Trends in Capillary Electrophoresis: 1997

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Abstract

This article is intended to present an overview of developments in the field of capillary electrophoresis (CE) and its application to the analysis of micro-environments. Instrumental developments in injection and detection methods and the separation chemistries are outlined. Emphasis is placed on methods and means that have significantly improved the capability of CE. Subsequently, several selected applications to the exploration of microenvironments such as CE-based sensors, CE on microchip, and single cell analysis are described. The recent advancements in these areas are highlighted.

Introduction

Electrophoresis was first addressed in a doctoral thesis by Arne Tiselius in 1930 and published in scientific literature in 1937 (1). It states that positive ions under an electrical field are attracted by the cathode, and negative ions are attracted by the anode. The electrophoretic mobility (μ_{ep}) is proportional to the charge-to-size ratio of the ions, which is expressed as:

$$\mu_{ep} = \frac{q}{6\pi\eta r} \qquad \text{Eq 1}$$

where q is the net charge of a solute, r is the hydrodynamic radius of the ion, and η is the viscosity of the medium. The migration velocity equals the product of μ_{ep} and an electrical field E ($V_{ep} = \mu_{ep}E$). A mixture of components with different μ_{ep} values is thus separated.

This principle was first applied to form a slab electrophoresis, typically for the analysis of macromolecules. Separation was performed on a plate covered

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with porous gel, and discrimination was based on molecular sieving. The analysis usually took several hours. This method had been dominant in the field of macromolecular analysis until the 1980's when capillary electrophoresis (CE) caught wide attention due to a paper by Jorgenson and Lukacs, in which a highly efficient separation and sensitive analysis of amino acids with CE was reported (2).

The performance of electrophoresis in a narrow fused-silica tube (internal diameters less than 75 µm) has granted electrophoresis extremely high power in terms of high separation efficiency, fast response time, and very low sample volume requirement (less than 50 nL). The low sampling volume makes CE an ideal microscale analytical technique for probing microenvironments. The detection of chemical components at the single-cell level and the analysis of single-enzyme molecules have been possible with CE. It is also effective for the analysis of a large range of compounds from small ions and neutral molecules to macromolecules. The application covers food, environmental, biochemical, and pharmaceutical analyses as well as pharmacological studies and shows great promise in biofluid analysis and clinical diagnosis. Many excellent reviews, books, and papers on this topic have been published in recent years (3–5). The introduction of the first commercial CE instrument in 1988 greatly sped up CE research.

Figure 1 is a schematic diagram of the basic components of a CE instrument. The ends of a narrow-bore, fused-silica capillary are placed in buffer reservoirs. The reservoirs are filled with the buffer electrolyte. The electrodes are immersed in the reservoirs to make electrical contact between the high-voltage power supply and the capillary. Sample is loaded into the capillary by replacing one of the reservoirs with a sample reservoir and applying either an electrical field (electromigration injection) or an external pressure (hydrostatic injection). After replacing the sample reservoir, the electrical field is applied, and the separation is performed. Detection can be made at the opposite end directly through the capillary wall or by coupling the capillary with other detection techniques such as mass spectroscopy or electrochemical detection through specially designed interfaces.

Upon contacting the buffer electrolyte, the acidic silanol groups at the surface of the fused-silica capillary undergo dissociation, which results in a negatively charged capillary wall and, consequently, the formation of electrical double layers. Close to the surface is a layer of cations that are adsorbed on the surface and essentially fixed. The remaining cations form a diffuse layer in which the cations exchange continuously with those in the rest of the solution. When an electrical field is applied across the capillary, the cations in the diffuse layer will move towards the cathode, causing the liquid within the capillary to move at a constant velocity. The process is called electroosmosis, and the electroosmotic mobility (μ_{eof}) is:

$$\mu_{eof} = \frac{\varepsilon\zeta}{4\pi\eta} \qquad \text{Eq 2}$$

where ε is the dielectric constant of the medium, η is the viscosity of the medium, and ζ is the zeta potential across the solid–liquid interface.

Therefore, within the capillary, ionized species will actually migrate with a velocity resulting from the sum of their intrinsic mobility and the mobility of electroosmotic flow (EOF):

$$V = (\mu_{ep} + \mu_{eof})\, E \qquad \text{Eq 3}$$

As a result, anions can be dragged to move in the same direction with the cations if the magnitude of the EOF is greater than the mobilities of anions. Neutral compounds will migrate together at the velocity of the EOF (EOF = $\mu_{eof} E$).

A large volume of work related to CE has been carried out in recent years, but this review is not intended to cover all the details. First we will give a general introduction to the field of CE, followed by its instrumental developments including injection and detection methods and then separation chemistries. Emphasis will be placed on methods and means that have significantly improved the capability of CE. Subsequently we will address several applications of CE to the exploration of microenvironments such as a single cell. Development of these techniques and methodologies may be critical to the solution of current important issues in micro total analysis systems and biosciences. It is also our intention to show a trend of CE development in the sense that electrophoresis-based methods have been moved from the slab through the capillary to the microchip. Very fast analyses (within a few seconds) can be pursued with CE on chips.

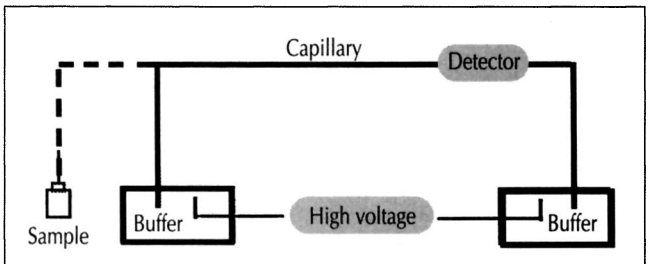

Figure 1. Schematic diagram of the basic components of a CE instrument.

Sample introduction techniques

Basically two types of injection techniques have been developed for CE: hydrodynamic and electromigration injections. They are available in almost every commercial instrument. Two reviews can be referred to for the technical details (6–7). Various modifications based on these techniques have been made in order to couple CE to other analytical techniques or to fulfill special analysis purposes. Interfaces were developed for coupling microdialysis (8), liquid chromatography (9), and supercritical chromatography to CE (10). However, these designs have not yet advanced beyond the research and development stage to commercial products.

Hydrodynamic injection

Hydrodynamic injection is the most widely used sample introduction method in CE. Sample is introduced to the capillary by a pressure difference maintained between the ends of the capillary. This is realized by imposing pressure at the injection end of the capillary, a vacuum at the exit end of the capillary, or the siphoning action obtained by elevating the injection reservoir relative to the exit reservoir. The volume of sample injected

is a function of the pressure difference across the capillary, the injection time, and the capillary internal radius. Either the injection time or the pressure difference is used in practice as a variable factor to adjust the injected amount of sample. With hydrodynamic injection, the quantity of sample loaded is independent of the sample matrix. Therefore, direct calibration with standard solutions is possible. It should be noted that hydrodanamic injection is incompatible with fixed, gel-filled capillaries and rigid sample matrices, which require electromigration injection.

Electromigration injection

Electromigration injection is performed by replacing the injection-end reservoir with the sample vial and applying a voltage across the capillary. Usually a field strength 3–5 times lower than that used for separation is applied. Analyte ions enter the capillary by both migration and the action of EOF. Therefore, discrimination occurs for ionic species; the more mobile ions are loaded to a greater extent than the less mobile ones. Neutral molecules are swept into the capillary by EOF. It should be pointed out that sample loading depends on the EOF and the mobility, which can change due to differences in the sample matrices. As a result, electromigration is not as reproducible as hydrodynamic injection. Quantitation with electromigration injection therefore requires an internal standard.

Other injection techniques

It has been observed that a typical CE separation lasts from 5 to 30 min, which is fast with respect to many other analytical techniques but slow relative to many chemical events. The capability of using CE to monitor a dynamic chemical system was exploited by developing an optically gated sample injection (11). The components in a mixture were first tagged with a fluorescent molecule and then introduced into the capillary. Near the entrance of the capillary, a laser is used to photodegrade the tag and thus render the material undetectable to a fluorescence detector that is located further along the capillary. A sample zone is generated by momentarily switching off the laser and thereby allowing a small amount of tagged material to pass intact. Because the sample modulation is optical rather than mechanical, temporally narrow plugs of material can be introduced into the capillary. The injection can be made while the capillary is maintained at the separation voltage. Analysis of a mixture of fluorescein isothiocyanate-labeled amino acids could be completed within 1.5 s.

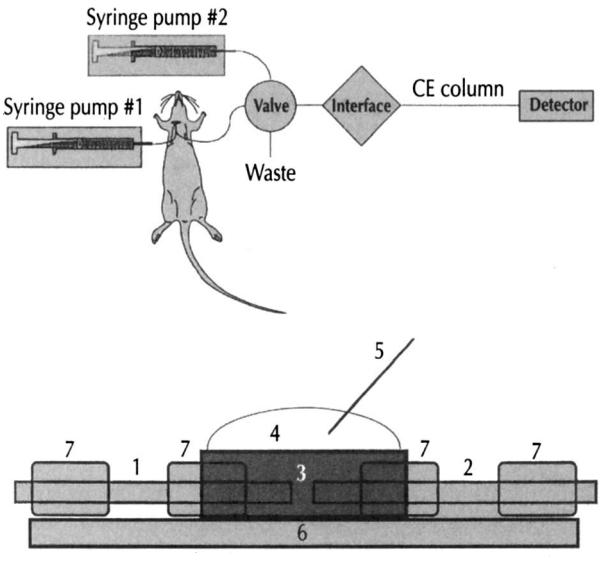

Figure 2. (A) Schematic of an on-line microdialysis CE system and (B) the injection interface. 1, transfer capillary; 2, CE capillary; 3, CE run buffer reservoir; 4, CE run buffer; 5, ground electrode; 6, microscope slide; 7, guiding tubing. *(Reproduced with permission from reference 8.)*

Moving boundary sample introduction (12) is an alternative to the previous zonal injection methods for monitoring reaction-based chemical analysis. The capillary is initially filled with the analyte solution while the fast-migrating reagent is maintained at the inlet reservoir. Upon application of an electrical field, the analyte and reagent will merge due to differences in their electrophoretic mobilities. The reaction proceeds within the capillary. The reaction product is transported rapidly to the detector under the influence of an applied potential. This method allows the chemical analysis to be performed and detected entirely on-column and is termed electrophoretically mediated microanalysis (EMMA). EMMA was demonstrated for the complexometric determination of inorganic ions (13) and analysis of both enzyme and substrates (14). For enzyme reaction, shutdown of the potential after the merging of enzyme and substrate zones allowed for the detection of single lactate dehydrogenase (LDH) with laser-induced fluorescence (LIF) (E. Yeung. Personal communication, 1994.). This method was recently applied for a 24-s kinetic determination of leucine aminopeptidase (15).

A microloop injection system was also developed for quantitative injection to CE (16). A wire loop was deployed at the tip of a capillary on which a thin film of liquid was formed. The loop was transferred to a sealed chamber, and a pneumatic pressure was applied to introduce the contents of the loop into the capillary. The sample surface tension and viscosity did not have a significant effect on the injected volume.

The coupling of microdialysis with CE enables direct in vivo monitoring of low-molecular-weight analytes present in the extracellular space of essential tissues. A diagram of the on-line microdialysis–CE system is presented in Figure 2 (8). The overall system is based on a rotary microinjection valve and a specially designed injection interface. The microdialysate sample flows directly from the experimental animal into the sample loop of the microinjection valve. The valve converts the continuous flow of the dialysate into discrete plugs, which are sent to the CE interface by the flow of the CE run buffer from pump 2. The sample plug is pumped out of the transfer line of the interface and reaches the injection end of the separation capillary. Electromigration injection is then performed.

Microdialysis–CE is most common for the monitoring of neurotransmitters such as neuroamines in the central nervous system. Recently its usefulness was also addressed for the monitoring of drugs and endogenous compounds in systemic fluids and tissues for pharmacokinetics studies (17). Coupling CE to microdialysis in these applications is advantageous due to the

fast separation allowed. A microdialysis–micellar electrokinetic chromatographic (MEKC) system provided an injection precision of 2% relative standard deviation and facilitated in vivo analysis with a 90-s temporal resolution (8).

Sensitivity enhancement

Enhancement in sensitivity represents a major challenge in current CE research. Modification on the injection methods offers one way to cope with the problem. Different strategies have been applied, such as isotachophoresis and electrophoretic stacking. Use of chromatographic packing materials and membranes as preconcentrators facilitates the introduction of samples in large amounts. On-line microreactors for sample treatment and derivatization were also developed. Among these techniques, isotachophoresis and electrostacking are easily implemented in commercial instruments.

Isotachophoresis

Isotachophoresis (ITP) involves the segregation of analytes into zones that are sandwiched between leading and tailing electrolytes (LE and TE). Because the current must be constant throughout the capillary, the electric field varies within each zone to maintain constant velocity. Note that the concentration in each zone is constant and is determined by the concentration of LEs, which is usually in the 0.1M range. Very dilute samples can thus be concentrated into bands that approach the concentration of the LE. For anion analysis, highly mobile chloride is often used as the LE, whereas ions with lower mobility than the analyte anions are chosen as the TE. For cation analysis, potassium and ammonium ions are usually used as the LEs; low-mobility cations such as various amino acids are usually used as the TEs. No EOF is allowed, so that capillaries with internal coating are required for this operation mode. It should be noted that only one type of ion, either cations or anions, can be concentrated and subsequently analyzed in one run. This technique can lead to concentration factors surpassing 10. Various methods have been developed to implement ITP in CE analysis (18–19).

Electrophoretic stacking

Mikkers et al. first reported the concentration of analyte from a sample matrix with a lower conductivity than the running buffer in 1979 (20). Chien and Burgi extensively practiced the principle (21). Since then, sample concentration with electrophoretic stacking has been increasingly employed to achieve sensitivity enhancement. So far, 10–1000-fold sensitivity enhancement has been reported with this technique. The principles and technical details have been reviewed by Chien and Burgi (21). When the conductivity of the sample is lower than the conductivity of the running buffer, there is a greater voltage drop across the sample plug. The voltage drop consequently leads to an amplified field in which analyte ions move with a greater velocity through the sample plug. When they reach the sample plug–running buffer interface, they encounter a much lower electric field, and the magnitude of their velocity decreases dramatically, resulting in a concentration of analyte ions in a thin layer at the surface. Anions and cations focus at different sides of the sample plug; neutral compounds experience no focusing.

A method to inject very large sample volumes (as much as two thirds of the capillary) was proposed by Chien and Burgi (22). After hydrodynamic injection of the sample, the capillary was returned to the buffer, and a voltage was applied in a reversed mode. The analyte anions experienced a stacking at the sample–running buffer interface. Simultaneously, electroosmotic flow pumped water plug out of the capillary. When the monitored current reached 90% of the value previously measured for the running buffer alone, the voltage was turned off, and the polarity was reversed. Automatic polarity switching is available in commercial CE instruments. To concentrate cations with this method, it was necessary to use a capillary with an immobilized positive charge. A procedure to concentrate anions and cations simultaneously was also developed (23).

However, a bias is associated with the polarity switching when the ratio of injection volume to column volume exceeds the ratio of electroosmotic flow to ion mobility. The solution to this problem was attempted by employing an electroosmotic flow modifier (e.g., diethylenetriamine [DETA]) as a pump (24). A 100-fold improvement in sensitivity for the analysis of inorganic anions was achieved without switching polarity.

Recently a head-column field-amplified sample injection was developed for the analysis of positively chargeable and hydrophobic compounds; a 1000-fold enhancement in sensitivity was obtained (25). The sample stacking performed in a binary system requires an initially introduced low-conductivity zone (water plug) with more than 1-mm length, a sample injection voltage less than 20 kV, and an injection time interval less than 60 s.

Chromatographic preconcentration methods

The topic of on-line preconcentration with chromatographic methods has received considerable attention recently. This technique enables the analysis of sample amounts in vast excess of the total capillary volume, leading to a significant improvement in CE detectability.

One way to implement the chromatographic preconcentration methods is to fill the inlet of a separation capillary with chromatographic packing materials (C_8 or C_{18} phase), which are fixed by porous glass frits. The procedure involves initial conditioning of the capillary, hydrodynamic introduction of sample, washing of adsorbed analytes, and transfer of the sample to the capillary. The method's feasibility has been demonstrated by chiral separation of low-concentration verapamil (VER); a detection limit 200 times lower was obtained. It was performed to determine VER in human plasma (26). A T-connected capillary technique to concentrate propranolol from serum was also reported. The initial segment of a capillary was filled with protein-coated octadecylsilane (ODS) silica gel. Up to 1 µL of sample could be injected without loss of resolution. The sensitivity was increased by two orders of magnitude (27).

A short capillary with a modified inner surface was also used as a preconcentrator, which was connected to a separation capillary (28–29). Dilute samples of herbicides loaded to the concentration capillary were bound to a C_{18}-coated surface by hydrophobic interaction. A 10–35-fold increase in concentration resulted (28). A modified inner surface with the reaction between iminodiacetic acid and zinc was reported for the

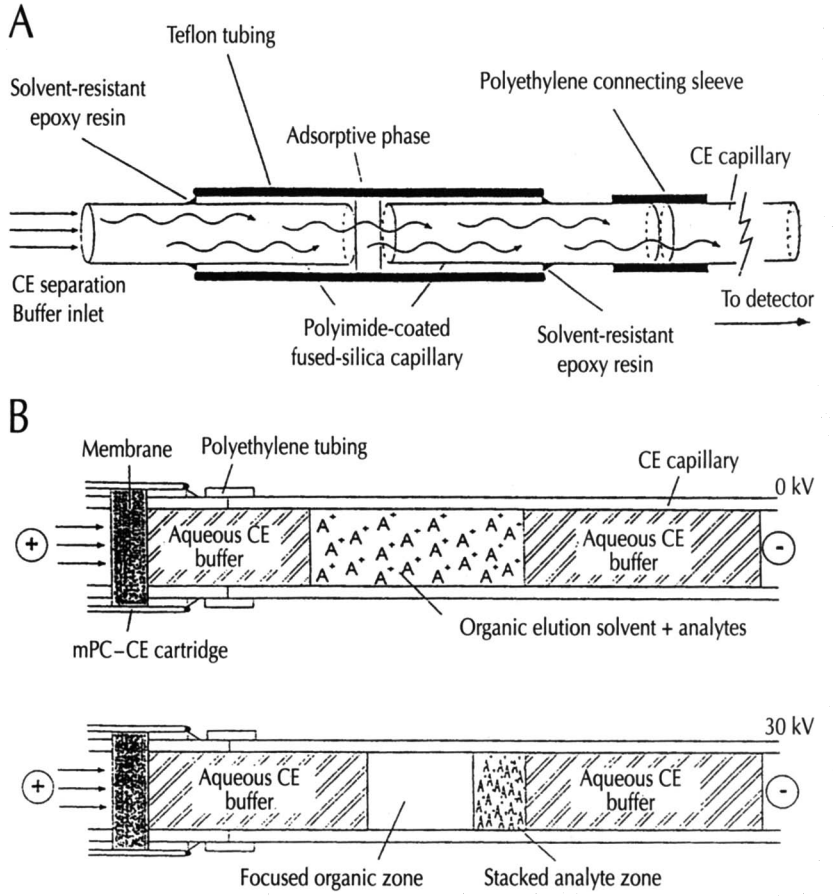

Figure 3. (A) Schematic of a membrane mPC–CE cartridge connected to a conventional CE capillary at the inlet. (B) Schematic of analyte stacking of compounds for use in conjunction with mPC–CE after analytes have been eluted from the membrane and the CE voltage has been applied. *(Reproduced with permission from reference 33.)*

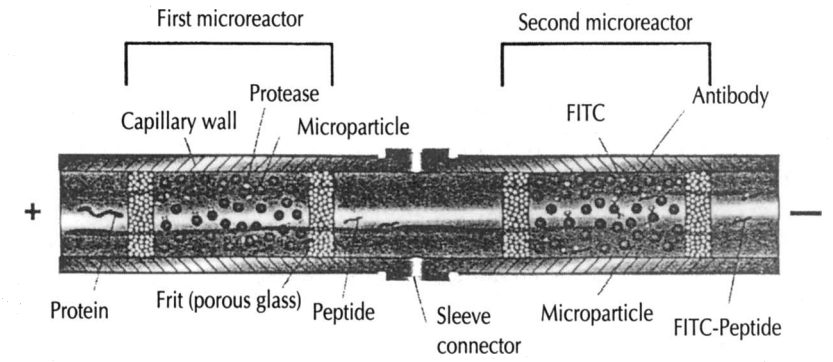

Figure 4. Schematic of two consecutive microreactors. Two microreactors are placed in tandem (and connected by the sleeve connector) to enable the cleavage of the prolyl hydroxylase α-subunit at the first microreactor (150-µm i.d.) and then the derivatization of the generated peptides at the second microreactor (75-µm i.d.). *(Reproduced with permission from reference 36.)*

concentration of metal binding proteins with 25-fold sensitivity enhancement (29). A selective immunoaffinity concentration on a protein G chromatographic support material resulted in a preconcentration enhancement of 1000-fold (30).

The use of an on-line impregnated membrane as a preconcentrator was reported by Naylor's group (31,32). The membrane was installed in a Teflon cartridge located between a short capillary and a separation capillary with Teflon tubing. The analytes loaded onto the capillary were absorbed by the membrane, which was then washed with separation buffer to remove salts. Ultimately, analytes were eluted from the membrane with a minimum of organic solvent, and the CE voltage was subsequently applied (see Figure 3). The membrane was used to remove salts and buffers that were used to solubilize biologically derived mixtures. Cleanup and preconcentration of the sample were performed entirely on-line. An added advantage of using the membrane concentrator was the sample stacking that occurred because the analyte was in a low conductivity solution relative to the separation buffer after being eluted from the membrane (33).

On-line microreactor

On-line coupling of chemical reactions has been performed for derivatization and enzymatic digestion of samples prior to CE analysis. An enzyme-catalyzed microreactor coupled to CE for peptide mapping was developed (34). The reactor could also be used to create fluorescent derivatives of primary amines (35). A schematic of two microreactors placed in tandem is presented in Figure 4 (36). Prolyl hydroxylase α-subunit experienced the cleavage at the first microreactor, and the resultant peptides were then derivated at the second microreactor. On-line microreaction analyte concentrators for consecutive protein digestion and peptide derivatization were reviewed recently (36).

Separation modes

Capillary zone electrophoresis

Capillary zone electrophoresis (CZE) is the basic operation mode of CE and has been employed in 60% of CE analyses. Analytes suitable for CZE include metal ions, inorganic anions, organometallics, small organic acids and bases, peptides, and proteins. A prerequisite to CZE application is that the analytes, which migrate under an applied field, have to be charged or chargeable. The difference in migration results mainly from the different charge densities of the ionic species, which are essentially affected by the pH of the buffer electrolyte. In most cases, an optimization can be achieved by adjusting the buffer pH. When the pH change does not facilitate a satisfactory separation, selectivity can be obtained by the addition of additives into the buffer electrolyte. The additives

include ion-pairing agents, complexing agents (polycarboxylic acids and chelating agents), organic solvents (37), polycyclic ethers and their derivatives, and various chiral selectors. Yang et al. established a theoretical migration model for inorganic cations (38). With this model, the effective μ_{ep} was described in terms of absolute μ_{ep}, complex formation constants, concentration of complexing reagents, and organic solvent in the buffer; the separation could be optimized. A review summarized the separation mechanisms and optimization methods for the analysis of small ions with CE (39).

Chiral discrimination with CZE is an important and tremendously successful application area (40–41). A racemate mixture can be resolved by the addition of chiral selectors to the buffer electrolyte. If these two compounds interact differentially with the chiral selector, their electrophoretic mobilities should be altered to different extents, and the separation should be achieved. Cyclodextrins (α-CD, β-CD, and γ-CD) are the most commonly used chiral selectors, although their derivatives (i.e., sulfated β-CD [44] and sulfobutyl ether β–CD [43]) were found to be superior to the neutral CD selectors for the separation of a variety of chiral compounds. Other chiral selectors are crown ethers and their derivatives, microcyclic antibiotics (44,45), chiral ligand-exchange complexes (46), noncyclic oligosaccharides and polysaccharides (47), and proteins (48). A theoretical model was developed to be used for the determination of thermodynamic parameters and separation optimization (49). The maximum electrophoretic mobility difference was predicted when the concentration of free selector equaled the reciprocal of the average binding constant. Selectivity was found to be in the reverse order of binding constants. A multiequilibria-based model was developed to account for separation of chiral acids and bases as a function of both pH and CD concentration (50,51).

CZE was also a possible separation mode for the analysis of carbohydrates, which was outlined in a number of recent reviews (52–54). These compounds are normally uncharged, so borate complexation was often carried out to impart charge into the compounds. Successful separation was demonstrated for the separation of mono- and oligosaccharides. Although some glycans can absorb ultraviolet (UV) light at around 200 nm, high-sensitivity detection requires labeling these analytes with a suitable chromophore or fluorophore. For this purpose, reductive amination with 2-aminopyridine and aminonaphthalenes was usually performed at the reducing end of the carbohydrates followed by reduction with sodium cyanoborahydrades. However, the derivatization caused a problem with the excess of reagent, which interfered with the CE analysis. This problem could be alleviated by using 2-aminoacridone as the derivative reagent, because the excess reagent and the derivatives could be separated with MEKC (55).

MEKC

MEKC was introduced by Terabe in 1984 (56). Books and reviews are available on this subject (57). It is distinguished for its capability to deal with the separation of electrically neutral molecules. The key for MEKC is the addition of a surfactant into the buffer electrolyte. When the concentration of surfactant is above its critical micelle concentration (CMC), aggregates of surfactant molecules form the micellar phase, which acts as a pseudo-stationary phase. The differences in distribution constants of charged and neutral compounds into the micellar phase lead to successful separation. MEKC is also used for the separation of ions because it provides selectivity in addition to electrophoresis and is typically useful for compounds with the same charges and similar structures, though these compounds may have the same or very similar electrophoretic velocities and are not separable with CZE. Interaction of the analytes with the micellar phase is determined by the hydrophobic properties of the analytes.

Important factors in surfactant selection are the length of hydrophobic alkyl chain, the electric charges, and the structure of the hydrophilic group. A longer alkyl chain increases the interaction between the hydrophobic analytes but rarely changes the separation factor. In addition, the long chain causes a high Kraft point so that high temperatures are required for the operation. Surfactants with short alkyl chains have high CMCs and high conductivity; therefore, Joule heating causes band-broadening. A dodecyl group is often preferred. A hydrophilic group affects the selectivity of micelles. Ionic surfactants with opposite charges of the analytes interact strongly, and the tendency increases as the hydrophobicity of the analytes increases. As a result, the capacity factor is too high to achieve sufficient resolution. Nonionic surfactants do not separate electrically neutral compounds. However, they tend to have weaker interaction with peptides than ionic surfactants and are therefore more suitable for the separation of peptides. Both nonionic and zwitterionic surfactants have low conductivities, which is advantageous in terms of low Joule heating and the possibility of using high concentrations of surfactants. For enantiomer discrimination, chiral surfactants are useful.

MEKC is suitable for light-to-medium hydrophobic compounds. Analyses have been performed with inorganic ions, phenols, organometallics, amino acids, carbohydrates, peptides, proteins, polynuclear aromatic hydrocarbons, and most herbicides and pesticides, which are difficult to handle with CZE. For some larger peptides and proteins, modifiers such as organic solvents, cyclodextrins, and urea have to be added to the buffer electrolyte in order to increase the aqueous solubility of the analytes by reducing the interaction with the micellar phase. A similar problem with overly strong association of highly hydrophobic compounds in MEKC was also encountered. The addition of organic solvents to the buffer electrolyte was a solution, but the concentration of organic solvents was very limited due to the damage of micelles. Yang et al. reported their solution with the use of an ionic polymer to replace the surfactant (58). The ionic polymer can be viewed as covalently bonded micelles with zero CMC. As a result, the separation allowed the use of high contents of organic solvents.

Capillary gel electrophoresis

Capillary gel electrophoresis (CGE) is performed in a polyacrylamide or agarose gel-filled capillary, and separation is based on molecular sieving by the porosity of the gel. It is a major separation mode for the analysis of proteins, oligonucleosides, and DNA fragments. These large biopolymers have a constant charge–size ratio and are thus not separated with

CZE and MEKC. Although it is no doubt a good alternative to traditionally used slab gel electrophoresis and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) in terms of highly efficient separation and short analysis time, several problems are associated with the separation mode (e.g., gel breakage, gel degradation by hydrolysis, particularly at the alkaline pH commonly used to separate the biopolymers, clogging of the injection end, and short lifetime of the capillary).

Non-gel sieving CE was introduced to overcome these problems (59). In non-gel sieving, water-soluble linear polymers such as methylcelluloses, dextrans, and polyethylene glycol are used in the separation buffer. The polymer solution can be replenished at the beginning of each analysis, enabling a capillary to be used for up to hundreds of runs with good reproducibility. A difference from slab gels is that entangled polymer solutions are preferred to crosslinked gels. The principles of size-based separation in polymer solutions have been reviewed recently (60).

Figure 5 shows a simultaneous separation of myoglobin molecular mass markers (molecular weight range: 2512–16949) using CGE (61). Separation was completed within 13 min in a buffer composed of 12% dextran and 10% glycerol (for slowing down the diffusion of peptides). This method was much simpler and saved much analysis time compared with slab gel electrophoresis (12 h).

Affinity CE

Affinity means that two or more components interact specifically in the form of hydrogen binding, electrostatic interaction, hydrophobicity, and van der Waals force. CE processes involving affinity effects between solute and buffer additive have been termed as affinity CE (ACE), which is the subject of a recent review (62).

One major application of ACE is the determination of physicochemical parameters. Binding constants are measured for metal–protein complexes (63), enzyme–inhibitor interaction (64), molecular recognition by carbohydrate (65), DNA–peptide interactions (66), antibody–antigen interactions (67), and protein–drug association (68), etc. A recent review highlighted the determination of drug–protein interactions by using CE (69). In addition, the association between micelles and solutes (70) and the association between cyclodextrins and chiral molecules (71) are also classified into this category, and the relevant constants are obtained with ACE. Furthermore, ACE is a useful separation mode for chiral drug discrimination (72–74) as well as for the study of combinatorial peptide libraries (75,76), protein surface structure, and structure–function relationships (77). The number of studies on ACE has increased in recent years.

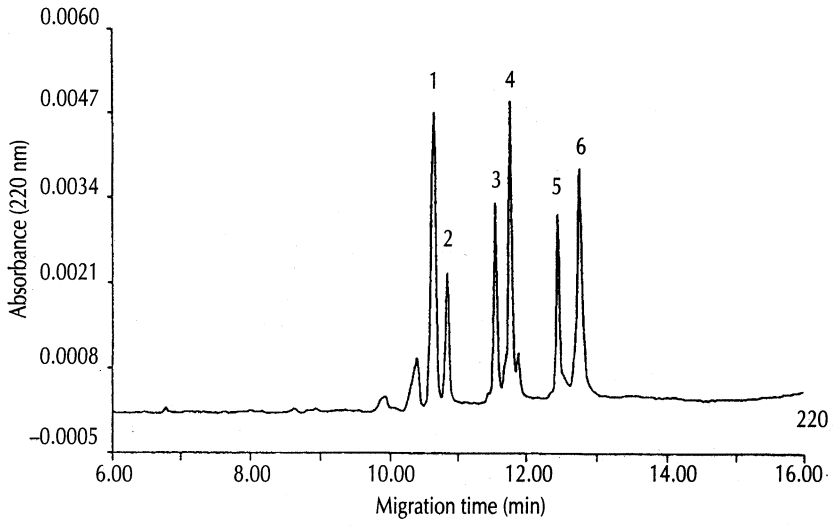

Figure 5. Electropherogram of peptides in non-gel-sieving CE. Experimental conditions: 24 cm x 50-µm i.d. uncoated capillary; myoglobin molecular weight, 2152–16,949 BDH; buffer, 0.4M *tris*-borate, 0.1% SDS, 10% glycerol, and 12% dextran (molecular weight: 2,000,000); pH 8.3; voltage, 12 kV; temperature, 20°C. Peaks: 1, Globin III (132–153); 2, Globin II (1–55); 3, Globin I, (56–131); 4, Globin I + III (56–153); 5, Globin I + II (1–131); 6, Globin (1–153). *(Reproduced with permission from reference 61.)*

Other separation modes

Isotachophoresis (ITP) is currently used more as an on-line concentration means than as a separation method. The principles of ITP and its application for on-line sample concentration have been described above.

Capillary isoelectric focusing (CIEF) involves the creation of a static pH gradient within the capillary that causes all analytes to migrate to their respective isoelectric points. Compounds may be eluted from the capillary or detected in situ in their respective zones. It is an alternative to conventional electrofocusing and chromatofocusing for the determination of isoelectric points (p*I*) of proteins. Yao et al. determined the p*I* values of acidic and basic proteins with CIEF (78).

Capillary electrochromatography (CEC) uses the fused-silica tubing, which is either packed with spherical stationary phase (packed CEC) or coated with some type of adsorbent (open tubular CEC) on its inner surface. Electroosmotic flow sweeps eluent and sample through the capillary, and separation occurs because of the partitioning of the analytes between the stationary phase and the mobile phase in addition to differences in electrophoretic mobility. The flatter flow profile contributes to improved separation efficiency compared with pressured-driven chromatography, but it is only significant for very low capacity factors. Some problems include bubble formation and poor reproducibility for electroosmotic flow and capacity factors. Approaches to eliminate bubbles include the use of low-conductivity buffers (79) and the introduction of a pressurized flow generated by an LC pump (80). Separation was demonstrated for basic amines and small biomolecules such as β-agonists. Although reports on this separation mode are still limited, CEC has been viewed as a potential alternative to capillary LC with better mass transfer kinetics and efficiency.

Detection methods

Lack of sensitivity has been a major limitation in CE. The small capillary dimensions encountered in CE and the minuscule zone volumes create a system in which sensitive

detection of solute zones without introducing zone dispersion is a major challenge. Also because of this reason, detection in an on-column configuration is more suitable for CE and easily implemented. Although UV detection is still the most commonly used detection mode for CE due to its relatively universal nature, fluorescence and mass spectrometry (MS) detectors have received increasing attention, and significant advancements have been made in recent years. Fluorescence detection has attained a detection limit of 10^{-11}M, which is the reason for its wide acceptance in biochemistry, pharmaceutical, and pharmacokinetics studies as well as biomedical research. Various electrochemical detection methods have also been studied extensively. Fluorescence detectors, electrochemical detectors, and CE–MS interfaces are commercially available. New detectors such as chemiluminescence detectors, refractive index, thermooptical absorbance detectors, and nuclear magnetic resonance (NMR) were also reported to be feasible for CE detection.

UV–visible absorbance detection

UV–visible (UV–vis) detection is applicable to a wide spectrum of compounds, including proteins. However, the usual concentration detection limit obtained with this detection mode is 10^{-4} to 10^{-5}M, which hardly fulfills the requirement in trace analysis. The design of a bubble cell capillary and Z-shaped detection cell aims to increase detectability by enhancing the optical path length (81). Sensitivity increases of 10-fold were usually claimed with the designs. Detection is also carried out through the use of diode array and fast scanning detection schemes.

For compounds without intrinsic absorbance, derivatization procedures in the form of precapillary, postcolumn, and on-line complexation were developed. On-column complexation with 4-(2-pyridylazo)resorcinol following sample stacking produced a detection limit of 1×10^{-8}M for Co^{2+}, Fe^{2+}, and Zn^{2+} (82). Alternatively, indirect detection has been utilized for the analysis of inorganic ions, amino acids, amines, polyamines, organic acids, aliphatic surfactants, and sugars, etc. The key to indirect detection is the selection of a suitable UV background-absorbing substance with a high absorbance capability and the same charge as the analytes. Imidazole and chromate have been very commonly used for the detection of inorganic cations and anions, respectively. Indirect detection of anions and cations with organic dye additives achieved a sensitivity 100 times better than the best previously reported data (83).

Fluorescence detection

Fluorescence detection is also easily adapted to CE in an on-column configuration. Fused silica is the most suitable material, due to its light cut-off near 170 nm and low luminescence properties. Excitation radiation, either from an arc lamp or a laser, is focused onto a section of the capillary where the polymer coating has been removed. The resulting fluorescence is collected at an angle of 90° relative to the excitation beam. Capillary diameters of 25–75 µm are compatible with this detection mode. Major studies in this category focus on the excitation sources and derivatization chemistry. Laser-induced fluorescence is much preferred because of its great gain in sensitivity. A trend can be observed to apply diode lasers, which are inexpensive, small, and long-lasting, and are expected to facilitate the construction of a miniaturized CE system. Laser-based CE detection (84) and diode laser detectors (85) were reviewed recently.

With a uniquely designed flat detection window, sheath flow cuvette (SFC) postcolumn detection cells reduce light scatter and background noise, facilitating a high sensitivity detection in LIF (86). Wavelength-resolved fluorescence detection (87,88) and spatial-scanning laser fluorescence detection (89) systems were also reported.

A fluorescence detection scheme is typically powerful for trace biomedical, biochemical, and pharmaceutical residue analysis. Compounds that frequently require this detection mode include amines, amino acids, peptides, proteins, nucleic acids, and carbohydrates. Because most of the compounds have no native fluorescence, various derivatization strategies have been developed, which were extensively reviewed (90,91).

With a novel detection approach (i.e., laser-induced resonance energy transfer detection), prior derivatization can be avoided. Direct determination of salicylic acid (2,4-dihydroxybenzoic acid), gentisic acid, and salicyluric acid in urine and detection of salicylate in serum was demonstrated (92). The method is highly sensitive; detection limits are in the range of 10^{-7}M. The method is applicable to complex matrices such as serum and urine without prior sample concentration and preparation.

Indirect fluorescence detection is also a method of choice to avoid complex derivatization. It is operated in a manner similar to indirect UV. Fluorophore is used as an absorbing background, and the detection of complexed metal ions and anions in single erythrocytes has been performed with success.

Electrochemical detection

The principle and technical details of electrochemical detection (EC) for CE have been described in two recent reviews (93,94). Potentiometric and amperometric detectors were widely employed. Typically, a cylindrical fiber is inserted into the capillary, or a disk electrode is brought up to the end of the capillary. Optimized designs use a conically etched capillary end. Means were developed to keep the detection circuit separate from the electrophoresis driving circuit. The EC detection is useful for electroactive substances such as amines, amino acids, peptides, and carbohydrates. The electroactive compounds can undergo oxidizing reactions on the surface of electrodes, causing a change in the electrode surface steady states that can be monitored. The advantages of electrochemical detection are its low cost, relatively high selectivity, and low detection limits. Detection of cellular contents in a single cell was attained with this detection mode.

MS

MS as a CE detector is quickly gaining interest in recent years and is the subject of a number of review articles (95–97). CE–MS is unique and powerful in the sense that the new system combines the capability of providing structural information, high sensitivity, the universal nature offered by MS, and the excellent selectivity provided by CE. It is therefore an analytical tool with great potential in studying complex biomolecules such as glycoproteins and nucleotides (98).

A key to coupling CE with MS is the design of an interface that permits the introduction of the CE-fractionated sample components to MS while maintaining the high sensitivity and precision of peak identification in the following evaporation, ionization, and mass analysis. Because the flow rate in CE is much lower than the total flow rate into the ionization sources, an additional flow is needed to make up the difference. Sheath-based and liquid junction interfaces are widely used for electrospray ionization (ESI) and continuous flow fast-atom bombardment (CF–FAB). A coaxial sheath-flow was advantageous in terms of the independent optimization of CE buffer and sheath flow composition–FAB matrix. Figure 6 shows the liquid junction and coaxial sheath-flow CE interfaces.

CF–FAB and ESI are the two most utilized ionization techniques for CE–MS. ESI has been widely adopted over the past years due to its high sensitivity and high mass range. Inductively coupled plasma as an ionization source enables elemental analysis with CE–MS (99). Because of the high sensitivity and high mass ranges of over 200,000, matrix-assisted laser desorption–ionization (MALDI) is also preferably used as an ionization source for CE, and the interface is mostly in off-line format. Ion trap is a major mass analyzer used for CE–MS because it is considered to be potentially promising for high-sensitivity and low-cost detection. Tandem MS is used to provide additional structural information. Fourier transformation ion cyclotron resonance (FTICR) with ESI has been shown to be a high-sensitivity, high-precision means of mass measurement; characterization of hemoglobin in a single erythrocyte (approximately 450 amol) was attainable (100).

Biofluids usually contain very low analytes, which poses a challenge to the current detection ability with MS (101). This problem was alleviated by using preconcentration based on ITP, electrostacking, and chromatographic methods (32,102) prior to CE–MS. CE–MS involving preconcentration with the chromatographic methods and membrane in drug metabolism studies was highlighted by Naylor et al. (32). Sequencing of a peptide less than 60 femtomoles was achieved with the method (103). In addition, CE–MS was shown to be particularly useful for scanning combinatorial peptide libraries (76).

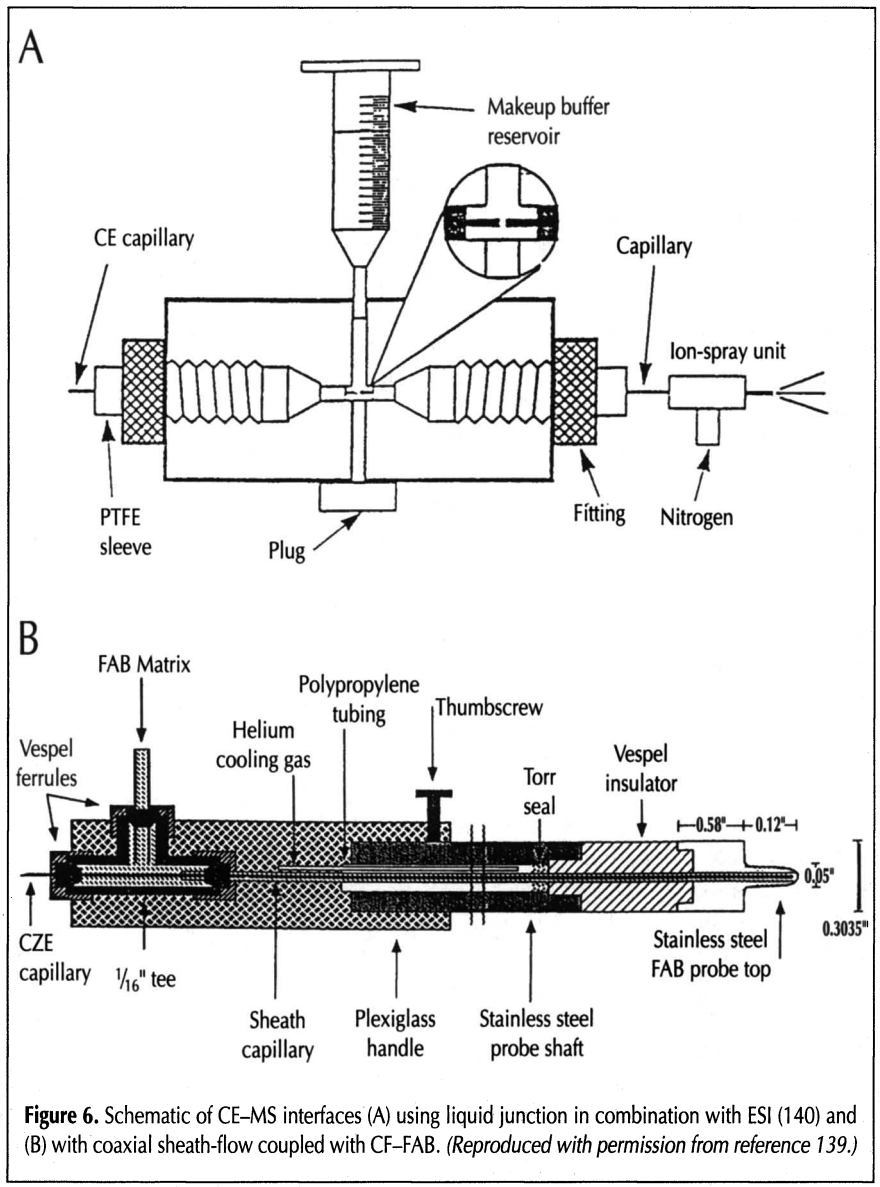

Figure 6. Schematic of CE–MS interfaces (A) using liquid junction in combination with ESI (140) and (B) with coaxial sheath-flow coupled with CF–FAB. *(Reproduced with permission from reference 139.)*

Selected applications in microscale analysis

The real power of CE in providing excellent separation efficiencies has been extensively exploited by developing suitable injection techniques and a variety of detection schemes as well as establishing various separation chemistries. It is a current trend to miniaturize the chemical analysis systems. As a result, even faster analysis can compete with sensors, and microenvironments can be effectively explored with CE-based separation techniques.

CE-based sensors

The concept of separation-based sensors was proposed by Ewing and coworkers in 1989 (104). They coupled CE to an electrochemical detector so that a neurotransmitter, dopamine, in a single nerve cell could be determined within approximately 6 min. The underlying principle of the sensor is to achieve selectivity by separation and sensitivity by detection.

Chemical sensors usually lack selectivity, which has hindered their application in "real world" analysis. The selectivity of these sensors relies on the selectivity provided by a chemical reagent phase, which is not readily adaptable to the detection of different species and not reusable. The use of CE to improve the selectivity of a chemical sensor was demonstrated by the construction of an integrated CE laser-induced fluorescence fiberoptic sensor (105). Sample was injected from the inlet of a short capillary (8 cm), and the outlet of the capillary was interfaced to a fiberoptic sensor for optical detection. With this system, up to 3000 theoretical

plates were obtained for the CZE analysis of charged fluorescent dyes and metal–8-hydroquinoline-5-sulfonic acid complexes based on in situ complexation as well as MEKC analysis of neutral fluorescent compounds.

Biosensors detect chemical species on the basis of molecular recognition. Entire living cells can be used as biosensors because many chemical species can evoke a response from a single cell. However, when more than one component in a sample matrix elicits a response, the resulting signal often cannot be interpreted. This problem can be solved by imparting high CE selectivity to the biosensor. A CE-based single-cell biosensor system was recently reported by Zare's group (106). Figure 7A presents a schematic diagram of the single-cell biosensor system used. A single cell was positioned underneath the outlet of a capillary with an inner diameter of 25 µm and an outer diameter of 360 µm. Electroosmosis delivered positive, neutral, and negative species that were separated by CE to the extracellular surface of living cells, which elicited a change in the cytosolic concentration of calcium ions in cultured rat PC-12 cells. Changes in the calcium concentration were monitored through fluorescent changes of calcium indicator fluo-3 with fluorescent microscopy. The CE single-cell biosensor system could identify acetylcholine (ACh), which is difficult to detect with traditional measurement methods. Figure 7B illustrates the identification of ACh in a complex biological matrix using the system.

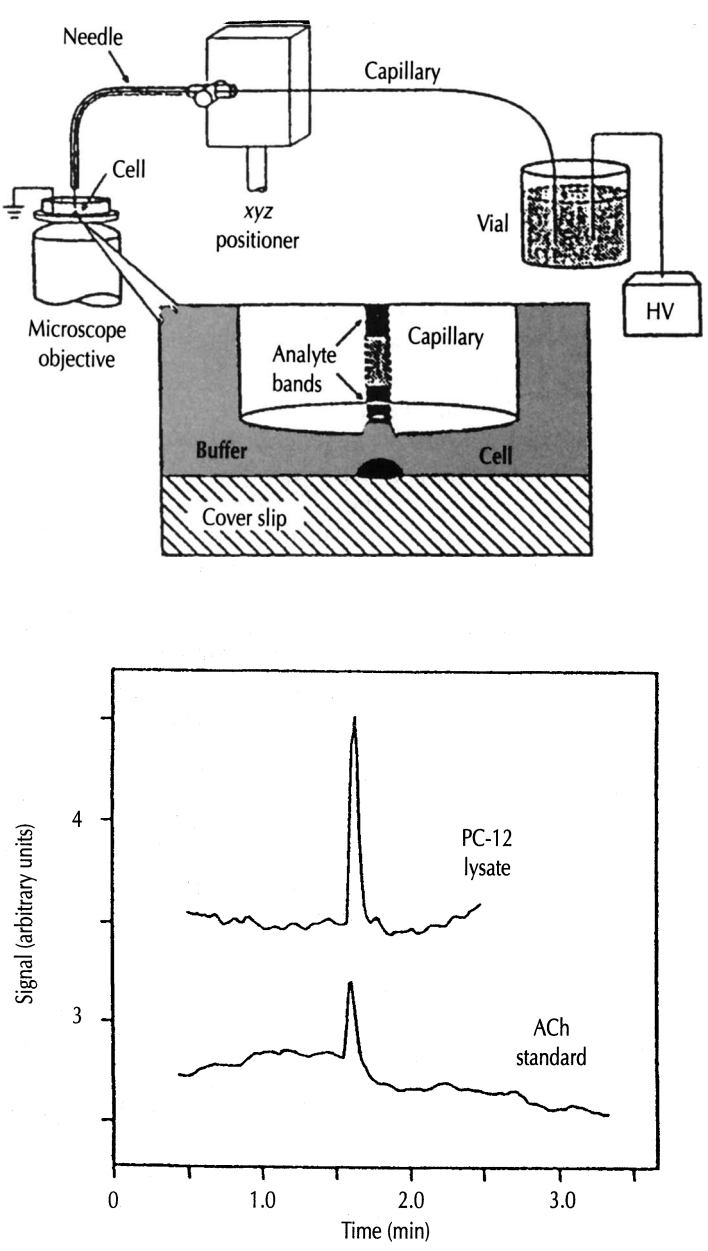

Figure 7. (A) A CE single-cell biosensor system. Detection was based on the monitoring of Ca^{2+} changes with the use of Ca^{2+}-sensitive dye fluo-3. Analytes were separated by CE, and the capillary effluent was directed to PC-12 cells cultured on a microscope cover slip approximately 20–40 µm from the channel outlet. The capillary was threaded through a syringe needle and was glued at the needle entrance and exit points to stabilize the position of the outlet. Species that evoked changes in Ca^{2+} were detected with an epi-illuminated fluorescence microscope. (B) Electropherogram of a sample derived from the lysate of PC-12 cells. ACh was identified by the comparison of the migration times of the unknown peak and the standard peak. Experimental conditions: 10-s gravity injections (more than 10 cm above the outlet). The lysate and ACh standard (0.8mM) were separated with a field of approximately 400 V/cm in a 25-µm-i.d. capillary. Fluo-3 AM ester was included in the measurement buffer. *(Reproduced with permission from reference 106.)*

A major limitation associated with the cell-based biosensor is loss of agonist-induced response caused by receptor desensitization. A cell-to-cell scanning in CE was proposed to deal with the problem (107). By scanning an array of immobilized cells underneath the CE column outlet, a new cell was used to assay each electrophoretically fractionated component. A successful detection of bradykinin after electrophoretic separation was obtained within 120 s by an NG-108-15 cell, which was known to undergo desensitization.

CE is compatible with the biosensor in terms of physiological compatibility (uses aqueous separation buffer), speed, and high separation efficiency. The ability to work with very low sample volume (down to picoliter volumes) is another advantage in studying microenvironments. On the other hand, incorporation of a biosensor to CE makes a highly selective system for sensing many important biological species that are not easily detected with conventional measurement methods. This area shows great promise in clinical diagnosis and biochemical studies.

CE on microchip

Harrison and coworkers pioneered the approach of performing CE on a microfabricated planar glass chip and published their

results in 1992 and 1993 (108,109). With this system, they demonstrated the analysis of fluorescently labeled amino acids in less than 5 s, with theoretical plates ranging from 40,000 to 75,000 for different amino acids. These are equivalent to results obtained in conventional fused-silica capillaries, but the total analysis time was as short as that attained with sensors. Subsequently, different groups showed that the system was feasible for various chemical analysis systems previously established with CE.

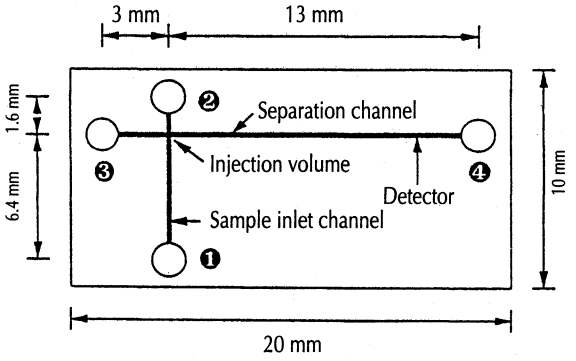

Figure 8. Layout of the microchip channels. *(Reproduced with permission from reference 111.)*

The microchip is fabricated by a standard photolithographic patterning followed by chemical etching (110). Figure 8 shows the layout of a 1 × 2-cm planar glass chip, which consists of two capillary channels intersecting at right angles (111). The short channel between reservoirs 1 and 2 is used to supply sample solutions, whereas the long channel between reservoirs 3 and 4 is used for separations. A cover plate with 0.3-mm holes to provide channel access points is thermally bonded to the glass plate in which channels were etched. Plastic pipette tips inserted into the holes of the cover plate act as the buffer reservoirs into which the buffer and platinum electrodes are placed. Injection is performed by first applying a voltage difference across the channel between reservoirs 1 and 2 and then switching to reservoirs 3 and 4 for the separation. The injection volume is equal to the volume of intersection. Detection is performed with fluorescence or laser-induced fluorescence at the site indicated in the figure.

Figure 9 presents recent results obtained in our laboratory (Y. Zhang, H.K. Lee, and S.F.Y. Li. Fluorescence detection in short capillary and chip using a variable-wavelength epifluorescence microscope. *Talanta,* in press.). The baseline separation of five porphyrin-free acids was achieved on a microfabricated glass chip with a separation length of 2.8 cm under an electrical field of 500 V/cm. Analysis was monitored by a variable-wavelength epifluorescence microscope in an on-column configuration.

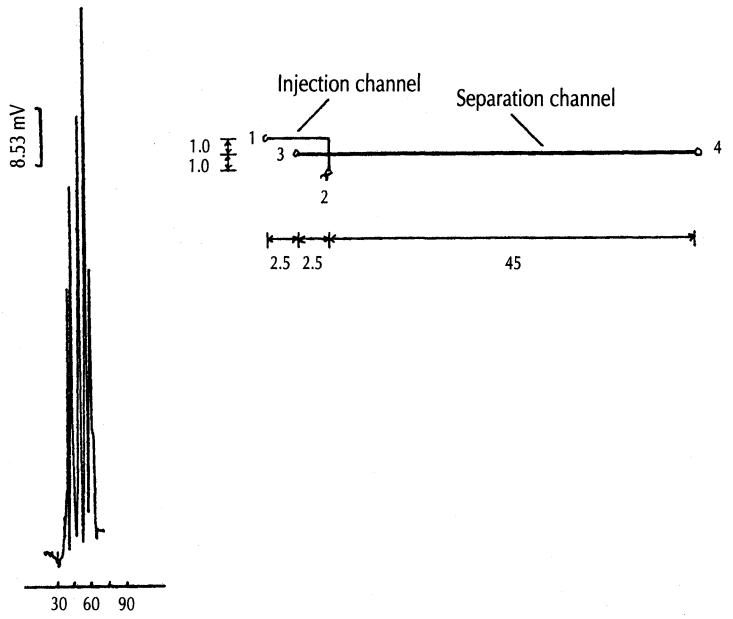

Figure 9. Electropherogram obtained for a mixture of porphyrins using a glass chip. Peaks (from left to right): coproporphyrin I, protoporphyrin IX, hexacarboxylporphyrin, heptacarboxylporphyrin I, and uroporphyrin. The concentration of each of the porphyrins was around 0.5μM. Experimental conditions: buffer, 0.05M phosphate, 0.025M borate, pH 7; separation channels, 50-μm width × 8-μm depth × 4.5-cm length, 2.8-cm effective length; electromigration injection by applying the voltage difference of 1 kV across reservoirs 1 and 2 for 4 s to drive the samples into the channel separation was carried out by applying voltage between reservoirs 3 and 4; detection was made by a lamp-based epifluorescence microscope with an excitation wavelength of 510–560 nm and emission wavelength higher than 590 nm. (Y. Zhang, H.K. Lee, and S.F.Y. Li. Fluorescence detection in short capillary and chip using a variable-wavelength epifluorescence microscope. *Talanta,* in press.)

Various existing CE operating modes, including CZE, MEKC (112), and CGE (113), could be performed on the microfabricated structures. Analytes studied include metal complexes (114), amino acids (109), PCR (115) and LCR (116) products, DNA for high-speed sequencing (117), and antisense oligonucleotides (118). An on-chip sample stacking procedure was developed, showing increased separation efficiency and enhanced detection limits (119).

MEKC on a glass chip with a cyclic channel system allowed the analysis of six fluorescein isothiocyanate labeled amino acids within a few seconds (112). Compared with conventional MEKC in fused-silica capillaries, analysis on a microchip is 1–2 orders of magnitude faster, with higher efficiency and at no expense of accuracy and precision. Human urine derivated with FITC and subsequently diluted 10-fold with water was analyzed. The cyclic channel system permitted an unbiased, dead-volume-free electromigration sample inlet system of about 12 pL.

The microfabricated silicon PCR reactor was directly linked to a CE chip through a microfabricated channel filled with hydroxyethylcellulose-sieving matrix, forming a microfabricated DNA analysis device (115). Electromigration injection was performed directly from the PCR chamber through the cross-injection channel to the CE on-chip devices.

A PCR-CE analysis of a β-globin target cloned in M13 cells was completed in under 20 min; CE analysis time was less than 120 s. Real-time monitoring of β-globin target amplification as a function of cycle number was also achieved.

In addition, use of quartz substrates is advantageous due to the superior optical properties of quartz, especially in the UV region (200–400 nm), which is suitable for the detection of a majority of compounds (114).

Most planar microstructures comprise separation channels of a few centimeters length with a width of 30–90 µm and a height on the order of 10 µm. Due to the small-channel cross-section, heat dissipation on the chips is better than in conventional capillaries. Therefore, higher electric fields can be applied, which provide analysis times on the second time scale and minimize longitudinal dispersion of the analytes. With this manifold, high-speed analysis and sampling volume as low as tens of picoliters are achievable. Furthermore, high sample throughput was realized by using capillary array electrophoresis chips (120). The microchip simultaneously serves as injector, microreactor, and detector (121), creating a "lab on a chip" concept (122). The sample injection, pretreatment, separation, and detection are performed simply by controlling the direction of electroosmotic pumping with switching applied voltages. Because microchips are potentially dispensable and inexpensive, the approach shows great promise in biomedical studies and clinical diagnosis. Research is in progress to design different geometries of the channels to provide high separation efficiency with optimized sample pretreatment and detection (121).

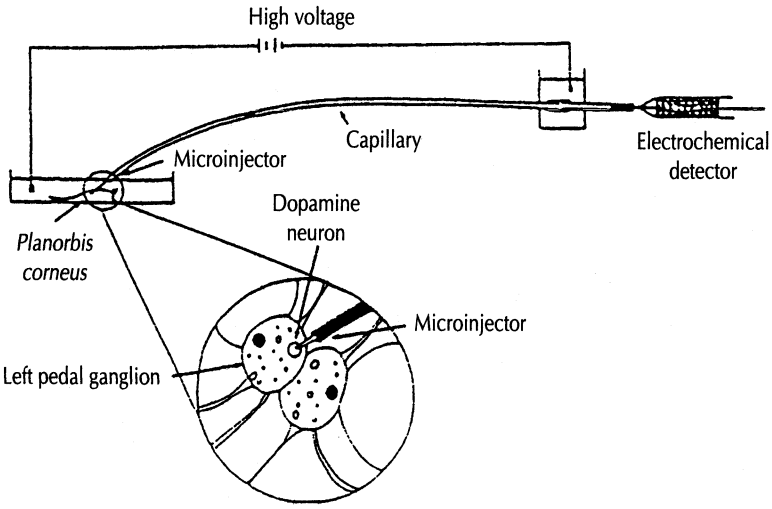

Figure 10. System used for the removal and separation of cytoplasmic samples from single nerve cells of *Planorbis corneus*. *(Reproduced with permission from reference 124.)*

Assay of single cells

Ewing and Wallingford pioneered the research of CE at the single-cell level (123). They successfully introduced subpicoliter cytoplasmic samples from a single cell to a capillary by a microinjector with an inner diameter of 5 µm. The microinjector was

Table I. Selected Single-Cell Analyses

Single cells	Analytes	Detection methods	Reference
Giant dopamine neuron and a large serotonin neuron of *Planorbis corneus*	Dopamine (DA) Serotonin (5-HT) Dihydroxyphenylacetic acid (DOPAC)	EC	123 124
Neurons from the snail *Helix aspersa*	Amino acids	LIF	131
Human erythrocytes	Glutathione (GSH) Na^+, K^+ Carbonic anhydrase (CAH) Hemoglobin A_0 Lactate and pyruvates Lactate dehydrogenase (LDH) Glucose-6-phosphate dehydrogenase (G6PDH) Hemoglobin	FL with derivatization indirect FL FL FL indirect FL FL LIF ESI–FTICR–MS	125 125 126 126 128 127 132 100
Nerve cells	Neuropeptides	Phosphor imaging detector	133
Pancreatic cells	Insulin	LIF	134
Rat peritoneal mast cells (RPMCs)	Serotonin and proteins	LIF	135
Xenopus laevis oocytes	Inorganic ions and tryptophan	UV	136
NG 108-15 cells	Glutathione and gamma-glutamylcysteine	FL	137
Lymphocytes from normal and leukemia cells	Lactate dehydrogenase (LDH) isoenzymes	LIF	138

formed by etching one end of a silica capillary with hydrofluoric acid. Figure 10 shows their system (124). Dopamine was detected with an electrochemical detector.

Yeung's group successfully detected inorganic ions (125), proteins (126), enzymes (127), and pyruvate and lactate (128) in even smaller human erythrocytes with laser-based detection schemes. A recent review presented various derivatization methods for the analysis of single cells with fluorescence detection (129). Table I lists the selected single-cell analyses.

One recent report first addressed the single cell analysis with electrospray ionization–Fourier transform ion-cyclotron resonance–mass spectrometry (ESI–FTICR–MS) (100). The use of MS as detector in CE-based single cell analysis may yield structural information of cellular constituents, which is significant in this area. Characterization of hemoglobin from a single human erythrocyte (approximate to 450 amole) was carried out.

CE is well-suited for probing microenvironments due to its typical features (i.e., the compatibility between the dimensions of the capillary [inner diameters of 2–200 µm] and the size of most cells [diameters of 5–500 µm], much smaller sample requirements than most other analytical methods, extremely high separation efficiency, high analysis speed, and possible on-capillary sample pretreatment [enzymatic digestion and derivatization, for example]). The study of single-cell pharmacokinetics with CE–LIF was reported very recently (130). Further developments in this domain are still expected.

Conclusion

CE is an analytical technique that has received great attention in recent years. The power of CE in providing excellent separation efficiencies has been extensively exploited by developing suitable injection techniques, detection schemes, and separation chemistries. In some fields, it has broken the limitations of existing analytical techniques such as HPLC, etc. Miniaturization of CE-based chemical analysis systems on microfabricated chips has become a trend. As a result, even faster analyses that can compete with sensors may be realized. Also, miniaturization of CE-based analytical methods provides techniques to probe microenvironments in which many unknown matters are still waiting to be unfolded, while existing methods have been very limited. More achievements in this area can be expected in the near future.

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References

1. A. Tiselus. *Trans. Faraday Soc.* **33:** 524 (1937).
2. J.W. Jorgenson and K.D. Lukacs. Zone electrophoresis in open-tubular glass capillaries. *Anal. Chem.* **53:** 1298–1302 (1981).
3. S.F.Y. Li. *Capillary Electrophoresis: Principles, Practice and Application.* Elsevier B.V., Amsterdam, 1992.
4. C.A. Monnig and R.T. Kennedy. Capillary electrophoresis. *Anal. Chem.* **66:** 280R–314R (1994).
5. R.L. St. Claire, III. Capillary electrophoresis. *Anal. Chem.* **68:** 569–586R (1996).
6. A.R. Wallingford and A.G. Ewing. Capillary electrophoresis. *Advances in Chromatography,* Vol. 29. J.C. Giddings, E. Grushaka, and P.R. Brown, Eds. Marcel Dekker, New York, NY, 1989, pp. 1–76.
7. J.D. Olecbno and J.A. Nolan. Injection methods in capillary electrophoresis. *Capillary Electrophoresis in Analytical Biotechniques.* P.G. Righetti, Ed. CRC, Boca Raton, FL, 1996, pp. 61–99.
8. B.L. Hogan, S.M. Lunte, J.F. Stobaugh, and C.E. Lunte. Online coupling of in–vivo microdialysis sampling with capillary electrophoresis. *Anal. Chem.* **66:** 596–602 (1994).
9. M.M. Bushey and J.W. Jorgenson. Automated instrumentation for comprehensive two-dimensional HPLC/CZE. *Anal. Chem.* **62:** 978–84 (1990).
10. C.R. Yonker and R.D. Smith. High performance and supercritical CE. *J. Chromatogr.* **517:** 573–81 (1990).
11. C.A. Monnig and J.W. Jorgenson. On-column sample gating for high-speed capillary zone electrophoresis. *Anal. Chem.* **63:** 802–807 (1991).
12. F.E. Regnier, D.H. Patterson, and B.J. Harmon. Electrophoretically mediated microanalysis (EMMA). *Trends in Anal. Chem.* **14:** 177–181 (1996).
13. S. Liu and P.K. Dasgupta. Flow-injection analysis in the capillary format using electroosmotic pumping. *Anal. Chim. Acta* **268:** 1–6 (1992).
14. B.J. Harmon, D.H. Patterson and F.E. Regnier. Mathematical treatment of electrophoretically mediated microanalysis. *Anal. Chem.* **65:** 2655–62 (1993).
15. B.J. Harmon, J. Leesong, and F.E. Regnier. Moving boundary electrophoretically mediated microanalysis. *J. Chromatogr. A.* **726:** 193–204 (1996).
16. P.K. Dasgupta and K. Surowiec. Quantitative injection from a microloop–reproducible volumetric sample introduction in capillary zone electrophoresis. *Anal. Chem.* **68:** 1164–68 (1996).
17. S.M. Lunte and C.E. Lunte. Microdialysis sampling for pharmacological studies: HPLC and CE analysis. *Advances in Chromatography,* Vol. 36. P.R. Brown and E. Grushaka, Eds. Marcel Dekker, New York, NY, 1996, pp. 383–432.
18. P. Bocek, M. Deml, P. Gebauer, and V. Dolnik. *Analytical Isotachophoresis.* VCH Verlagsgesellschaft, Weinheim, 1988.
19. C. Schwer and F. Lottspeich. Analytical and micropreparative separation of peptides by capillary zone electrophoresis using discoutinous buffer systems. *J. Chromatogr.* **623:** 345–55 (1992).
20. F.E.P. Mikkers, F.M. Everaerts, and T.P.E. Verheggen. Concentration distributions in free zone electrophoresis. *J. Chromatogr.* **169:** 1–10 (1979).
21. R.L. Chien and D.S. Burgi. On-column sample concentration using field amplification in capillary zone electrophoresis. *Anal. Chem.* **64:** 489A–496A (1992).
22. D.S. Burgi and R.L. Chien. Improvement in the method of sample stacking for gravity injection in capillary zone electrophoresis. *Anal. Biochem.* **202:** 306–309 (1992).
23. R.L. Chien and D.S. Burgi. Field-amplified polarity-switching sample injection in high-performance capillary electrophoresis. *J. Chromatogr.* **559:** 156–61 (1991).
24. D.S. Burgi. Large volume stacking of anions in capillary electrophoresis using an electroosmotic flow modifier as a pump. *Anal. Chem.* **65:** 3726–29 (1993).
25. C.X. Zhang and W. Thormann. Head-column field-amplified sample stacking in binary-system capillary electrophoresis—A robust approach providing over 1000-fold sensitivity enhancement. *Anal. Chem.* **68:** 2523–32 (1996).
26. J.Y. He, A. Shibukawas, M. Zeng, T. Sawada, S. Amane, and

T. Nakagawa. On-capillary sample preconcentration incorporated in chiral capillary electrophoresis. *Anal. Sci.* **12:** 177–81 (1996).
27. I. Morita and J. Sawada. Capillary electrophoresis with on-line sample pre-treatment for the analysis of biological samples with direct injection. *J. Chromatogr.* **641:** 375–81 (1993).
28. J. Cai and Z. El Rassi. On-line preconcentration of triazine herbicides with tandem octadecyl capillaries–capillary electrophoresis. *J. Liq. Chromatogr.* **15:** 1179–92 (1992).
29. J. Cai and Z. El Rassi. Selective on-line preconcentration of proteins by tandem metal chelate capillaries–capillary zone electrophoresis. *J. Liq. Chromatogr.* **16:** 2007–24 (1993).
30. L.J. Cole and R.T. Kennedy. Selective preconcentration for capillary zone electrophoresis using protein-G immunoaffinity capillary chromatography. *Electrophoresis* **16:** 549–56 (1995).
31. A.J. Tomlinson, L.M. Benson, R.P. Oda, and S. Naylor. Online preconcentration capillary electrophoresis–mass spectrometry (PC–CE–MS). *J. High Res. Chromatogr.* **18:** 381–83 (1995).
32. A.J. Tomlinson, L.M. Benson, N. Guzman, and S. Naylor. Preconcentration and microreaction technology on-line with capillary electrophoresis. *J. Chromatogr. A.* **744:** 3–15 (1996).
33. S. Naylor, L.M. Benson, and A.J. Tomlinson. Application of capillary electrophoresis and related techniques to drug metabolism studies. *J. Chromatogr. A.* **735:** 415–38 (1996).
34. L. Licklider and W.G. Kuhr. Optimization of on-line peptide-mapping by capillary zone electrophoresis. *Anal. Chem.* **66:** 4400–07 (1994).
35. S.Y. Zhou, H. Zuo, J.F. Stobaugh, C.E. Lunte, and S.M. Lunte. Continuous in-vivo monitoring of amino acid neurotransmitters by microdialysis sampling with on-line derivatization and capillary electrophoresis separation. *Anal. Chem.* **67:** 594–99 (1995).
36. N.A. Guzman. Consecutive protein digestion and peptide derivatization employing an on-line analfie concentrator to map proteins using capillary electrophoresis. *Capillary Electrophoresis in Analytical Biotechnology.* P.G. Righetti, Ed. CRC Press, Boca Raton, FL, 1996, pp. 101–21.
37. S.H. Hansen, J. Tiornelund and I. Bjornsdottir. Selectivity enhancement in capillary electrophoresis using non-aqueous media. Trends in *Anal. Chem.* **15:** 175–80 (1996).
38. Q. Yang, Y. Zhuang, J. Smeyers-Verbeke, and D.L. Massart. Interpretation of migration behaviour of inorganic cations in capillary ion electrophoresis based on an equilibrium model. *J. Chromatogr. A* **706:** 503–15 (1995).
39. M. Jimidar, Q. Yang, J. Smeyers-Verbeke, and D.L. Massart. Method development and optimisation for small ion capillary electrophoresis. *Trends in Anal. Chem.* **15:** 91–102 (1996).
40. S. Fanali. Identification of chiral drug isomers by capillary electrophoresis. *J. Chromatogr.* **735:** 77–121 (1996).
41. M. Novotny, H. Soini, and M. Stefansson. Chiral separation through capillary electromigration methods. *Anal. Chem.* **66:** 646A–655A (1994).
42. A.M. Stalcup and K.H. Gahm. Application of sulfated cyclodextrins to chiral separations by capillary zone electrophoresis. *Anal. Chem.* **68:** 1360–68 (1996).
43. R.J. Tait, D.O. Thompsion, V.J. Stella, and J.F. Stobaugh. Sulfobutyl ether β-cyclodextrin as a chiral discriminator for use with capillary electrophoresis. *Anal. Chem.* **66:** 4013–18 (1994).
44. D.W. Armstrong, Y. Tang, S. Chen, Y. Zhou, C. Bagwill, and J.R. Chen. Macrocyclic antibiotics as a new class of chiral selectors for liquid chromatography. *Anal. Chem.* **66:** 147384 (1994).
45. M.P. Gasper, A. Berethod, U.B. Nair, and D.W. Armstrong. Comparison and modeling study of vancomycin, ristocetin A and teicoplanin for CE enantiomer separation. *Anal. Chem.* **68:** 2501–2514 (1996).
46. P. Gozel, E. Gassmann, H. Michelsen, and R.N. Zare. Electrokinetic resolution of amino acid enantiomers with copper(II)–aspartame support electrolyte. *Anal. Chem.* **59:** 44–49 (1987).
47. H. Nishi and S. Terabe. Enantiomeric separation of diltiazem, clentiazem and its related compounds by capillary electrophoresis using polysaccharides. *J. Chromatogr. Sci.* **33:** 698–703 (1995).
48. R. Isaksson, C. Pettersson, G. Pettersson, S. Jonsson, J. Stalberg, J. Hermansson, and I. Marle. Cellulases as chiral selectors. *Trends in Anal. Chem.* **10:** 431–38 (1994).
49. S.G. Penn, E.T. Bergstrom, and D.M. Goodall. Capillary electrophoresis with chiral selectors: Optimisation of separation and determination of thermodynamic parameters for binding of tioconazole enantiomers to cyclodextrins. *Anal. Chem.* **66:** 2866–73 (1994).
50. Y.Y. Rawjee, R.L. Williams, and G. Vigh. Capillary electrophoretic chiral separations with cyclodextrin additives. 1. Acids—Chiral selectivity as a function of pH and the concentration of beta-cyclodextrin for fenoprofen and ibuprofen. *J. Chromatogr.* **635:** 291–306 (1993).
51. Y.Y. Rawjee and G. Vigh. A peak resolution model for the capillary electrophoretic separation of the enantiomers of weak acids with hydroxypropyl beta-cyclodextrin-containing background electrolytes. *Anal. Chem.* **66:** 619–27 (1994).
52. S. Honda. Separation of neutral carbohydrates by capillary electrophoresis. *J. Chromatogr. A.* **720:** 337–51 (1996).
53. A. Paulus and A. Klockowe. Detection of carbohydrates in capillary electrophoresis. *J. Chromatogr. A* **720:** 353–76 (1996).
54. R.J. Linhardt and A. Pervin. Separation of negatively charges carbohydrates by capillary electrophoresis. *J. Chromatogr.* **720:** 323–35 (1996).
55. M. Greenaway, G.N. Okafo. P. Camilleri, and D. Dhanak. A sensitive and selective method for the analysis of complex mixtures of sugars and linear oligosaccharides. *J. Chem. Soc. Chem. Commun.* 1691–92 (1994).
56. S. Terabe, K. Otsuka, K. Ichikawa, Y. Tsuchiya, and T. Ando. Electrokinetic separation with micellar solutions and open-tubular capillaries. *Anal. Chem.* **56:** 111–13 (1984).
57. S. Terabe, N. Chen, and K. Otsuka. Micellar electrokinetic chromatography. *Advances in Electrophoresis,* Vol. 7. A. Chrambach, M.J. Dunn, and R.J. Radola, Eds. VCH Weinheim, 1994, pp. 87.
58. S. Yang, J.G. Bumgarner, and M.G. Khaledi. Separation of highly hydrophobic compounds in MEKC with an ionic polymer. *J. High Res. Chromatogr.* **18:** 443–45 (1995).
59. M.D. Zhu, D.L. Hansen, S.S. Burd, and F. Gannon. Factors affecting free zone electrophoresis and isoelectric focusing in capillary electrophoresis. *J. Chromatogr.* **480:** 311–19 (1989).
60. Principles of size-based separation in polymer solutions. *Capillary Electrophoresis in Analytical Biochemistry.* P.G. Righetti, Ed. CRC Press, Boca Raton, FL, 1996, pp. 477–508.
61. Y. Zhang, H.K. Lee, and S.F.Y. Li. Separation of myoglobin molecular mass markers using non-gel sieving capillary electrophoresis. J. Chromatogr. A 744: 249–357 (1996).
62. Y.H. Chu, L.Z. Avila, J.M. Gao, and G.M. Whitesides. Affinity capillary electrophoresis. *Account of Chemical Research* **28:** 461–68 (1995).
63. K. Haupt, F. Roy, and M.A. Vijayalakshmi. Immobilized metal ion affinity capillary electrophoresis of proteins—A model for affinity capillary electrophoresis using soluble polymer supported ligands. *Anal. Biochem.* **234:** 149–54 (1996).
64. J.M. Gao, S. Qiao, and G.M. Whitesides. Increasing binding constants of ligands to carbonic-anhydrase by using creasy tails. *J. Med. Chem.* **38:** 2292–2301 (1995).
65. K. Shimura and K. Kasai. Determination of the affinity constants of concanavalin-A for monosaccharides by fluorescence affinity probe capillary electrophoresis. *Anal. Biochem.* **227:** 186–94 (1995).
66. N.H.H. Heegaard and F.A. Robey. Use of capillary zone electrophoresis for the analysis of DNA-binding to a peptide derived from amyloid P component. *J. Liq. Chromatogr.* **16:** 1923–39 (1993).
67. M. Defrutos, S.K. Paliwal, and F.E. Regnier. Analytical immunology. *Methods in Engymology,* Vol. 270, Academic Press, New York, NY, 1996, pp. 82–101.
68. T. Ohara, A. Shibukawa, and T. Nakagawa. Capillary electrophoresis/frontal analysis for microanalysis of enantioselective

protein binding of a basic drug. *Anal. Chem.* **67:** 3520–25 (1995).
69. J. Oravcova, B. Bohs, and W. Lindner. Drug–protein binding studies—New trends in analytical and experimental methodology. *J. Chromatogr. B* **677:** 1–28 (1996).
70. M.A. Garcia, M.L. Marina, and J.C. Diez-Masa. Determination of solute–micelle association constants for a group of benzene derivatives and polycyclic aromatic hydrocarbons with sodium dodecyl sulphate by micellar electrokinetic chromatography. *J. Chromatogr. A* **732:** 345–59 (1996).
71. V. Letermination, M. Taverna, D. Wouessidjewe, D. Duchene, and D. Ferrier. Determination of the binding constant of salbutamol to unmodified and ethylated cyclodextrins by affinity capillary electrophoresis. *J. Chromatogr. A* **735:** 321–31 (1996).
72. J. Yang and D.S. Hage. Chiral separation in capillary electrophoresis using human serum–albumin as a buffer additive. *Anal. Chem.* **66:** 2719–25 (1994).
73. Y. Tanaka and S. Terabe. Partial separation zone technique for the separation of enantiomers by affinity electrokinetic chromatography with proteins as chiral pseudo-stationary phases. *J. Chromatogr. A* **694:** 277–84 (1995).
74. S. Sun, G.E. Barker, R.A. Hartwick, N. Grinberg, and R. Kaliszan. Chiral separation using an immobilised protein–dextran polymer network in affinity capillary electrophoresis. *J. Chromatogr. A* **652:** 247–52 (1993).
75. Y.H. Chu, D.P. Kirby, and B.L. Karger. Free solution identification of candidate peptides from combinatorial libraries by affinity capillary electrophoresis–mass spectrometry. *Journal of the American Chemical Society* **117:** 5419–20 (1995).
76. Y.H. Chu, Y.M. Dunayevskiy, D.P. Kirby, P. Vouros, and B.L. Karger. Affinity capillary electrophoresis mass-spectrometry for screening combinatory libraries. *J. Am. Chem. Soc.* **118:** 7827–35 (1996).
77. J.A. Boutin, P. Hennig, P.H. Lambert, S. Bertin, L. Petit, J.P. Mahieu, B. Serkiz, J.P. Volland, and J.L. Fauchere. Combinatorial peptide libraries–robotic synthesis and analysis by nuclear-magnetic-resonance, mass spectrometry, tandem mass spectrometry and high performance capillary electrophoresis techniques. *Anal. Biochem.* **234:** 126–41 (1996).
78. Y.J. Yao, K.S. Khoo, M.C.M. Chung, and S.F.Y. Li. Determination of isoelectric points of acidic and basic proteins by capillary electrophoresis. *J. Chromatogr. A* **680:** 431–35 (1994).
79. R.J. Boughtflower, T. Underwood, and C.J. Paterson. Capillary electrochromatography–Some important considerations in the preparation of packed capillaries and the choice of mobile phase buffers. *Chromatographia* **40:** 329–35 (1995).
80. T. Eimer, K.K. Unger, and J. van der Greef. Selectivity turning in pressurized-flow electrochromatography. *Trends in Anal. Chem.* **15:** 463–67 (1996).
81. Technical Note: High-sensitivity detection cell for HP^{3D} capillary electrophoresis system. Hewlett-Packard, 1997.
82. F.B. Regan, M.P. Meaney, and S.M. Lunte. Determination of metal ions by capillary electrophoresis using on-column complexation with 4-(2-pyridylazo) resorcinol following trace enrichment by peak stacking. *J. Chromatogr. B* **657:** 409–17 (1994).
83. Z. Mala and R. Vespalee, and P. Bocek. Capillary zone electrophoresis with indirect photometric detection in the visible range. *Electrophoresis* **15:** 1526–30 (1994).
84. T.T. Lee and E.S. Yeung. *Capillary electrophoresis detectors–lasers. Methods in Enzymology,* Vol 270, Academic Press, New York, NY, 1996, pp. 419–49.
85. A.J.G. Mank, H. Lingeman, and C. Gooijer. Diode laser-based detection in LC and CE. *Trends in Anal. Chem.* **15:** 1–11 (1996).
86. S. Takahashi, K. Murakami, T. Anazawa, and H. Kambara. *Anal. Chem.* **66:** 1021–26 (1994).
87. A.T. Timperman, K. Khatib, and J.V. Sweedler. Wavelength-resolved fluorescence detection in capillary electrophoresis. *Anal. Chem.* **67:** 139–44 (1995).
88. A.T. Timperman and J.V. Sweedler. Capillary electrophoresis with wavelength-resolved fluorescence detection. *Analyst* **121: 45R–52R** (1996).
89. S.C. Beale and S.J. Sudmeier. Spatial-scanning laser fluorescence detection for capillary electrophoresis. *Anal. Chem.* **67:** 3367–71 (1995).
90. E.S. Yeung. Optical detectors for capillary electrophoresis. *Adv. Chromatography,* Vol. 35. P.R. Brown and E. Grushka, Eds. Marcel Dekker, New York, NY, 1995, pp. 1–51.
91. H.E. Schwartz, K.J. Ulfelder, F.T.A. Chen, and S.L. Pentoney, Jr. The utility of laserinduced fluorescence detection in application of capillary electrophoresis. *J. Capillary Electrophoresis* **1:** 36–54 (1994).
92. J.R. Petersen, M.G. Bissell, and A.A. Mohammad. Laser-induced resonance energy transfer—A novel approach towards achieving high sensitivity in capillary electrophoresis–I. Clinical diagnostic application. *J. Chromatogr. A* **744:** 37–44 (1996).
93. A.G. Ewing, J.M. Mesaros, and P.F. Gavin. Electrochemical detection in microcolumn separations. *Anal. Chem.* **66:** 527A–537A (1994).
94. J.G. Chen, S.J. Woltman, and S.G. Weber. Electrochemical detection of biomolecules in liquid chromatography and capillary electrophoresis. *Adv. in Chromatography,* P.R. Brown and E. Grushka, Eds., Marcel Dekker, New York, NY, 1996, pp. 273–313.
95. J. Cai. and J. Henion. Capillary electrophoresis–mass spectrometry. *J. Chromatogr. A* **703:** 667–92 (1995).
96. R.D. Smith, H.R. Udseth, J.H. Wahl, D.R. Goodlett, and S.A. Horstadler. Capillary electrophoresis–mass spectrometry. *Methods in Enzymology,* Vol. 271, Academic Press, New York, 1996, pp. 448–486.
97. K.B. Tomer, L.J. Deterding, and C.E. Parker. Capillary electrophoresis coupled with mass spectrometry. *Adv. in Chromatography,* Vol. 35. P.R. Brown and E. Grushka, Eds. Marcel Dekker, New York, NY, 1995, pp. 53–99.
98. A.S. Cohen, D.L. Smisek, and B.H. Wang. Emerging technologies for sequencing antisense oligonucleotides–capillary electrophoresis and mass spectrometry. *Adv. in Chromatography,* Vol. 36. P.R. Brown and E. Grushka, Eds. Marcel Dekker, New York, NY, 1996, pp. 127–62.
99. J.W. Olesik, J.A. Kinzer, and S.V. Olesik. Capillary electrophoresis–inductively coupled plasma spectrometry for rapid elemental speciation. *Anal. Chem.* **67:** 1–12 (1995).
100. S.A. Hofstadler, J.C. Severs, R.D. Smith, F.D. Swanek, and A.G. Ewing. Analysis of single cells with capillary electrophoresis–electrospray ionisation–Fourier transform ion cyclotron resonance–mass spectrometry. *Rapid Communication in Mass Spectrometry* **10:** 919–22 (1996).
101. E. Vellum, H. Dollekamp, and C.C. Blessum. Capillary electrophoresis for clinical problemsolving–analysis of urinary diagnostic metabolites and serum proteins. *J. Chromatogr. B* **683:** 55-65 (1996).
102. L.M. Benson, A.J. Tomlinson, A.N. Mayeno, G.J. Gleich, D. Wells, and S. Naylot. Membrane preconcentration capillary electrophoresis mass spectrometry (mPC–CE–MS) analysis of 3-phenylamino-1,2-propanediol (PAP) metabolites. *J. High Res. Chromatogr.* **19:** 291–94 (1996).
103. A.J. Tomlinson, S. Jameson, and S. Naylor. Strategy for isolating and sequencing biological derived MHC class-I peptides. *J. Chromatogr. A* **744:** 273–78 (1996).
104. A.G. Ewing, R.A. Wallingford, and T.M. Olefirowicz. Capillary electrophoresis. *Anal. Chem.* **61:** 292A–303A (1989).
105. M.J. Sepaniak, T. Vo-Dinh, D.L. Stokes, V. Tropina, and J.E. Dickens. Demonstration of an integrated capillary electrophoresis–laser-induced fluorescence fiber-optic sensor. *Talanta* **43:** 1889–1901 (1996).
106. J.B. Shear, H.A. Fishman, N.L. Albritton, D. Garigan, R.N. Zare, and R.H. Scheller. Single cells as biosensors for chemical separation. *Science* **267:** 74–77 (1995).
107. H.A. Fishman, O. Orwar, N.L. Albritton, B.P. Modi, J.B. Shear, R.H. Scheller, and R.N. Zare. Cell-to-cell scanning in capillary electrophoresis. *Anal. Chem.* **68:** 1181–86 (1996).

108. A. Manz, D.J. Harrison, E.M.J. Verpoorte, J.C. Fettinger, A. Paulus, H. Ludi, and H.M. Widmer. Planar chips technology for miniaturization and integration of separation techniques into monitoring systems–Capillary electrophoresis on a chip. *J. Chromatogr.* **593:** 253–58 (1992).
109. D.J. Harrison, K. Fluri, K. Seiler, Z.H. Fan, C.S. Effenhauser, and A. Manz. Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science* **261:** 895–97 (1993).
110. G.T.A. Kovacs, K. Petersen, and M. Albin. Silicon micromachining: Sensors to systems. *Anal. Chem.* **68:** 407A–412A (1996).
111. Z.H. Fan and D.J. Harrison. Micromachining of capillary electrophoresis injectors and separators on glass chips and evaluation of flow at capillary intersections. *Anal. Chem.* **66:** 177–84 (1994).
112. F. von Heeren, E. Verpoorte, A. Manz, and W. Thormann. Micellar electrokinetic chromatography separations and analyses of biological samples on a cyclic planar microstructure. *Anal. Chem.* **68:** 2044–53 (1996).
113. F. Vonheeren, E. Verpoorte, A. Manz, and W. Thormann. Characterization of electrophoretic sample injection and separation in a gel-filled cyclic planar microstructure. *J. Micro. Sep.* **8:** 373–81 (1996).
114. S.C. Jacobson, A.W. Moore, and J.M. Ramsey. Fused quartz substrates for microchip electrophoresis. *Anal. Chem.* **67:** 2059–63 (1995).
115. A.T. Woolley, D. Hadley, P. Landre, A.J. DeMello, R.A. Mathies, and M.A. Northrup. Functional integration of PCR amplification and capillary electrophoresis in a microfabricated DNA analysis device. *Anal. Chem.* **68:** 4081–86 (1996).
116. J. Cheng, M.A. Shoffner, K.R. Mitchelson, L.J. Kricka, and P. Wilding. Analysis of ligase chain-reaction products amplified in a silicon-glass chip using capillary electrophoresis. *J. Chromatogr. A* **732:** 151–58 (1996).
117. A.T. Woolley and R.A. Mathies. Ultra-high-speed DNA-sequencing using capillary electrophoresis chips. *Anal. Chem.* **67:** 3676–80 (1995).
118. C.S. Effenhauser, A. Paulus, A. Manz, and H.M. Widmer. High-speed separation of antisense oligonucleotides on a micromachined capillary electrophoresis device. *Anal. Chem.* **66:** 2949–53 (1994).
119. S.C. Jacobson and J.M. Ramsey. Microchip electrophoresis with sample stacking. *Electrophoresis* **16:** 481–86 (1995).
120. A.T. Woolley and R.A. Mathies. Ultra-high-speed DNA fragment separations using microfabricated capillary array electrophoresis chips. *Proc. Natl. Acad. Sci. USA* **91:** 11348–52 (1994).
121. K. Fluri, G. Fitzpatrick, N. Chien, and D.J. Harrison. Integrated capillary electrophoresis devices with an efficient postcolumn reactor in planar quartz and glass chips. *Anal. Chem.* **68:** 4285–90 (1996).
122. D.J. Harrison, K. Fluri, N. Chien, T. Tang, and Z.H. Fan. Micromachining chemical and biochemical analysis and reaction systems on glass substrates. *Sensors and Actuators B Chemical* **33:** 105–109 (1996).
123. R.A. Wallingford and A.G. Ewing. Capillary zone electrophoresis with electrochemical detection in 12.7 µm diameter columns. *Anal. Chem.* **60:** 1972–75 (1988).
124. T.M. Olefirowicz and A.G. Ewing. Dopamine concentration in the cytoplasmic compartment of single neurons determination by capillary electrophoresis. *J. Neurosci. Meth.* **34:** 11–15 (1990).
125. B.L. Hogan and E.S. Yeung. Determination of intracellular species at the level of a single erythrocyte via capillary electrophoresis with direct and indirect fluorescence detection. *Anal. Chem.* **64:** 2841–45 (1992).
126. T.T. Lee and E S Yeung. Quantitative determination of native proteins in individual human erythrocytes by capillary zone electrophoresis with laser-induced fluorescence detection. *Anal. Chem.* **64:** 3045–51 (1992).
127. Q. Xue and E.S. Yeung. Variability of intracellular lactate–dehydrogenase isoenzymes in single human erythrocytes. *Anal. Chem.* **66:** 1175–78 (1994).
128. Q. Xue and E.S. Yeung. Indirect fluorescence determination of lactate and pyruvate in single erythrocytes by capillary electrophoresis. *J. Chromatogr. A* **661:** 287–95 (1994).
129. P.B. Hietpas and A.G. Ewing. On-column and postcolumn derivatization for capillary electrophoresis with laser-induced fluorescence for the analysis of single cells. *J. Liq. Chromatogr.* **18:** 3557–76 (1995).
130. W. Tong and E.S. Yeung. Monitoring single-cell pharmacokinetics by capillary electrophoresis and laser-induced native fluorescence. *J. Chromatogr. B* **689:** 321–26 (1997).
131. R.T. Kennedy, M.D. Oates, B.R. Cooper, B. Nickerson, and J.W. Jorgenson. Microcolumn separations and the analysis of single cells. *Science* **246:** 57–63 (1989).
132. Z. Rosenweig and E.S. Yeung. Laser-based particle-counting microimmunoassay for the analysis of single human erythrocytes. *Anal. Chem.* **66:** 1771–76 (1994).
133. S.E. Tracht, L. Cruz, C.M. Stobbawiley, and J.V. Sweedler. Detection of radionuclides in capillary electrophoresis using a phosphor-imaging detector. *Anal. Chem.* **68:** 3922–27 (1996).
134. W. Tong and E.S. Yeung. Determination of insulin in single pancreatic cells by capillary electrophoresis and laser-induced native fluorescence. *J. Chromatogr. B* **685:** 35–40 (1996).
135. S.J. Lillard, E.S. Yeung, and M.A. McCloskey. Monitoring exocytosis and release from individual mast-cells by capillary electrophoresis with laser-induced native fluorescence detection. *Anal. Chem.* **68:** 2897–2904 (1996).
136. S. Nussberger, F. Foret, S.C. Herbert, B. Karger, and M.A. Hediger. Nonradioactive monitoring of organic and inorganic solute transport into single *Xenopus* oocytes by capillary electrophoresis. *Biophysics Journal* **70:** 998–1005 (1996).
137. O. Orwar, H.A. Fishman, N.E. Ziv, R.H. Scheller, and R.N. Zare. Use of 2,3-naphthalenedicarboxaldehyde derivatization for single-cell analysis of glutathione by capillary electrophoresis and histochemical localization ion by fluorescence microscopy. *Anal. Chem.* **67:** 4261–68 (1995).
138. Q.F. Xue and E.S. Yeung. Determination of lactate-dehydrogen isoenzymes in single lymphocytes from normal and leukemia-cell lines. *J. Chromatogr. B* **677:** 233–40 (1996).
139. M.A. Moselev, L.J. Deterding, K.B. Tomer, and J.W. Jorgenson. Determination of bioactive peptides using capillary zone electrophoresis/mass spectrometry. *Anal. Chem.* **63:** 10914 (1991).
140. W. Nichols, J. Zweigenbaum, F. Garcia, M. Johansson, and J. Henion. CE–MS for industrial applications using a liquid junction with ion-spray and CF-FAB mass spectrometry. *LC–GC* **10:** 676–86 (1992).

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