers a number of advantages over traditional IC techniques such as efficiency, specificity, and column lifetime. These advantages are particularly important in explosive residue analysis, where there is little prior knowledge about the sample or its matrix. The same instrument can be used for both anion and cation analysis with a simple change in buffer system, and the use of different wavelengths in the anion analysis improves specificity. The high efficiency of the CE techniques helps to minimize peak interferences and allows a wide range of ions to be analyzed simultaneously.

When CE and IC are combined, the differences between separation mode and action provide assistance to the analyst in determining the nature of the sample. Use of CE and IC in tandem greatly reduces problems caused by interfering ions and allows easy peak confirmation.

Forensic analysis of organic explosive residues

Many of the organic chemicals used to manufacture explosives are similar to those used in making smokeless powder. CE analysis of these materials can be conducted using the same methods developed for GSR analysis. A separation of 15 potential explosive components was achieved using the borate/SDS buffer system described above.[19] This method has been utilized to separate and identify explosive residues extracted from soil contaminated by explosives.[39] Further optimization of the method has enabled the separation of 24 explosive compounds including the isomers of aminodinitrotoluene, dinitrotoluene, and nitrotoluene.[40] The results were compared to those obtained using HPLC protocols, and were found to provide superior resolution, efficiency, and speed of analysis. Analysis times of under 10 min are routine. The method is ideal for the analysis of bulk samples of high explosives and smokeless powder pipe bomb residues; however, it remains to be seen if detection limits in CE will be sufficient for high-explosives post-blast analysis.

Forensic DNA analysis

Forensic DNA typing has become a powerful tool for associating suspects to and excluding suspects from the scene of a crime or identifying human remains from accidents or war.[41,42] All forensic DNA typing involving electrophoretic separations has used agarose or polyacrylamide slab gel electrophoresis.[41] Certain states are now requiring all convicted sex offenders to submit blood samples for DNA typing, and the Armed Forces Institute of Pathology (AFIP) is preparing a database for use in identifying military personnel. The above requirements place a tremendous burden on laboratories performing DNA analyses by traditional techniques. For this reason, there is an interest in improving the speed of these analyses. With its tremendous potential for high-speed DNA analysis, CE can have a role in the future of DNA typing.

Unlike proteins and other biopolymers, DNA maintains a constant charge-to-mass ratio regardless of length. Proper separation of different-sized fragments by CE requires the use of a sieving matrix.[43] Several approaches have been taken to separate DNA fragments by sieving including polyacrylamide gel-filled capillaries,[44,45] agarose gel-filled capillaries,[46] and soluble polymers.[43,47-49]

Single base pair (bp) resolution of single-stranded DNA molecules, such as primers and sequencing fragments, is best achieved by separation on polyacrylamide gel-filled capillaries.[50] Unfortunately, special techniques are required to maintain these columns because they are prone to problems such as bubble formation and contamination.[51,52] Soluble polymers such as linear polyacrylamide and hydroxyethyl cellulose (HEC) (Aldrich, Milwaukee, WI) can be dissolved in a liquid buffer for less demanding applications in which single base pair resolution is not necessary.[47] The buffer can be pumped in and out of the capillary, eliminating many of the technical problems with gel-filled capillaries, and ensuring a clean separation medium prior to each individual analysis.[53]

Proper sample preparation is also important in DNA separations by CE. Products of the polymerase chain reaction (PCR) (Hoffmann-LaRoche, Nutley, NJ) contain high levels of chloride ions.[47,54] These salt ions possess a high charge-to-mass ratio and are preferentially introduced onto the capillary during electrokinetic injection, thus reducing sensitivity.[45,47] To eliminate this problem, various workers have introduced different procedures for salt removal including float dialysis, ultracentrifugation, and ethanol precipitation.[47,48,54] Of these procedures, float dialysis appears to be the most efficient.

Sample cleanup can be cumbersome when many samples are being analyzed, and material can be lost by binding to purification membranes.[55] Hydrodynamic injection may enable post-PCR cleanup to be avoided.[55,56] However, hydrodynamic injections do not introduce as much sample onto the capillary and can only be used with soluble polymer buffers.

On-line detection of nucleic acids in CE is performed with either UV absorbance or laser-induced fluorescence (LIF).[47,57] For fluorescent analysis, intercalating dyes such as the cyanine dyes thiazole orange, oxazole yellow, or their dimers are added to the buffer. These dyes only fluoresce when bound to DNA,[58] permitting detection of DNA at low nanogram-per-milliliter concentrations.[57,59,60]

One type of forensic DNA analysis involves the exploitation of polymorphic areas containing tandemly repeated DNA sequences. Sequences that vary according to length (i.e., the number of tandem repeats) are referred to as variable-number tandem repeats (VNTRs).[61] Constant regions located on either side of the sequence variable region allow annealing of PCR primers and subsequent amplification[62] of these specific DNA regions to a detectable quantity.[63] It is particularly useful in situations common to forensics in which samples can be degraded or are available in limited quantities.[61,64,65] DNA has been successfully amplified from blood, bones, semen, semen stains, saliva, and hair.[61,64]

The PCR products generated from forensic DNA samples are presently analyzed by polyacrylamide gel electrophoresis (PAGE) and detected by fluorescent labeling or silver staining.[62,66] These products are then sized by comparison to standards in parallel lanes or by use of fluorescent-tagged standards in the same lane. In order to duplicate this type of analysis by CE, strict control of analysis parameters must be maintained.

The use of capillary electrophoresis as a possible alternative to gel electrophoresis in the analysis of VNTRs is being examined by several groups.[45,47,57,60,67] Samples in CE must be analyzed sequentially rather than in parallel; thus internal standards must be added to the PCR-amplified sample to account for run-to-run variations.

Currently, several approaches are being explored for analysis of PCR-amplified VNTRs. These include a variety of column types and injection methods. McCord et al.[47] have described a procedure in which a tris-borate buffer containing 0.75% HEC was combined with the intercalating dye ethidium bromide for the analysis of the VNTRs D1S80 and SE33. These VNTR systems contain 16-bp and 4-bp repeats, respectively. The analysis was carried out using 100 µm i.d. DB-17-coated columns and UV detection at 260 nm.

Williams et al.[45] have described a procedure involving sample dialysis followed by electrokinetic injection with capillary gel electrophoresis separation and UV detection at 260 nm. Their approach showed excellent resolution, and by maintaining careful control of instrument parameters, they were able to achieve over 100 sample injections per column. A standard deviation of less than 0.4 bp was obtained for repetitive analysis of alleles from the HUMTH01 (4-bp repeat) VNTR system.[45] Alleles were sized by comparison of migration times to a 100-bp ladder (i.e., DNA fragments of 100 bp, 200 bp, 300 bp, etc.).

A possible improvement in soluble polymer buffer systems was described by Pearce and Watson,[68] who utilized a phosphate buffer containing 1.25% hydroxypropylmethyl cellulose at pH 5.7. The authors noted that the lower pH should help maintain the capillary coating. Their system was used in the analysis of the ApoB VNTR system, as well as to check for primer degradation.[68]

Several groups have noted that there are specific advantages to the use of laser fluorescence in the CE analysis of PCR-amplified DNA.[57,59,60,69] Fluorescence detection has been shown to be 400 times more sensitive than UV.[59] This high sensitivity permits the use of smaller column diameters and reduced injection times, both of which result in improved resolution. High sensitivity also permits hydrodynamic injection to be used, potentially eliminating the need for salt removal prior to injection.[55] A number of different VNTRs were analyzed by this technique including D1S80, MBP, HUMTH01, VWa, and ApoB.[57,60,67,69]

Figure 8 shows an example of the analysis of a HUMTH01 standard mixture of alleles by laser fluorescence. This system is currently being examined for use in human identification.[62,70] HUMTH01 alleles, ranging in size from 179 bp to 203 bp with a 4-bp repeat, can be amplified faithfully by PCR.[62] The figure demonstrates

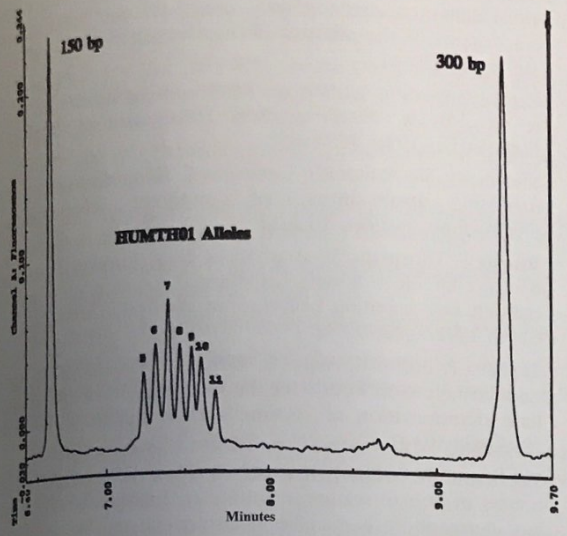

FIGURE 8 *Rapid separation of HUMTH01 allelic ladder. The 150-bp and 300-bp markers allow the size determination of each allele. Conditions: 0.05 mm × 37 cm DB-17 column; 1% HEC, 100 mM tris-borate, 2 mM EDTA, pH 8.1; 50 ng/mL YO-PRO-1 (Molecular Probes Inc., Eugene, OR); pressure injection at 0.5 psi for 5 sec water, 45 sec sample; voltage gradient: 0–5.2 min at -15 kV (14.5 μA), 5.2–10 min at -5 kV (4.8 μA); 25 °C; LIF detection, 520 nm.*

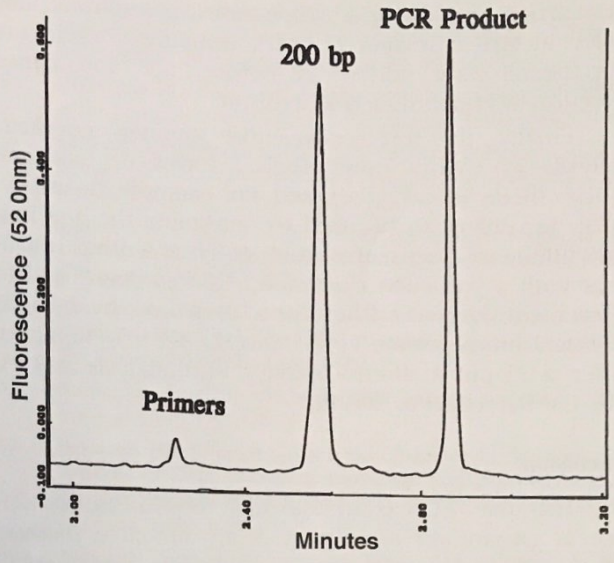

FIGURE 9 *Quantitation of a 414-bp PCR product amplified from mitochondrial DNA. Conditions: 0.05 mm × 27 cm DB-17 column; 1% HEC, 100 mM tris-borate, 2 mM EDTA, pH 8.1, 50 ng/mL YO-PRO-1; pressure injection at 0.5 psi for 45 sec; -15 kV (22 μA); 25 °C; LIF detection, 520 nm.*

baseline resolution of the seven major HUMTH01 alleles in under 10 min. By comparison, horizontal PAGE requires over 2 hr to obtain the same resolution. The 150-bp and 300-bp peaks act as internal standards and are used to produce an estimate of the size of each allele. This rapid separation was obtained by using a short, narrow capillary combined with a step voltage gradient.

Differences in DNA base sequence among individuals also have forensic relevance. One hypervariable system under investigation is the control region of mitochondrial DNA.[71,72] Following extraction of mitochondrial DNA from hair, bone, teeth, or other tissues, the control region can be successfully amplified via PCR. The PCR product can then be sequenced and the suspect's sequence compared to the DNA from the crime scene.[72]

CE is currently being used to quantitate and evaluate quality of DNA generated by PCR.[54,55,73] *Figure 9* is an electropherogram showing a 200-bp internal standard and a PCR product obtained from amplifying mitochondrial DNA. CE can provide a rapid check for DNA quality and quantity prior to sequencing. In addition, only small amounts of the PCR mixture are consumed by the analysis, leaving the bulk of the sample for future analyses.[55]

Routine sequencing of DNA by CE will only become a reality once multiple capillary systems are commercially available.[74] Current sequencing gels can support 24 to 36 lanes, yielding much greater throughput than currently available CE instrumentation.[66] However, multiple capillary CE systems, using as many as 100 capillaries, have the potential to dramatically improve the present sequencing rates and typing rates for VNTRs.[75]

The future of forensic DNA analysis by capillary electrophoresis is promising. PCR product sizing and quantitation are being actively investigated by several groups. Areas under investigation include improving the precision and accuracy of DNA sizing, separating multiple nonoverlapping VNTRs, and optimizing run time, resolution, and throughput. CE separations of DNA may never completely replace the established slab gel electrophoresis techniques, but capillary electrophoresis has shown the potential for useful forensic DNA analysis. If this situation can continue to improve, the routine application to forensic DNA casework is only a matter of time.

Other forensic applications

Forensic scientists are required to examine biological samples other than DNA to provide evidence in cases involving homicide, rape, assault, etc. Semen and saliva are two fluids that the forensic serologist is frequently asked to identify. CE has been used to identify saliva from stains as old as six months.[76] The results were obtained using a 1 mol/L phosphate buffer at pH 7.0 with 1 mol/L trimethyl-ammonium-propanesulfonate added to prevent wall adsorption. UV absorbance detection was performed at 214 nm. The same researchers have also used CE to differentiate seminal plasma from saliva and serum plasma by quantifying the low-molecular-weight (below 30,000 D) proteins.[77] This analysis

was carried out using a 100 mmol/L phosphoric acid buffer at pH 4.5 with 1 mol/L trimethyl-ammonium-propanesulfonate added as before. UV absorbance detection was conducted at 214 nm.

Finally, the forensic scientist routinely conducts analyses on many nonbiological forms of evidence besides those already discussed. For example, the analysis of pen inks can be used to determine the age and authenticity of documents, and match a writing instrument with a particular document. CE has been used to differentiate between fiber-tipped pen inks by using a 3:1 ammonium acetate (100 mmol/L, pH 4.5):methanol buffer, a 25 µm × 20 cm capillary, and analysis at 8 kV with UV detection at 206 nm.[78]

Conclusion

The use of CE as an analytical tool in forensic science is expanding rapidly. It is an attractive method because of its potential for rapid analysis, use of small sample quantities, high-efficiency separations, and relative economy. CE is also readily adapted to a wide variety of applications, as the preceding discussion demonstrates, making it one of the most flexible and economical analytical tools available. Continued research will certainly expand the utility of CE in forensic science.

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