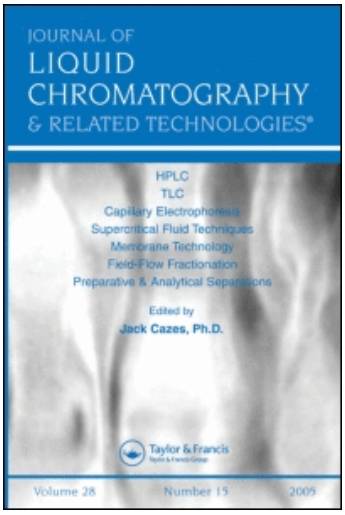

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

THIRTY-FIVE YEARS OF CAPILLARY ELECTROPHORESIS: ADVANCES AND PERSPECTIVES

Haleem J. Issaq[a]
[a] Analytical Chemistry Laboratory, SAIC-Frederick, Inc., National Cancer Institute at Frederick, Frederick, MD, U.S.A.

Online publication date: 30 May 2002

To cite this Article Issaq, Haleem J.(2005) 'THIRTY-FIVE YEARS OF CAPILLARY ELECTROPHORESIS: ADVANCES AND PERSPECTIVES', Journal of Liquid Chromatography & Related Technologies, 25: 8, 1153 — 1170
To link to this Article: DOI: 10.1081/JLC-120004015
URL: http://dx.doi.org/10.1081/JLC-120004015

MARCEL DEKKER, INC. • 270 MADISON AVENUE • NEW YORK, NY 10016

J. LIQ. CHROM. & REL. TECHNOL., 25(8), 1153–1170 (2002)

THIRTY-FIVE YEARS OF CAPILLARY ELECTROPHORESIS: ADVANCES AND PERSPECTIVES*

Haleem J. Issaq

Analytical Chemistry Laboratory, SAIC-Frederick, Inc.,
National Cancer Institute at Frederick, P.O. Box B,
Frederick, MD 21702
E-mail: issaqh@mail.ncifcrf.gov

ABSTRACT

Since the publication by Hjerten of his study on free zone electrophoresis thirty-five years ago and the publication by Jorgenson and Lukacs of their study in 1981, CE instrumentation and applications have become widespread. CE today includes capillary zone electrophoresis, micellar electrokinetic chromatography, capillary gel electrophoresis, capillary isoelectric focusing, isotacophoresis, and capillary electrochromatography. Array capillary electrophoresis and array microchip separations are also important aspects of modern CE instrumentation. CE has become a versatile analytical technique, which is successfully used for the separation of small ions, neutral

*This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institutes of Health, under Contract No. NO1-CO-12400. By acceptance of this article, the publisher or recipient acknowledges the right of the U.S. Government to retain a nonexclusive, royalty-free license and to any copyright covering the article. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

molecules, and large biomolecules and for the study of physicochemical parameters. It is being utilized in widely different fields, such as analytical, forensic, clinical, and organic chemistry, in addition to natural products, the pharmaceutical industry, chiral separations, molecular biology, and other fields.

INTRODUCTION

Electrophoresis as an analytical technique was first introduced by Tiselius in 1930.[1] In his thesis he described the separation of blood plasma proteins, namely albumin, from α, β, and γ-globulin, using electrophoresis. Thirty-five years ago Hjerten showed that it was possible to carry out electrophoretic separations in a 300 μm glass tube and to detect the separated compounds by ultraviolet absorption.[2] Although other researchers used electrophoresis in glass and Teflon tubes,[3–5] CE did not become popular until 1981 when Jorgenson and Lukacs published their work in which they demonstrated the simplicity of the instrumental setup and the high resolving power of CZE.[6] This simple and efficient instrumental setup with on-column UV/Vis detection, which is the basis of most commercial instruments on the market today, used a narrow internal diameter fused silica capillary, 75 μm i.d., high voltage, up to 30 kV for the separation of ionic species. The results shown were astonishing: sharp narrow peaks; 400,000 theoretical plates/meter (TPM), and not 10,000 TPM as in HPLC; also, short analysis times, minutes compared to hours by slab gel electrophoresis (SGE). In 1984, Terabe added a micelle, SDS, to the running buffer solution and was able to resolve a mixture of neutral compounds.[7] This was the beginning of micellar electrokinetic chromatography (MECC) and the application of CE to the separation of ionic as well as neutral species. Later, a chiral reagent, cyclodextrin, was added to the running buffer to resolve enantiomeric mixtures.

Robert Gorley introduced the first commercial instrument in 1988. Applied Biosystems, Beckman, Spectraphysics, Hewlett Packard, Waters, and others soon followed with a number of additional commercial instruments. However, the rush to introduce CE instruments did not produce the required profits for a company to continue operating. Thus, today, only a few companies market CE instruments. Also, companies have shifted into designing CE instruments for specific tasks, such as array electrophoresis instruments for DNA sequencing, and the use of the electrophoretic-driven microchip technology.

The first HPCE conference was held in Boston in 1989, and Kuhr published the first fundamental review of CE as an analytical technique in Analytical Chemistry in 1990.[8] Also, in 1990, The Frederick Conference on CE (FCCE) was born; the emphasis of the FCCE was the application of CE to biological and biochemical problems. FCCE held a special session devoted to graduate students

and postdoctoral fellows to allow these young scientists to present their work. In the last ten years, our laboratory has been involved in the development and application of CE for the separation of small, as well as large biomolecules.[10] For a more recent historical review of advances in CE instrumentation, the reader is referred to the excellent write-up by Camilleri.[9]

The theory of electrophoresis is not really as recent as one might be led to believe from the historical review presented thus far. Long before Tiselius' use of electrophoresis as an analytical technique, it was known that an electric field applied over a solution could be used to separate charged particles. According to a review in Chromatographia Supplement, "In the 19th century, physical chemists had applied electrophoretic methods to separate or fractionate colloids. The basis for the description of the electrophoresis of ions was laid by F. Kohlrausch in 1897 in an article published in Annalen der Physik und Chemie".[11]

CE has been used for the separation of inorganic ions and organic acids; organic molecules; amino acids, peptides, and proteins; natural products; nucleic acids; illicit drugs; chiral mixtures; and for physicochemical studies such as protein/drug and protein/DNA interactions. This review is not meant to be a comprehensive one; rather to show the speed of CE advancement, applicability, and future directions.

Is Capillary Electrophoresis the Ultimate Separation Technique?

The questions that have been asked over the last fifteen years are why use capillary electrophoresis when other separation techniques are available? What is so special about it and what are its advantages and limitations?

The field of separation science, prior to CE, was dominated mainly by three techniques; thin layer chromatography (TLC), gas chromatography (GC), and high performance liquid chromatography (HPLC). Even though CE has met a lot of success in the last 20 years, GC and HPLC are still dominant techniques; however, CE has a few distinct advantages over other separation techniques. The advantage of CE over GC, HPLC, TLC, and slab gel electrophoresis (SGE) is its simplicity and applicability for the separation of widely different compounds, inorganic ions, organic molecules, and large biomolecules, using the same instrument and, in most cases, the same column, while changing only the composition of the running buffer. This cannot be said about any of the other separation techniques. In addition, CE possesses the highest resolving power of any liquid separation technique, due to its plug flow and minimal diffusion. CE, unlike field flow fractionation (FFF), counter current chromatography (CCC), and super critical fluid chromatography (SFC), succeeded because of its wide application to the separation of different groups of compounds, while the others did not, and also, because of its application to the separation of biomolecules

such as proteins and nucleic acids. In order for a new technique to succeed, it has to have certain applications that cannot be done or cannot be accomplished as easily or as economically by existing techniques. In addition, using array capillary electrophoresis, one can analyze many samples simultaneously in minutes, which is why CE is such a viable separation technique.

The question that one would ask is the following: "*Is CE the ultimate separation technique?*" Let us first define what we mean by *ultimate separation technique*. At the 1996 Pittsburgh Conference I gave an invited presentation dealing with the above topic. I defined *ultimate separation technique* as one that should meet the following criteria: (a) can be applied to the separation, detection, and quantitation of a wide variety of mixtures; (b) should have high resolving power; (c) is fast; (d) is not destructive; (e) is economical to operate; (f) can be automated; (g) is reproducible; (h) is simple and easy to operate; (i) is environmentally friendly; (j) is multidimensional; (k) can be used for microanalytical, as well as preparative techniques; and (l) can be used for the analysis of multi-samples simultaneously using the same instrument. Not one of the existing separation techniques fully meets the above criteria; however, CE comes very close.

It should be noted that CE is only an analytical and not a preparative technique. Also, quantitation is easier and reproducibility is better by GC and HPLC. Compared to SGE, CE is faster, easier, simpler, can be automated, and gives quantitative data. Unlike SGE and TLC where two-dimensional separations are achieved easily, in CE, like HPLC and GC, such separations cannot be performed. However, CE can be used online with other instrumental techniques such as HPLC, mass spectrometry (MS), and nuclear magnetic resonance (NMR). The amount of material needed for a CE experiment is very small, nanoliters of sample and microliters of buffer, compared to the other separation techniques, which require microliters of sample and milliliters of solvent. The resulting CE waste, mostly an aqueous buffer with a small percentage of an organic modifier, is relatively safe and can be discarded without any danger to the environment. This is not true when TLC or HPLC is used where large amounts of organic solvent waste are produced, or GC where volatile compounds escape into the environment.

CE Modes

In addition to CZE, where a bare fused silica capillary and a simple buffer are used, and MECC, where a micelle is added to the buffer to effect the separation of neutral molecules, CE encompasses other techniques, which are electrokinetically driven. These include isoelectric focusing (IEF), where the neutrally coated capillary is filled with an ampholite to create a pH gradient, used mainly for the separation of proteins based on their isoelectric point; capillary gel

electrophoresis (CGE), where the capillary is filled with an entangled polymer and separation is based on size. Capillary electrochromatography (CEC), where the column is packed with derivatized, normally C-18, particles or ion exchange particles, is mainly a size-exclusion mechanism. In CEC, which is an HPLC-like procedure, electroosmotic flow is used to drive the mobile phase through the column in place of a pump. The result is a higher resolution than when HPLC with a pump is used.

Instrumentation

Although the instrumental setup presented by Jorgenson and Lukacs[6] was different from the original design by Hjerten,[2] no major changes in the basic design of CE instrumentation have been made since then. The improvements in commercial CE instrumentation in the last 20 years include: the introduction of capillary liquid and air cooling systems, LIF, and electrochemicals for sensitive detection, and array electrophoresis employing 96 capillaries. The advances are mostly in methodology and applications. The advances in methodology include the development and application of MECC, CGE, IEF, ITP, and capillary electrochromatography (CEC) to the separation of widely different groups of compounds. The development of sensitive detection schemes and reproducible columns propelled CE into the forefront of analytical techniques. However, sample introduction into the capillary needs to be developed in order to achieve reliable quantitative data. In GC and HPLC, a known volume of known concentration of a standard solution is measured and injected onto the column. In CE, the sample is introduced electrokinetically or hydrodynamically. The amount injected is not immediately known; it is determined after a few calculations which require the knowledge of capillary internal diameter, buffer viscosity, total capillary length, buffer density, time of injection, applied voltage, electrophoretic mobility of the analyte, and other parameters. In short, sample introduction in CE is not as simple or straightforward as in GC or HPLC.

CE Detection

Modes of detection that have been developed for HPLC are used in CE. UV/Vis, LIF, mass spectrometry, electrochemical detection, conductivity, fluorescence, indirect UV/Vis, indirect fluorescence, and nuclear magnetic resonance have been employed for the detection of different groups of ions and molecules. These topics will not be discussed here because they can be found in any book dealing with CE. We will only mention that LIF and electrochemical detection using micro-electrodes are the most sensitive detection techniques. The

sensitivity of LIF was demonstrated by works from the laboratories of Edward Yeung and Norman Dovichi, where they reported in separate studies on the detection of a single molecule.

CE Applications

Hjerten in his initial review of free zone electrophoresis,[2] demonstrated the applicability of CZE to the separation of widely different mixtures, such as inorganic ions; bases, i.e., cytosine, adenine, guanine, and uracil; nucleosides, cytidine, adenosine, guanosine, and uridine; nucleotides, uridine-5'-phosphate, guanosine-5'-phosphate, adenosine-5'-phosphate, and cytidine-5'-phosphate; proteins in normal human serum; nucleic acids, such as native and thermally denatured T2 DNA; viruses; cells and subcellular particles. Hjerten also used indirect detection for detecting substances that have no UV absorption.

Inorganic Ions and Organic Acids

Hjerten presented data showing the separation of Bi from Cu ions.[2] Capillary electrophoresis is well suited for the separation of inorganic ions using indirect UV detection. A notable one is the separation of 36 anions in 3 minutes.[12] CE has also been used for the separation of nitrate from nitrite in water and urine,[13,14] pyridinecarboxylic acid isomers and related compounds,[15] and the separation of heterocyclic nitrosoamino acid conformers at subambient temperatures.[16]

Amino Acids, Peptides, and Proteins

Capillary electrophoresis is well suited for the separation of amino acids, as was demonstrated by Jorgenson and Lukacs in their first publication.[6] Since then, many studies that dealt with the separation of amino acids and their enantiomers, peptides, and proteins have been published. We cite here a few of those studies.

Micellar electrokinetic chromatography is the method of choice for the separation of a mixture of a large number of amino acids because the 20 amino acids possess different chemical properties—basic, acidic, polar, hydrophilic, and hydrophobic—that make their separation by CZE according to their charge density using a simple buffer difficult. Therefore, a micelle is added to the buffer to effect the separation. Sensitive detection is achieved by laser-induced fluorescence (LIF) after derivatization of the amino acids with a fluorescent

tag. In our laboratory, amino acids were resolved by MECC and detected with pulsed UV laser-induced fluorescence after derivatization with 9-fluorenyl methyl chloroformate.[17] The three aromatic amino acids, tryptophan, phenylalanine, and tyrosine, can be detected with UV-LIF without derivatization with a fluorescent tag.[18] A review by Issaq and Chan discussed the CE separation and LIF detection of amino acids and their enantiomers.[18]

Capillary electrophoresis has been successfully used for the separation of peptides, to check the purity of synthetic peptides, to predict the mobility of peptides;[19,20] and for peptide mapping of proteins after enzymatic digestion.[21] Peptides are detected after separation by UV absorption at 214 nm or by laser-induced fluorescence (LIF) after derivatization with a fluorescent reagent.[22] Pulsed UV-LIF was used for the detection of peptides without derivatization if the peptides contained one of the three aromatic amino acids.[23] For a comprehensive review of CE of peptides, see Ref. (24).

The CZE separation of proteins in untreated fused silica capillaries has been done at low pH to minimize the adsorption of proteins to the capillary wall. The majority of protein CE separation studies used neutrally coated capillaries or buffer modifiers to eliminate or suppress the protein-wall interaction. Reported attempts to eliminate the adsorption of proteins to the capillary wall include physically coating the inner capillary wall with methyl cellulose,[3] polyacrylamide,[3,25] polyethyleneimine,[26] polyethyleneglycol,[27] non-ionic surfactant coatings,[28] or via silane derivatization.[3,29,30] Another procedure for minimizing the adsorption of proteins is through the use of additives to the buffer, i.e., dynamic coating. For a comprehensive review of protein separations, the reader is referred to Chapter 9 in Ref. (24).

Natural Products

MECC is a well-suited technique for the separation of natural products, since the majority of the compounds are neutral molecules. Natural products are the organic and inorganic compounds found in nature: in plants (leaves, needles, bark, roots, flowers and seeds); in marine organisms (plants, animals, and microbes); in the microbial fauna found in highly diverse and sometimes extreme environments and in the soil. The pharmaceutical industry in its drug discovery effort has relied heavily on natural products, if not as a source of drugs, then as sources of novel bioactive chemotypes that can be developed into new drugs; an excellent example is penicillin. CE and MECC were used for the separation of widely different compounds from natural materials, including antibiotics, flavonoids, alkaloids, illicit drugs, toxins, Chinese herbal preparations, humic substances, and many other compounds. Two reviews of the subject have been published.[31,32]

Illicit drugs, cocaine and heroin, are considered natural products in this review. The earliest work on the use of CE for the separation of illicit drugs was reported by Weinberger and Lurie for the separation of heroin impurities employing a micellar buffer and UV detection.[33] In another study, Lurie et al. used MECC for the separation and UV/LIF at 248 nm, using a KrF pulse laser, for the detection of impurities in heroin and cocaine.[34,35] An improvement of 1,000-fold in sensitivity was observed when the UV/LIF detector was used, compared to a UV detector employing a deuterium lamp.

The advantages of CE and MECC for the analysis of natural products is their high resolving power and applicability to a versatile group of compounds. In our laboratory, MECC was used for the separation of a bark and needle extract for the assay of taxol, an anticancer agent.[36] For the application of CE for the separation of toxins, see Ref. (37).

Nucleic Acids

Capillary gel electrophoresis is a powerful analytical technique for the separation of nucleic acids based on their size. The separation of double-stranded DNA fragments, single-stranded DNA, and polymerase chain reaction (PCR) products is achieved by employing capillaries filled with a replaceable liquid gel, which acts as a sieving medium that can be replaced after each analysis. Hydroxyethyl cellulose, hydroxymethyl cellulose, methyl cellulose, polyvinyl alcohol, liquid polyacrylamide, and others have been used as liquid polymers. For a comprehensive review of the subject, history, theory, and applications, the reader may consult Ref. (38).

Issaq et al.[39] examined the effect of different parameters on migration time, resolution, and speed of analysis of DNA fragments and PCR products. These parameters include column length, applied voltage, gel type, and concentration, and buffer ionic strength. The results show that a 1 cm capillary at an applied voltage of 185 V/cm filled with commercial gel was adequate for the separation of small DNA fragments and PCR products. Array capillary electrophoresis with gel-filled capillaries has been used for DNA sequencing.[40] Also, microchip array technology has been used for DNA sequencing.[41]

Clinical Applications

Applications of CE in the clinical laboratory have been reported.[42] In our laboratory CE has been used for the separation of homovanillic and vanillylmandelic acids in baby [human?] urine,[43] hydroxyproline in human urine and serum,[44] and polysaccharides from human urine.[45] Also, we have

reported the separation of retinoic acid isomers,[46] caffeine metabolites,[47] and determination of estrogens by CE with electrochemical detection and microdialysis sampling.[48] Those interested in the CE analysis of drugs should consult Ref. (49).

Chiral Separations

Three approaches can be used to separate a chiral mixture: (a) adding a chiral compound, such as cyclodextrin (CD), to the running buffer; (b) reacting the amino acids with a chiral reagent, such as 1-(9-fluorenyl) ethyl chloroformate (FLEC), to form two stereoisomers that can be easily resolved using a non-chiral column;[50] or using a chiral column. Gassmann et al.[51] were the first to report on chiral separations by CE. They resolved a mixture of dansylated D-and L-amino acids by adding Cu(II)-L-histidine to the running buffer. Since then, many studies using different forms of cyclodextrins, their derivatives, and other chiral reagents have been published.[17,18,50,52–57] For a detailed discussion of chiral separations by CE and MECC, the reader is advised to consult reference 56.

Reversed Flow-MECC

One of the weaknesses of MECC is its application to the separation of hydrophobic compounds. RF-MECC is a procedure that was developed for the separation of these compounds.[58] The principle is very simple: electrokinetic chromatography with negatively charged CD or SDS added to the buffer was conducted in polyacrylamide-coated fused-silica capillaries under suppression of electroosmotic flow with reversed polarity; i.e., the flow to the detector is from the negative electrode to the positive electrode. In this procedure, the most hydrophobic solutes are eluted first, unlike MECC where the most hydrophobic solutes elute last as a broad peak. This procedure was used for the separation of a mixture of polycyclic aromatic hydrocarbons and other hydrophobic compounds,[58–61] and a racemic mixture of amino acids, dipeptides, aflatoxins, and chlorophenols.[57]

Physicochemical Studies

Another question has been: Is CE a separation technique only or can it be used to determine physicochemical parameters? A search of the literature reveals that CE can indeed be used to determine physicochemical parameters. CE has been used for the determination of:[62] (a) pK values of weak electrolytes and amphoteric compounds; (b) pI values of proteins by cIEF; (c) mobility and its relation to charge/mass ratio in peptides and proteins; (d) mobility of nucleic

acids; (e) viscosity; (f) binding and dissociation constants; (g) the approximate molecular weight of proteins; (h) the approximate number of base pairs of DNA fragments and PCR products; (i) detection of DNA mutants;[63–65] and other applications. CE was used in our laboratory to determine protein/drug interaction and protein/DNA interaction;[25] to determine the partition coefficient and hydrophobicity of organic compounds;[59] to estimate the number of base pairs in PCR products and DNA fragments;[66] and to evaluate monomer–dimer equilibria of peptides.

Capillary Electrochromatography

The column type (bare or coated), along with its contents, determines the mode of CE separation. A bare fused silica capillary and a simple buffer are used for CZE. The addition of a micelle to the buffer to resolve mainly neutral compounds is known as MECC, while reversed flow-MECC uses a neutrally coated fused silica capillary and a micelle buffer for the separation of hydrophobic compounds. The addition of a chiral reagent to the buffer, simple or micelle, is used for the separation of racemic mixtures. The filling of a neutrally coated capillary with an ampholite is used for the determination of the pI of proteins by IEF. Filling the neutrally coated capillary with a liquid gel, entangled polymers, is used in CGE for the separation of DNA fragments and PCR products. In 1974, Pretorius and coworkers[67] showed that electroosmotic flow, not a pump, can be used to drive the mobile phase through a 1-mm glass LC column packed with 75–175 μm particles. Jorgenson and Lukacs[68] demonstrated the feasibility of using electroosmotic flow and 170 μm i.d. capillaries packed with 10 μm particles, for the CEC separation of a mixture. When CEC is compared to HPLC, better overall resolution, higher efficiency, and lower back pressure, which will allow the use of up to 1 μm silica particles, are obtained with CEC. To date, although CEC has been used for the separation of different groups of compounds, it still has not been fully utilized due to the problems of generating reproducible column inlet and outlet frits and the packing of the capillary column.

Gradient Elution in CE, CEC, and Microfluidics

Gradient elution is routinely used in high performance liquid chromatography (HPLC) to achieve the complete resolution of a mixture that can not be resolved using isocratic elution. Unlike isocratic elution, where the mobile phase composition remains constant throughout the experiment, in gradient elution the mobile phase composition changes with time. The change could be continuous or stepwise, known as *step gradient*. In continuous gradient, the analyst can pick one

of three general shapes; linear, concave, or convex. Gradient elution in HPLC is achieved using two pumps, two solvents, and a solvent mixer. Step gradient is accomplished by periodic change of the mobile phase composition at predetermined times to achieve the resolution of a mixture. In capillary electrophoresis (CE), electroosmotic flow is used in place of a mechanical pump and controls the flow of the mobile phase, in most cases, an aqueous buffer. Electroosmotic flow is controlled by many parameters including buffer type, concentration, ionic strength, pH, and buffer modifiers such as organic solvents, surfactant and polymers, capillary surface chemistry, and applied voltage. Another approach to control electroosmotic flow is by applying an external electric field, which can change the direction and rate of electroosmotic flow by external voltage.

A manual step gradient was used by Balchunas and Sepaniak[69] to separate a mixture of amines by micellar electrokinetic chromatography (MECC). Stepwise gradients were produced by pipetting aliquots of a gradient solvent to the inlet reservoir, which was filled with 2.5 mL of running buffer. A small magnetic stirring bar was used to ensure thorough mixing of the added gradient solvent with the starting mobile phase. The gradient elution solvent was manually added, in four 0.5 mL increments spaced 5 minutes apart, 5 minutes after start of the experiment.

Bocek and his group[70] developed a method for controlling the composition of the operational electrolyte directly in the separation capillary in isotachophoresis (ITP) and capillary zone electrophoresis (CZE). The method is based on feeding the capillary with two different ion species from two separate electrode chambers by simultaneous electromigration. The composition and pH of the electrolyte in the separation capillary is, thus, controlled by setting the ratio of two electric currents. This procedure can be used, in addition to generating mobile phase gradients, for generating pH gradients.[71] Sustacek et al.[72] dynamically modified the pH of the running buffer by steady addition of a modifying buffer. Sepaniak et al.[73–75] produced continuous gradients of different shapes—linear, concave, or convex—by using a negative polarity configuration in which the inlet reservoir is at ground potential and the outlet reservoir is at negative high potential. This configuration allows two syringe pumps to pump solutions into and out of the inlet reservoir. Tsuda[76] used a solvent program delivery system, similar to that used in HPLC, to generate pH gradients in CZE. A pH gradient derived from temperature changes has also been reported.[77] Chang and Yeung[78] used two different techniques, dynamic pH gradient and electroosmotic flow gradient to control selectivity in CZE. Dynamic pH gradient from pH 3.0 to 5.2 was generated by HPLC gradient pump. Electroosmotic flow gradient was produced by changing the reservoirs containing different concentrations of cetylammonium bromide for injection and running.

Capillary electrochromatography (CEC) is a separation technique, which combines the advantages of micro HPLC and CE. In CEC, the HPLC pump is replaced by electroosmotic flow. Behnke and Bayer[79] developed a microbore system for gradient elution using 50–100 μm fused silica capillaries packed with 5 μm octadecyl reversed phase silica gel and voltage gradients, up to 30,000 volts across the length of the capillary. A modular CE system was combined with a gradient HPLC system to generate gradient CEC. Enhanced column efficiency and resolution were realized. Zare and his coworkers[80] used two high voltage power supplies and a packed fused silica capillary, to generate an electro-osmotically driven gradient flow in an automated manner. A mixture of polycyclic aromatic hydrocarbons was resolved in the gradient mode, which were not separated when the isocratic mode was employed. Others[81–84] used gradient elution in combination with CEC to resolve different mixtures. Taylor et al.[82] and Taylor and Teale[83] designed a sampling interface for CEC using a gradient HPLC system to deliver samples and mobile phase to the column. The interface, which consists of a steel tee connector and restriction capillary, was connected to the 30 kV power supply that was continuously purged with mobile phase from the HPLC system and a constant voltage of 25 kV applied throughout the analysis. This instrumental setup was used for the CEC separation of ten corticosteroids.[83]

Multiple, intersecting narrow channels can be formed on a glass chip to form a manifold of flow channels in which CE can be used to resolve a mixture of solutes in seconds. Harrison and coworkers[85] showed that judicious application of voltages to multiple channels within a manifold can be used to control the mixing of solutions, and to direct the flow at the intersection of channels. The authors concluded that such a system, in which the applied voltages can be used to control the flow, can be used for sample dilution, pH adjustment, derivatization, complexation, or masking of interferences. Ramsey and coworkers[86] used a microchip device with electrokinetically controlled solvent mixing for isocratic and gradient elution in MECC. Isocratic and gradient conditions are controlled by proper setting of voltages applied to the buffer reservoirs of the microchip. The precision of such control was successfully tested for gradients of various shapes—linear, concave or convex—by mixing pure buffer and buffer doped with a fluorescent dye. By making use of the electroosmotic flow and employing computer control, very precise manipulation of the solvent was possible and allowed fast and efficient optimization of separation problems.

FUTURE DIRECTIONS

The future of capillary electrophoresis looks very promising. During the last ten years, CE has been evaluated and many theoretical studies have been

carried out. Our group studied the effect of buffer and buffer modifiers on resolution, mobility, and Joules heating[87–98] and, as mentioned above, applied CE not only as a separation technique but to answer physicochemical questions. Today CE is an established and useful microanalytical technique with wide-spread applications. As a matter of fact, CE has grown phenomenally: at least 15 books and hundreds of articles have been published; two specific conferences, HPCE and the Frederick CE Conference, have been held in the United States; and many sessions in major meetings have been devoted to CE theory, instrumentation, and applications. There is no doubt that CE is an acceptable analytical technique. CE has found a strong footing in molecular biology; e.g., determination of DNA fragments, PCR products, and the sequencing of the human genome, in addition to studies of single cells, protein separations, and peptide mapping all use CE.

The future of CE is very bright, as the number of studies, development, and applications continue to multiply. In the next decade, CE will move into ultrafast separations—in seconds, not minutes—using microchip technology; and high efficiency, high-throughput analyses employing narrower and shorter capillaries (5–10 μm i.d. and 1–5 cm long) will be used in array format to achieve high-throughput analyses by CE. Instruments with 96 and 384 capillaries will be produced, not only to sequence DNA and the human genome, but to be used for efficient and simultaneous multisample analysis. The era of analyzing one sample at a time will be out of fashion. We will witness fundamental changes in laboratory instrumentation. For example, market studies already project high growth for "lab-on-a-chip" technologies. A key component of such technology involves forcing fluids to move through a chip's channels; the principles involved are borrowed from CE.

As an analytical technique, CE will become an established method in the pharmaceutical industry, the clinical laboratory, and the forensic laboratory, where high speed and accurate results are required. In the forensic laboratory, CE will be used for DNA typing, which at the present is being done by slab gel electrophoresis, and for gun powder analysis. CE is well suited for the forensic laboratory because of its versatility, small sample requirements, and speed of analysis. Apart from its use as an analytical tool, we envision a continued and expanded role for CE in the determination of physicochemical parameters. Multidimensional separations employing separation–separation and separation–spectroscopic techniques will be routinely used for the separation and identification of complex mixtures, biological and otherwise.

CONCLUSION

It is abundantly clear that CE has gained a respectable place in different fields where analysis is carried out. In this review, we could not possibly include

all the applications that have been done using CE. The reader should refer to the more than 15 books and score of reviews, especially the biannual reviews in Analytical Chemistry, that have been published on the applications of CE.

REFERENCES

1. Tiselius, A. *The Moving Boundary Method of Studying the Electrophoresis of Proteins* (Thesis), Nova Acta Regiae Sociates Scientiarum Upsaliensis 1930, Ser. IV, Vol. 7, Number 4.
2. Hjerten, S. Chromatogr. Rev. **1967**, *9*, 122.
3. Neuhoff, V.; Schill, W.B. Sternbach. Biochem. J. **1970**, *117*, 623.
4. Virtanen, R. Acta Polytech. Scand. (Helsinki) **1974**, *123*, 1.
5. Mikkers, F.E.; Everaerts, F.M.; Verhegen, T.P.E.M. J. Chromatogr. **1979**, *169*, 11.
6. Jorgenson, J.; Lukacs, K.D. Anal. Chem. **1981**, *53*, 1298.
7. Terabe, S.; Otsuka, K.; Ichikawa, K.; Tsuchuya, A.; Ando, T. Anal. Chem. **1984**, *56*, 111.
8. Kuhr, W.G. Anal. Chem. **1990**, *62*, 403R.
9. Camilleri, P. In *Capillary Electrophoresis, Theory and Practice*, 2nd Ed.; Camilleri, P., Ed.; CRC Press: Boca Raton, FL, 1997; 1–22.
10. Issaq, H.J. Electrophoresis **2000**, *21*, 1921.
11. Kohlrausch, F. Ann. Phys. Chem. **1897**, *62*, 14.
12. Jones, W.R.; Jandik, P. J. Chromatogr. **1992**, *608*, 385.
13. Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Capillary Electrophoresis **1994**, *1*, 116.
14. Janini, G.M.; Chan, K.C.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. B **1994**, *657*, 419.
15. Janini, G.M.; Chan, K.C.; Barnes, J.A.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. **1993**, *653*, 321.
16. Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. High Resolut. Chromatogr. **1994**, *17*, 753.
17. Chan, K.C.; Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. **1993**, *653*, 93.
18. Issaq, H.J.; Chan, K.C. Electrophoresis **1995**, *16*, 467.
19. Metral, C.J.; Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. High Resolut. Chromatogr. **1999**, *22*, 373.
20. Janini, G.M.; Metral, C.J.; Issaq, H.J.; Muschik, G.M. J. Chromatogr. A **1999**, *848*, 417.
21. Issaq, H.J.; Chan, K.C.; Janini, G.M.; Muschik, G.M. Electrophoresis **1999**, *20*, 1533.

22. Janini, G.M.; Lukso, J.; Issaq, H.J. J. High Resolut. Chromatogr. **1994**, *17*, 102.
23. Chan, K.C.; Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1993**, *16*, 1877.
24. deGoor, T.V.; Apffel, A.; Chakel, T.; Hancock, W. In *Capillary Electrophoresis of Peptides in Handbook of Capillary Electrophoresis*, 2nd Ed.; Landers, J.P., Ed.; CRC Press: Boca Raton, FL, 1998; 213–258.
25. Janini, G.M.; Fisher, R.J.; Henderson, L.E.; Issaq, H.J. J. Liq. Chromatogr. **1995**, *18*, 3617.
26. Towns, J.K.; Regnier, F.E. J. Chromatogr. **1990**, *516*, 69.
27. Bruin, G.M.; Chang, J.P.; Kuhlman, R.H.; Zegers, K.; Kraak, J.C.; Poppe, H. J. Chromatogr. **1989**, *471*, 429.
28. Towns, J.K.; Regnier, F.E. Anal. Chem. **1991**, *63*, 1126.
29. Jorgenson, J.W.; Lukacs, K.D. Science (Washington, DC) **1983**, *222*, 266.
30. Gilges, M.; Usmann, H.; Kleemiss, H.; Motsch, S.R.; Schomburg, G. J. High Resolut. Chromatogr. **1992**, *15*, 452.
31. Issaq, H.J. Electrophoresis **1997**, *18*, 2438.
32. Issaq, H.J. Electrophoresis **1999**, *20*, 3190.
33. Weinberger, R.; Lurie, I.S. Anal. Chem. **1991**, *63*, 823.
34. Lurie, I.S.; Chan, K.C.; Spartley, T.K.; Casale, J.F.; Issaq, H.J. J. Chromatogr. B **1995**, *669*, 3.
35. Lurie, I.S.; Hays, P.A.; Casale, J.F.; Moore, J.M.; Castel, D.M.; Chan, K.C.; Issaq, H.J. Electrophoresis **1998**, *19*, 51.
36. Chan, K.C.; Alvarado, A.B.; McGuire, M.T.; Muschik, G.M.; Issaq, H.J.; Snader, K.M. J. Chromatogr. B **1994**, *657*, 301.
37. Weinberger, R. Electrophoresis **2001**, *22*, 3639.
38. Righetti, P.G.; Gelfi, C. In *Capillary Electrophoresis of DNA in Analytical Biotechnology*; Righetti, P.G., Ed.; CRC Press: Boca Raton, FL, 1996; 431–476.
39. Issaq, H.J.; Chan, K.C.; Muschik, G.M. Electrophoresis **1997**, *18*, 1153.
40. Dovichi, N.J. Capillary Gel Electrophoresis for Large Scale DNA Sequencing: Separation and Detection. In *Handbook of Capillary Electrophoresis*, 2nd Ed.; Landers, J.P., Ed.; CRC Press: Boca Raton, FL, 1996; 545–565.
41. Huang, X.C.; Quesada, M.A.; Mathies, R.A. Anal. Chem. **1992**, *64*, 967.
42. Oda, R.P.; Bush, V.J.; Landers, J.P. Clinical Applications of Capillary Electrophoresis. In *Handbook of Capillary Electrophoresis*, 2nd Ed.; Landers, J.P., Ed.; CRC Press: Boca Raton, FL, 1996; 639–673.
43. Issaq, H.J.; Delviks, K.; Janini, G.M.; Muschik, G.M. J. Liq. Chromatogr. **1992**, *15*, 3193.
44. Chan, K.C.; Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. **1993**, *653*, 93.

45. Chan, K.C.; Issaq, H.J. *unpublished results.*
46. Chan, K.C.; Lewis, K.C.; Phang, J.M.; Issaq, H.J. J. High Resolut. Chromatogr. **1993**, *16*, 558.
47. Atamna, I.Z.; Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1991**, *14*, 427.
48. Perkins, M.; Zhong, M.; Issaq, H.J.; Davies, M.; Lunte, S. Amer. Assoc. Pharm. Scientists Meeting, Boston, MA, Nov 6, 1997.
49. Cohen, A.S., Terabe, S., Deyl, Z., Eds.; In *Capillary Electrophoresis of Drugs*; Elsevier: Amsterdam, 1996.
50. Chan, K.C.; Muschik, G.M.; Issaq, H.J. Electrophoresis **1995**, *16*, 504.
51. Gassmann, E.; Kuo, J.E.; Zare, R.N. Science **1985**, *230*, 813.
52. Terabe, S. Trends Anal. Chem. **1989**, *8*, 129.
53. Terabe, S.; Ozuaki, H.; Otsuka, K.; Ando, T. J. Chromatogr. **1985**, *332*, 211.
54. Gutman, A.; Paulus, A.; Cohen, A.S.; Grinberg, N., Karger, B.L. J. Chromatogr. **1988**, *448*, 41.
55. Fanali, S. J. Chromatogr. **1989**, *474*, 441.
56. Otsuka, K.; Terabe, S. Chiral Separations by Capillary Electrophoresis and Electrokinetic Chromatography. In *Capillary Electrophoresis Technology*; Guzman, N.A., Ed.; Marcel Dekker, Inc.: New York, 1993; 617–619.
57. Janini, G.M.; Muschik, G.M.; Issaq, H.J. Electrophoresis **1996**, *17*, 1575.
58. Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. B **1996**, *683*, 29.
59. Janini, G.M.; Muschik, G.M.; Issaq, H.J. J. High Resolut. Chromatogr. **1995**, *18*, 171.
60. Janini, G.M.; Muschik, G.M.; Issaq, H.J. In *Capillary Electrophoresis*, Frederick Conference, Frederick, MD, USA, October 23–25, 1995.
61. Issaq, H.J.; Janini, G.M.; Muschik, G.M. HPCE 96, Orlando, FL, USA, January 21–25, 1996.
62. Issaq, H.J. Eastern Analytical Symposium, Somerset, NJ, USA, November 15–20, 1998.
63. Khrapko, K.; Hanekamp, J.S.; Thilly, W.G.; Belenkii, A.; Foret, F.; Karger, B.L. Null. Acid Res. **1994**, *22*, 364.
64. Gelfi, C.; Righetti, P.G.; Trav, M.; Father, S. Electrophoresis **1997**, 724.
65. Li-Sucholeiki, X.-C.; Khrapko, K.; Andre, P.C.; Marcelino, L.A.; Karger, B.L.; Thilly, W.G. Electrophoresis **1999**, *20*, 1224.
66. Issaq, H.J.; Janini, G.M.; Chan, K.C.; Muschik, G.M. Eastern Analytical Symposium, Somerset, NJ, USA, November 16–21, 1997.
67. Pretorius, V.; Hopkins, B.J.; Shieke, J.D. J. Chromatogr. **1974**, *99*, 23.
68. Jorgenson, J.E.; Lukacs, K.D. J. Chromatogr. **1981**, *218*, 209.
69. Balchunas, A.T.; Sepaniak, M.J. Anal. Chem. **1988**, *60*, 617.
70. Popsichal, J.; Deml, M.; Gebauer, P.; Bocek, P. J. Chromatogr. **1989**, *470*, 43.
71. Bocek, P.; Deml, M.; Popsichal, J.; Sudor, J. J. Chromatogr. **1989**, *470*, 309.

72. Sustacek, V.; Foret, F.; Bocek, P. J. Chromatogr. **1989**, *480*, 271.
73. Sepaniak, M.J.; Swaile, D.F.; Powell, A.C. J. Chromatogr. **1989**, *480*, 185.
74. Powell, A.C.; Sepaniak, M.J. J. Microcol. Sep. **1990**, *2*, 278.
75. Powell, A.C.; Sepaniak, M.J. Anal. Instrum. **1993**, *21*, 25.
76. Tsuda, T. Anal. Chem. **1992**, *64*, 386.
77. Wang, C.W.; Yeung, E.S. Anal. Chem. **1992**, *64*, 502.
78. Chang, H.-T.; Yeung, E.S. J. Chromatogr. **1992**, *608*, 65.
79. Behnke, B.; Bayer, E. J. Chromatogr. **1994**, *680*, 93.
80. Yan, C.; Dadoo, R.; Zare, R.N.; Rakestraw, D.J.; Anex, D.S. Anal. Chem. **1996**, *68*, 2726.
81. Schmeer, K.; Behnke, B.; Bayer, E. Anal. Chem. **1995**, *67*, 3656.
82. Taylor, M.R.; Teale, P.; Westwood, S.A.; Perrett, D. Anal. Chem. **1997**, *69*, 2554.
83. Taylor, M.R.; Teale, P. J. Chromatogr. A **1997**, *768*, 89.
84. Gfrorer, P.; Schewitz, J.; Psecker, K.; Tseng, L.-H.; Albert, K.; Bayer, E. Electrophoresis **1999**, *20*, 3.
85. Seller, K.; Fan, Z.H.; Fluri, K.; Harrison, J. Anal. Chem. **1994**, *66*, 3485.
86. Kutter, J.P.; Jacobson, S.J.; Ramsey, J.M. Anal. Chem. **1997**, *69*, 516.
87. Issaq, H.J.; Atamna, I.Z.; Metral, C.J.; Muschik, G.M.; J. Liq. Chromatogr. **1990**, *13*, 1247.
88. Atamna, I.Z.; Metral, C.J.; Muschik, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1990**, *13*, 2517.
89. Atamna, I.Z.; Metral, C.J.; Muschik, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1990**, *13*, 3201.
90. Issaq, H.J.; Atamna, I.Z.; Muschik, G.M.; Janini, G.M. Chromatographia **1991**, *32*, 155.
91. Atamna, I.Z.; Issaq, H.J.; Muschik, G.M.; Janini, G.M. J. Chromatogr. **1991**, *588*, 315.
92. Janini, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1992**, *15*, 927.
93. Issaq, H.J.; Janini, G.M.; Atamna, I.Z.; Muschik, G.M.; Lukszo, J. Liq. Chromatogr. **1992**, *15*, 1129.
94. Janini, G.M.; Issaq, H.J. In *The Buffer in Capillary Zone Electrophoresis in Capillary Electrophoresis: Theory, Methodology and Applications*; Guzman, N.A., Ed.; Marcel Dekker, Inc.: New York, 1993; 119–160.
95. Janini, G.M.; Chan, K.C.; Barnes, J.A.; Muschik, G.M.; Issaq, H.J. Chromatographia **1993**, *35*, 497.
96. Janini, G.M.; Chan, K.C.; Muschik, G.M.; Issaq, H.J. J. Liq. Chromatogr. **1993**, *16*, 3591.
97. Janini, G.M.; Chan, K.C.; Barnes, J.A.; Muschik, G.M.; Issaq, H.J. J. Chromatogr. **1993**, *653*, 321.
98. Issaq, H.J.; Janini, G.M.; Chan, K.C.; Rassi, Z.E. Approaches for the Optimization of Experimental Parameters in Capillary Electrophoresis. In

Advances in Chromatography; Brown, P.R., Grushka, E., Eds.; Marcel Dekker, Inc.: New York, 1995; Vol. 35, 101–170.

Received January 5, 2002
Accepted January 23, 2002
Manuscript 5721