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Review

Overview of capillary electrophoresis and capillary electrochromatography

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Abstract

This paper provides an overview on the current status of capillary electrophoresis (CE) and capillary electrochromatography (CEC). The focus is largely on the current application areas of CE where routine methods are now in place. These application areas include the analysis of DNA, clinical and forensic samples, carbohydrates, inorganic anions and metal ions, pharmaceuticals, enantiomeric species and proteins and peptides. More specific areas such the determination of physical properties, microchip CE and instrumentation developments are also covered. The application, advantages and limitations of CEC are covered. Recent review articles and textbooks are frequently cited to provide readers with a source of information regarding pioneering work and theoretical treatments.

Keywords: Reviews; Capillary electrophoresis; Electrochromatography

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1. Introduction

The ability to obtain high separation efficiencies by the application of a voltage across a capillary was highlighted in the early 1980s. The late 1980s and early 1990s saw the advent of commercial capillary electrophoresis (CE) instrumentation and the transformation of a research technique into a routine technology in many industrial environments. The late 1990s have seen a broadening of the range of separation mechanisms in CE and instrumentation developments aimed at addressing practitioner's needs. The late 1990s have also witnessed an expansion of interest in the possibilities that capillary electrochromatography (CEC) may offer to the analytical scientist. The universal drive towards miniaturisation has had its impact in CE and considerable research effort is currently invested in developing and exploiting microchip CE devices.

There are thousands of CE instruments installed in laboratories worldwide and applications of CE have been reported in every major industry. Currently there are in excess of 7000 publications describing CE applications and developments. Currently there are about 300–400 publications for CEC but this number is sharply increasing. The purpose of this article is to present the current status of CE and CEC and to attempt to predict the likely trends and developments as we move into the next millennium of separation science.

It is interesting to look back over the development and implementation of CE. The early applications of CE focussed on the separation of biomolecules such as proteins and peptides where electrophoresis was the traditional method of analysis and use of CE was viewed as an extension of previous methodology. The analysis of small molecules such as pharmaceutical and agrochemicals was not routinely performed by electrophoresis and high-performance liquid chromatography (HPLC) was firmly established in these areas as the workhorse technique. CE has gradually become established as an alternative and support technique for HPLC and currently over 50% of the CE instruments worldwide are located in pharmaceutical companies.

Electrophoresis has not typically been applied to the analysis of water-insoluble neutral species and chromatographic techniques were normally employed in this area. Recognition of this limitation led to the development of micellar electrokinetic capillary chromatography. Capillary electrochromatography (CEC) has also shown itself to be capable of generating highly efficient and rapid separations of insoluble neutral compounds.

CE has been successfully used in a wide range of application areas. The most frequent is the analysis of pharmaceuticals. DNA separations are also popular and these are generally conducted by capillary gel electrophoresis in which gel- or polymer-filled capillaries act as sieving media to resolve the different lengths of DNA. Proteins and peptides are also popular analytes and methodologies in this area include sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) and capillary isoelectric focusing (cIEF). A number of suppliers sell reagents and kits; especially in the areas of SDS–PAGE and cIEF.

The widespread uptake of a new technique depends on a great many factors which may support or oppose its progress. For example, despite some initial resistance, HPLC has become the standard technique in many industries as it offered compelling advantages over other techniques available at the time, e.g., column and thin-layer chromatography. HPLC offered an automated, precise, sensitive and reliable means of analysing a very wide range of analytes. Industrial laboratories have therefore invested heavily in HPLC equipment and staff training. Many industrial analysts had to develop optimised HPLC methods and working practises to prove to their companies the worth of HPLC. Early HPLC equipment and practises were improved and refined. Working in 1980 we packed our own HPLC columns and performed manual syringe injections. This situa-

tion should be compared to present day sophisticated HPLC autosamplers and the wide range of suppliers of inexpensive HPLC columns. So where is CE in terms of itself development when compared to HPLC? It can be broadly considered to be in a refining stage – with routine implementation in some areas. In areas such as chiral analysis, DNA analysis, clinical applications and metal ion/inorganic anion analysis CE can often be shown to be a clear improvement over rival techniques in terms of efficiency, reliability and cost and routine methods have been established. As with all techniques CE has had to become ''approved'' in the eyes of the establishment. CE methods are now accepted in law courts, forensic analysis laboratories, submissions to drug regulatory authorities and pollution monitoring agencies.

So why has there been an interest in CE and CEC? HPLC is a well established technique in many of the areas in which CE and CEC are applied. Do these techniques offer advantages over traditional analytical methods or are they simply a more modern alternative means of analysis? There are a number of commercial and efficiency possibilities that CE and CEC have to offer over HPLC in particular. These can include reduced method development time, reduced operating costs and solvent consumption, higher separation efficiencies and analysis of solutes with limited UV chromophores. The major disadvantages cited against CE are mainly instrument related and include poorer injection precision and sensitivity when compared to HPLC. However, in industrial environments, CE development is restricted more by lack of training and experience, and the huge commercial investments that have already occurred in HPLC by these companies.

The future of CE seems assured as it now forms an established segment of analytical chemistry undergraduate and postgraduate courses. There are also a number [1–12] of general textbooks and monographs covering specific aspects of CE such as the analysis of carbohydrates, DNA and pharmaceuticals. Articles relating to CE appear in all major analytical journals and there is a specific *Journal of Capillary Electrophoresis* (http://www.isc-uk.demon.co.uk/jce.html). There are also a number of well established meetings focusing on CE and CEC, the most notable being the ''HPCE series'' (http://www.uni-saarland.de/matfak/fb12/HPCE2000/). There are a number of sources of information available on the internet relating to CE. These website links are summarised in a website (http://www.ceandcec.com/). A number of educational primer booklets and CDs are also available from CE instrument suppliers upon request. These primers provide general overviews on CE or cover specific application areas or techniques. The recent extensive review by Watzig et al. [13] excellently summarises method development approaches in CE and provides over 800 references and is recommended reading.

This paper focuses on the application areas of CE and is by no means exhaustive given the enormous scope of CE and CEC. Wherever possible reference is made to recent review articles where interested readers can find information relating to the pioneering work in that area and background theoretical treatments.

2. Method development options

Initially simple buffer solutions were employed to separate ionic water-soluble solutes. However, as CE has developed, the range of solute types has greatly expanded, and the chemistry used to obtain separations has become more elaborate. Recent reviews [13–15] summarise the range of selectivity manipulation options available in CE. These include organic modifiers, surfactant additions, ion-pair reagents, cyclodextrins [16], polymer additives, addition of complexing agents and combinations of these possibilities. To this extent the method development possibilities are now as extensive as those in HPLC. CE initially suffered from a limited ability to separate poorly water-soluble compounds but this has been addressed by use of non-aqueous CE, micellar electrokinetic chromatography (MEKC) and microemulsion electrokinetic chromatography (MEEKC). A range of commercially available internally coated capillaries has also become available which further enhances the selectivity possibilities. These coatings can enhance, eliminate or reverse the electroosmotic flow (EOF) that is generated in the capillary when the voltage is applied. The coating can also greatly reduce band broadening which may sometimes occur due to solute–wall interactions.

Developments in instrumentation have also occurred in recent years; most notably improvements in the reliability and sensitivity of instruments, which has helped in routine uptake of the technique. Instrumentation developments are covered in Section 13.

2.1. Micellar electrokinetic chromatography

Neutral solutions that cannot be resolved by CE using simple buffer. Therefore micellar surfactant solutions are used in MEKC to separate neutral components chromatographically. The surfactants are used at sufficiently high concentrations that the surfactant molecules group together to form micelles. Neutral solutes partition with these micelles in a chromatographic fashion and are separated based on their retention factors similar to reversed-phase HPLC. Generally anionic (negativity charged) micelles are formed using solutions of SDS above 10 m*M* concentration. However, different selectivities can be obtained [13] if different surfactants are used. Alternative surfactants include anionic surfactants such as bile salts, cationic surfactants such as cetyltrimethylammonium bromide and neutral surfactants such as Tween. Alternatively a charged additive such as an anionic cyclodextrin can be used instead of a surfactant to separate neutral solutes.

Water-insoluble compounds have high retention factors and organic solvents are added to the MEKC buffer to enhance their resolution. Selectivity can also be manipulated [13] by factors such as surfactant concentration, addition of urea, cyclodextrins, ion-pair reagents, temperature, pH, buffer type and concentration. Several of these factors may be optimised for a complex separation and experimental design schemes have been employed in certain instances.

A recent development in MEKC has been [17] to perform the separations in the absence of EOF. This may be achieved using coated capillaries or at low pH. This is particularly useful for acidic species which would be ionised at high pH and would not interact with a negativity charged SDS micelle.

2.2. Microemulsion electrokinetic chromatography

This technique is similar to MEKC and separates neutral solutes [18] based on their chromatographic retention factors. The solutes partition between the aqueous phase and oil droplets, which are moving through the solution. Fig. 1 shows a schematic of the separation principle. The microemulsion composition most frequently used involves a high pH buffer, octane, butan-1-ol and SDS. The water-immiscible octane forms minute oil droplets that are coated with SDS and butan-1-ol. The SDS reduces the surface tension of the solution which allows the microemulsion to form and the presence of the butan-1-ol stabilises the emulsion. The oil droplet is negatively charged and migrates against the EOF. Poorly water-

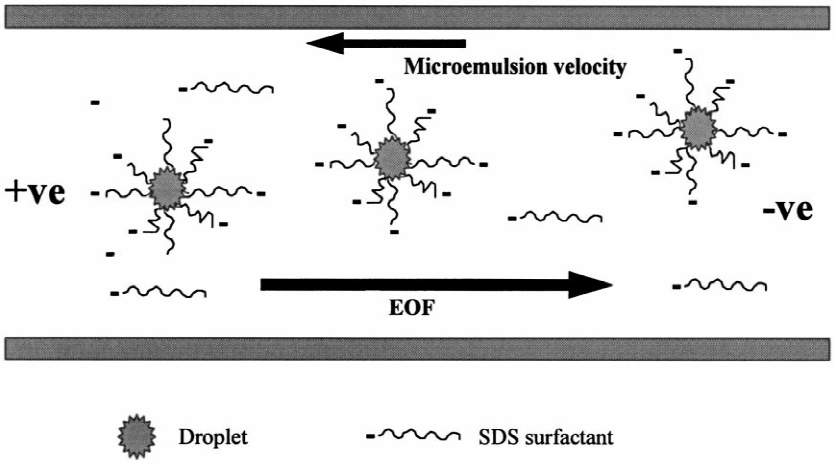

Fig. 1. Schematic of microemulsion electrokinetic chromatography.

soluble compounds partition strongly into the droplet and are highly retained with large retention factors. Ionised solutes can both partition with the oil droplet, migrate due to their charge, and may have an ion-pair interaction with the charged droplet. Selectivity can be altered by similar factors to those described above for MEKC. In addition the type and concentration of the oil can also be varied. The reports of the use of MEEKC are limited [18] compared to MEKC but the technique is highly applicable [18] to a broad range of solutes and can be considered as an alternative to MEKC, especially for water-insoluble compounds.

2.3. Non-aqueous capillary electrophoresis

The use of organic solvents as the electrolyte is a relatively recent development in CE with the majority of reports appearing after 1996. Typically [19] electrolytes are prepared in 100% organic solvents such as methanol or acetonitrile. Use of non-aqueous solvents affects the solvation of the ions and also alters the pK_a values of the solute. Therefore the selectivity can be adjusted by varying the organic solvent used or by using solvent mixtures. This ability allows fine-tuning of selectivity for charged components. Selectivity can also be altered [19] by the use of additives such as ion-pair reagents [20], cyclodextrins and surfactants into the non-aqueous electrolyte.

2.4. Electrolyte additives

A range of additives have been employed [21] to alter the separation selectivity in CE. These additives have some complexation interaction with the solute which affects its migration properties. Ion-pair reagents have been used [22] to form ion-pair complexes with charged solutes. Cyclodextrins have been employed [16,23] in both separation of chiral and non-chiral solutes. The use of cyclodextrins for chiral separations will be discussed in Section 8. Selectivity can often be altered [24] by using combinations of cyclodextrins added to the buffer. Cyclodextrins are useful for resolution of closely related species such as positional isomers which have similar electrophoretic mobilities but different retention factors. Organic acids or metal ions can also be added to the buffer to form solute-complexes with appropriate alteration in selectivity. Proteins may also be added [25] into the electrolyte to alter selectivity, or to study protein–drug binding.

3. Analysis of DNA and nucleic acids

The analysis of DNA has traditionally been performed by slab-gel electrophoresis methods. CE was viewed as having potential benefits over these methods as higher voltages could be applied as the Joule heating was effectively dissipated. On-capillary detection is also possible in CE which eliminates the need for staining and destaining procedures used in slab-gel. Sophisticated CE autosamplers are available which can quantitatively inject and analyse a large number of samples in an unattended sequence. However, slab-gel electrophoresis has an advantage in that it is a parallel technique and several samples and standards can therefore be run simultaneously. High detection sensitivities can be obtained in slab-gel methods by use of staining techniques (e.g., silver staining). Developments in CE technology have addressed these technical issues and commercially available capillary array instruments and highly sensitive laser-induced fluorescence (LIF) detectors have been introduced.

The analysis of DNA by CE has therefore been [26,27] an area of intense interest. Readers with a particular interest in the CE analysis of DNA and other nucleic acids are referred to the monograph [6] on the subject area. This activity has impact on a great many industries [27] including forensic analysis, biotechnology product development, pharmaceuticals, human genetics, quantitative gene dosage, microbiology and virology. Considerable research effort has centred on the development of novel separation media for the resolution of DNA mixtures. DNA of different lengths has similar electrophoretic mobilities as each increase in size is accompanied by a corresponding increase in the number of negative charges. Therefore separation by mobility differences is not the preferred approach and the majority of separations are achieved using a sieving mechanism. The capillary is filled with a matrix of synthetic or natural polymer. The various DNA fragments migrate through this matrix and become

entangled with, or trapped in, the matrix. The migration of the large DNA fragment is retarded to a greater extent and which results in a size-based separation mechanism. This is analogous to gel permeation chromatography.

A variety of polymers are used [6,28] to generate the sieving media include agarose, polyacrylamide, and solutions of celluloses such as hydroxyethylcellulose. Other developments include temperature dependent polymers [29]. Initial work in CE had focussed on the use of gels filled with cross-linked polyacrylamide gels. These early gel-filled capillaries suffered from problems such as shrinkage, drying-out and the inability to remove any contaminant that may be introduced into the capillary. The capillaries therefore had finite operating times and had particular operating requirements such as electrokinetic injection due to their high back-pressures and the need to store the capillaries filled with electrolyte in the fridge between injections. The use of liquid gels or polymer solutions has therefore been widely welcomed as these can offer improved routine performance and reliability.

In order to identify large pieces of DNA they are often reacted with a specific enzyme to generate a digest mixture of small DNA fragments. These fragments give a characteristic profile and CE is commonly used for this type of profiling. Use of pulsed a.c. voltages can allow [6,27] separations of DNA mixtures that are unresolved using d.c. voltage. The various DNA fragments stretch and recoil during the application of a.c. voltage. The rate at which they regain their original conformation affects their migration rate and can allow resolutions to be achieved. In particular, highly dilute polymer solutions can be used [30] to resolve larger DNA fragments – these solutions generally separate by entanglement rather than entrapment of the DNA.

Several CE instrument companies offer standard kits to perform DNA separations. Normally these are buffered solutions of polyacrylamide or cellulose and are used in conjunction with coated capillaries. The capillaries are usually coated to eliminate the EOF. The DNA is negatively charged and negative voltages are therefore used in the separations.

DNA analysis by CE can be used [31,32] in clinical chemistry for aspects such as prenatal screening for point mutant in DNA which are indicative for disorders such as cystic fibrosis and thalassemia. Nucleotides have been quantified [6] in different matrices, including tissue and cell extracts and several DNA and RNA sources. Therapeutic antisense oligonucleotides are of interest to many pharmaceutical companies and CE can be used [32] to characterise and quantify these materials. Larger portions of DNA can also exist in various topoisomers such as open-circular, nicked and super-coiled forms. When these various topoisomers degrade they can interconvert or revert to linear DNA. CE can be used [6] to resolve and quantify these species which is again of interest to pharmaceutical companies involved in this area.

Capillary arrays are used [33,34] in DNA sequencing of samples. Tens or hundreds of capillaries are analysed simultaneously [33,35] to produce high throughout DNA sequencers. These arrays are often coupled with highly sensitive laser induced fluorimeters to achieve highly efficient separations. Interchelator dyes are often added to the buffer solution to form fluorescent complexes with the DNA.

4. Clinical, biomedical and forensic applications

The high separation efficiencies obtained in CE make it an attractive separation technique in clinical applications [36–39] where the sample matrix may be extremely complicated and contain a number of endogenous components which have to be resolved from the solute of interest. A recent volume of the *Journal of Chromatography B* has covered the use of CE for forensic and clinical toxicology [40].

The rugged nature of the CE capillary allows it to be rinsed and cleaned between injections with relatively harsh solutions such as NaOH. This can allow direct injection of biofluids with possible reductions in sample pretreatment requirements which are attractive in clinical chemistry where sample throughput is high. The major disadvantage of CE in this area is its lack of concentration sensitivity compared to HPLC. Various approaches to this sensitivity limitation have been developed [41]; most notably the use of pre-concentration devices [36] which will be discussed later in this section. Other approaches to increasing method sensitivity include [42] protein precipitation, liquid–liquid extraction or solid-phase

extraction pretreatment, and optimised CE sample injection techniques such as field amplified sample injection.

Pre-concentrator devices have been developed [41] for analyte pre-concentration on-line with the CE capillary. These devices have been used primarily for non-specific analyte pre-concentration using packing material of the C_{18} type. Alternatively, the use of very specific antibody-containing cartridges and enzyme-immobilized microreactors have been demonstrated. The pre-concentrators are usually physically attached to the injection end of the capillary.

CE is widely used [43] for analysing proteins in physiological matrices, such as serum, urine, and cerebrospinal fluid and is challenging the use of traditional gel electrophoresis methods. CE especially benefits from the high sample throughput possibilities due to high capacity autosamplers.

The profiling of small compounds such as amino acids and organic acids in the serum, urine or tissues of patients can provide useful indication of patient disease state. CE has therefore found use [44,45] in the clinic for this type of application. CE has benefits of high resolution, simplicity, versatility, low operating costs and the possibility of direct sample injection without complex sample pretreatment. Another example in this area [46] is the determination of lipoproteins and apolipoproteins in the diagnosis of disorders of lipoprotein metabolism.

The use of microchip CE devices is particularly attractive for clinical applications [47] as this may offer rapid analysis of several samples simultaneously. Mass produced microchip devices may be cheap enough to become consumables. The application of microchip CE systems to human serum protein analysis, immunoassay, and DNA studies has been reviewed [47], along with various other clinical applications.

A number of papers have described [40,48,49] the use of CE in forensic analysis and clinical toxicology. Validated methods have been used to profile forensic specimens and to determine drugs of abuse in forensic samples. CE has been used to determine illicit and/or misused drugs in biological samples [50] including blood, saliva and hair. Often drug extraction, drug concentration (stacking) and sensitive detection methods are needed [50] to obtain the sensitivities required in this type of analysis. Drug identification can be confirmed by on-column multi-wavelength absorbance or CE coupled to mass spectrometry (MS).

5. Carbohydrates

At first thought CE would be considered inappropriate for carbohydrate analysis. Many carbohydrates have no ionisable functions or UV chromophores. However, CE has found great application [1,51] in this area. Carbohydrates can be detected using low UV wavelength detection, indirect UV detection or by formation of UV absorbing or fluorescent carbohydrate derivatives. Many carbohydrates possess acidic hydroxyl groups which ionise at high pH values (pH 10–12) and can therefore be successfully analysed as anions. Alternatively chelation of the carbohydrate with a suitable ion can be used to surmount both the problem of an uncharged analyte and limited chromophore. For example borate buffer is widely used for the analysis of carbohydrates. Borate complexes with adjacent hydroxyl groups on carbohydrates to form a negatively charged complex which has UV activity at low UV wavelengths. Readers are referred to the recently published book on the analysis of carbohydrates by CE [1] and a comprehensive review [51] on the subject. CE has been used to analyse various carbohydrates including mono- and oligosaccharides, glycoproteins, glycopeptides and glycosaminoglycans. Cyclodextrins can also be analysed by CE [52] by a variety of approaches.

6. Metal ions and small organic/inorganic anions

This area has recently been the subject of a recent volume of the *Journal of Chromatography A* [53] which provides an up-to-date overview of the background principles and applications in this popular area of CE analysis. This type of analysis was routinely performed by ion chromatography. CE is highly appropriate for these determinations as the test solutions are usually aqueous and the solutes are small, often highly charged ions. Generally the solutes have no chromophores and are detected in

CE using indirect UV detection. Standard separation conditions have been developed by commercial supplier companies and metal ion and inorganic anion test kits are commercially available.

6.1. Metal ions

Recent reviews [54,55] summarise the present status and background of metal ion analysis by CE which are generally determined using indirect UV detection. A small positively charged UV active species such as imidazole is added to the indirect detection buffer. Selectivity can be altered [56] by the addition of complexing agents such as small organic acids or crown others. Generally a low pH buffer and uncoated capillaries are used. Low ppm (mg/l) detection limits are typically obtained but these can be considerably improved using electrokinetic injection if the sample has a low conductivity matrix.

6.2. Inorganic anions and simple organic acids

This subject is covered by a recent review [57] which contains 293 references. Small inorganic anions and simple organic acids have high negative mobilities, which oppose the typical EOF direction of an uncoated capillary. This counter-migration would result in long migration times and highly diffused peaks. Therefore capillaries are commonly coated to reverse the typical EOF direction. Application of a negative voltage to the coated capillary then results in both the direction of the EOF and solute migration towards the detector which produces fast and efficient separations. Typically [58] a cationic surfactant such as tetradecyltrimethylammonium bromide (TTAB) or cetyltrimethylammonium bromide (CTAB) is added to the electrolyte. A bilayer of the positively charged TTAB absorbs onto the capillary wall which results in the capillary becoming positively charged and causes the EOF direction to become reversed. Indirect UV detection is achieved by the use of UV active anions such as chromate phthalate, or pyridine dicarboxylic acid, Fig. 2 shows separation of a range of simple anions using pyridine dicarboxylic acid as the UV absorbing species.

7. Pharmaceuticals

The analysis of pharmaceuticals is predominantly performed using HPLC and several application areas have been developed including main component analysis, determination of drug-related impurities, chiral separations, trace level determinations and quantitation of metal ions and inorganic anions. These application areas are equally possible by CE. Therefore CE is generally considered as a complementary or alternative technique to HPLC for pharmaceutical analysis. CE methods have been successfully validated and have been included in submissions to regulatory authorities. CE is also included [4] in pharmacopoeias. Extensive details of the use of CE in drug analysis can be found in recent monographs [4,10] on the analysis of drugs by CE. General review papers are also available [59,60] and recent issues of *Electrophoresis* [61] and the *Journal of Biochemical and Biophysical Methods* [62] were devoted to drug analysis by CE.

The majority of pharmaceuticals are bases and are protonated (positively charged) at low pH. Therefore a low pH buffer such as phosphate pH 2.5 can be applied to analysis of a range of basic drugs. For example a low pH phosphate buffer has been used [63] in forensic analysis to analyse 550 basic drugs using a single set of operating conditions. Acidic drugs can be resolved [4] using pH 7–10 borate or phosphate buffers. Neutral compounds, or mixtures of charged and neutral compounds, generally require the use of MEKC or MEEKC [18] methods. CE has been applied to all major drug classes and specific reviews have been published on the application of CE to the analysis of antibiotics [64] and sulphonamides [65].

Validation of pharmaceutical analysis CE methods [66] is a similar activity to HPLC method validation and involves assessment of validation parameters such as accuracy, precision, specificity, linearity and sensitivity.

A recent review [67] has covered the use of CE for purity control of pharmaceuticals. Generally HPLC remains the technique of choice in this area and CE is often used as a supporting orthogonal method. The major difficulty that CE faces in this area is that drug-related impurities generally have similar charge-to-mass ratios and are therefore dif-

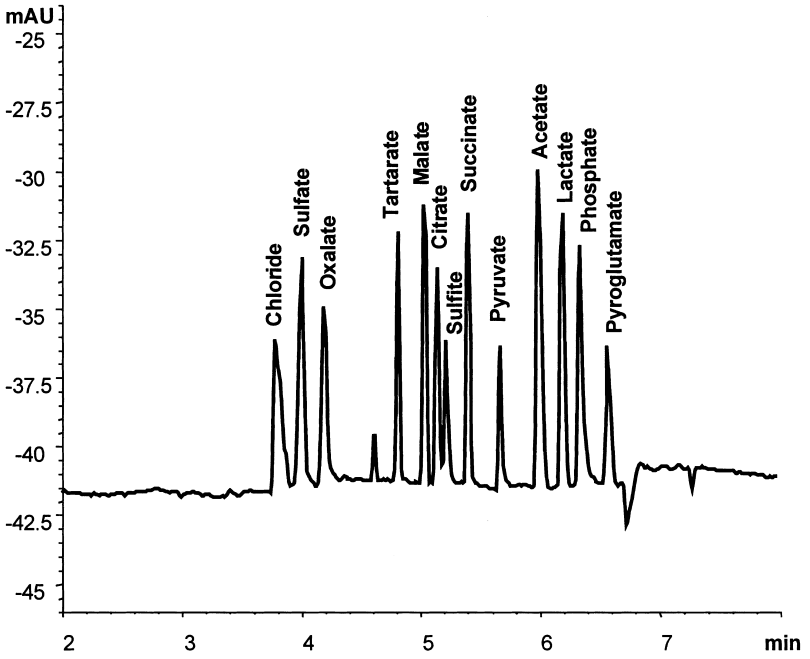

Fig. 2. Separation of a range of organic and inorganic anions using indirect UV detection. 5 m*M* 2,6-Pyridinedicarboxylic acid, 0.5 m*M* cetyltrimethylammonium bromide, pH 5.6; sample, each 25 mg/l; capillary, 80.5 cm (effective length 72 cm)×75 μm I.D.; injection, 100 mbar s, 20°C, −25 kV (reversed polarity); detection sig., 350/20 nm, ref., 200/10 nm. Reproduced with kind permission of Dr. D. Heiger of Hewlett-Packard Inc.

ficult to resolve from each other and from the main drug peak. Additionally if the impurity is neutral it will not be quantified. Improved selectivity and analyte range can usually be obtained using MEKC or MEEKC methods.

Peptide-based drugs are particular suited to analysis by CE [68] as they are ionised at both low and high pH values. The separations can also be conducted [68] using cIEF. Chiral analysis of peptide drugs is also readily achieved by CE.

The CE analysis of both water-soluble and insoluble vitamins has been reported [66] using a variety of approaches. The majority of vitamins are acidic and can separated using high pH borate or phosphate buffers. MEEKC [18] or MEKC methods are required to resolve the fat-soluble neutral vitamins.

8. Chiral separations

The separation of enantiomers by CE has proven to be one the most successful application areas. Chiral separations can be performed by HPLC but CE can offer significant advantages in terms of speed of method development and cost of analysis. The chiral selectors used in CE mimic those used in HPLC and include natural and derivatised cyclodextrins, carbohydrates, proteins, antibiotics and crown ethers. Specific chiral additives, in particular sulphated cyclodextrins and synthetic chiral surfactants, have been developed for use in CE. Chiral CE methods have been successfully validated and are in routine use in many industrial laboratories. For instance these methods have been used to measure the chiral purity of drugs and pesticides or to measure the chiral ratio of drugs in biofluids. Chiral separations have been the theme of a special issues of *Electrophoresis* [69]. The principles of chiral CE separations have been reviewed extensively [70,71].

Cyclodextrins are by far the most widely used chiral additive in CE as they are relatively cheap, water-soluble, and have low UV activity which permits use of low UV wavelengths for sensitive detection. Fig. 3 shows the structure of a β-cyclo-

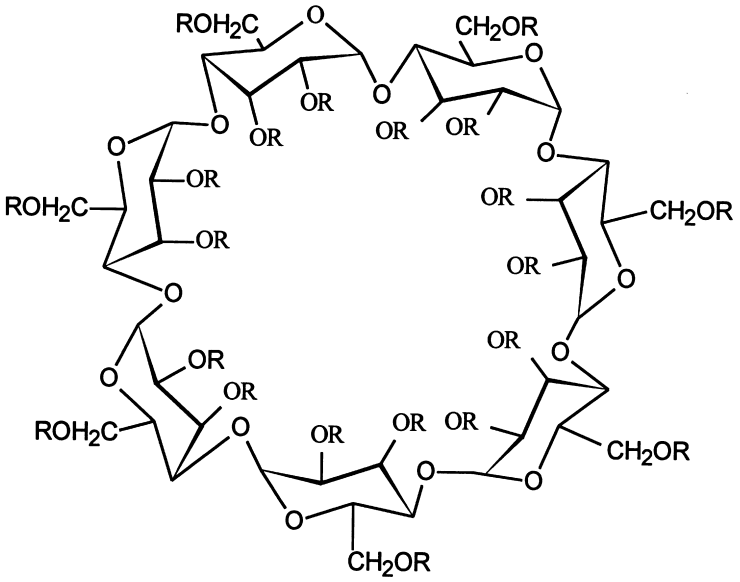

Fig. 3. Chemical structure of β-cyclodextrin.

dextrin, which is composed of seven fused sugar units. In the naturally occurring β-cyclodextrin the R groups on Fig. 3 are hydroxyl groups. These hydroxyl groups can be chemically converted to groups such as methyl and hydroxypropyl. The background to the use of cyclodextrins in chiral CE has been the subject of two recent review papers [72,73]. Generally separation conditions are developed which gives an acceptable peak shape and analysis time for the solute. A range of different types and concentrations of cyclodextrin are then added to the electrolyte in an attempt to develop the chiral separation. This approach can often lead to very rapid method development times. This is important, especially in the pharmaceutical industry where there is often a large number of compounds being examined.

There are a number of ionisable cyclodextrins used [74] in chiral CE which include aminated, sulphated and sulphobutylated. These are chemically modified natural cyclodextrins where the R groups in Fig. 3 are replaced by an ionisable function. These were originally developed to allow chiral resolution of neutral compounds but they can often produce enhanced resolutions for ionised solutes when compared to uncharged cyclodextrins. Highly purified single-isomer forms of charged cyclodextrins are available [75], which can produce exceptionally large chiral resolutions and can be applicable to a wide range of solutes.

Crown ethers are used [76,77] for the separation of chiral compounds having a primary amino function. They have been shown to be especially useful for amino acids and basic drugs which are separated at low pH. The crown ether forms a hydrogen-bond stabilised complex with the protonated primary amines. If the complexes of individual enantiomers have different stabilities then a chiral resolution can be achieved. The most commonly used crown ether is 18-crown-6-tetracarboxylic acid [18C6H(4)]. Combinations of crown ethers and cyclodextrins have been shown [78] to give the possibility of enhanced chiral resolutions.

Natural or synthetic chiral surfactants can be added [79] to the separation buffer in order to achieve chiral separations in CE. The most frequently used natural surfactant type are bile salts such as sodium cholate. Bile salts have a steroidal-type structure and form sheet-like micelles and are particularly useful for chiral separations of larger, less water-soluble chiral solutes. Synthetic surfactants such as *N*-dodecoxycarbonylvalines [80] are specifically manufactured to generate compounds which are able to form highly stereoselective micelles with low UV absorbances.

A number of noncyclic oligo- and polysaccharides such as dextran and heparin have been used [81] as chiral additives in CE. Various proteins have also been used [82] as chirally selective additives in CE separations. The various enantiomers bind to the proteins to different extents which alters their migration rate and results in a chiral separation. Examples of proteins employed in chiral CE include [82] bovine serum albumin and acid glycoprotein (AGP).

Macrocyclic antibiotics such as Rifamycin and Vancomycin possess many chiral centres and have been used [83,84] to give high enantioselectivities in chiral CE. These compounds do however possess a large amount of aromaticity. Use of optimised operating procedures or indirect UV detection is therefore necessary [83,84] to overcome this UV absorbance difficulty.

The most common application of chiral CE is the chiral purity testing of drug substance material. Typically a single enantiomer material is manufactured and a method is used to determine levels of the undesired enantiomer. CE methods can routinely detect levels of the undesired enantiomer in the region of 0.1–1% area/area. Other applications include the monitoring of the metabolism of chiral drugs where the chiral purity of the drug or metabolites is determined [85] in biological fluids.

9. Proteins/peptides

Proteins have traditionally been analysed by electrophoresis using slab-gel techniques. Separations may be based on protein physicochemical properties such as size, charge, isoelectric point (the pH at which the positive and negative charges of a zwitterionic solute exactly neutralise each other) and immunoresponses. The performance of CE has recently been [86] compared to the established electrophoresis techniques. The principal advantages of CE over previous gel methods relate to the time and labour saved by the automated CE instrumentation. Standard method kits are available for CE which are often used in combination with coated capillaries.

CE techniques for the analysis of protein include [87,88] IEF, MEKC, CE–MS and capillary gel electrophoresis. Recent reviews have specifically covered [89,90] the application of IEF to protein analysis. Applications include purity determination of the protein, and the assay of the protein level contained in dosage forms. Proteins are often identified by enzymatic cleavage of proteins and the subsequent examination of the peptides generated [91] by CE.

Adsorptions of proteins onto the capillary wall can cause peak tailing and loss of separation and a variety of approaches have [92] been developed to overcome this problem including use of buffer pH extremes, coated capillaries and zwitterionic buffer additives.

Detection sensitivity of proteins/peptides can be improved by sample pretreatment, sample derivatisation and LIF. MS is also applied in protein/peptide analysis [93] as this can yield significant additional information relating to the structure of the separated protein. Use of CE–MS–MS gives yet further structural information.

CE has particularly been applied [94] to the analysis of recombinant proteins which are cloned and expressed in large quantities using bacterial, yeast or mammalian systems. CE is used to analyse these proteins [95] following their purification using column chromatography. In particular levels of mutant or variant protein forms and glycoforms are assessed. CE has been applied to the analysis of recombinant glycoproteins [96] and hydrophobic proteins [97]. Workers at Genentech and co-workers have been successful [98] in their routine application of CE to protein and peptide analysis.

10. Affinity capillary electrophoresis and immunoassays

Affinity capillary electrophoresis (ACE) is a broad term referring to the separation by CE of substances that participate in specific or non-specific affinity interactions during their electrophoretic movement along the capillary. The interacting molecules can either be electrolyte additives or substances immobilized to a solid support. Examples of studies undertaken include [99,100] antigen–antibody, hapten–antibody, lectin–sugar, drug–protein and enzyme–substrate complexes. Binding constants can be measured using this approach and a recent review [101] has covered this aspect in detail focusing on drug–

protein binding studies. These drug–protein studies can also be used [101] to determine the binding differences between individuals enantiomers.

An extension of this type of analysis is the screening of combinatorial libraries. These libraries are used in drug discovery and are generally robotically prepared complex mixtures of synthetic solutes such as peptides. The libraries are tested for pharmacological activity by exposure to a target substrate such as an enzyme. These libraries can be assessed [102] by CE by injecting the enzyme and library mixture at different ends of the capillary. Separation conditions are employed such that the enzyme and library compounds move in different directions along the capillary. If there is no interaction between the compounds then they separate in the same order as when no enzyme is injected. If a solute interacts with the enzyme then its migration time will be extended (Fig. 4). Use of MS allows [103] the identification of the solute having an interaction. The interacting compounds can then be individually prepared and tested for pharmacological activity

There are several immunoresponse based assays possible [104,105] by CE. Many CE techniques have been used in this area [104,105] including cIEF, gel-electrophoresis, MEKC and ACE. Sensitive detectors such as LIF and fully automated instruments offer advantages over traditional methods of analysis. Increased sample throughput may be possible using multicapillary array or microchips with multiple channels.

11. Determination of solute physical properties

The dissociation constant of a compound can also be determined [106] from migration time data. The solute is analysed using electrolytes covering a range of pH values. The mobility of the solute can be calculated directly from the migration time and EOF times. A plot of the mobility versus pH is constructed and the pH at a point corresponding to 50% of the mobility values can be calculated which equates to the solute pK_a value.

The hydrophobicity of a drug is an important property as it governs the transport properties of the

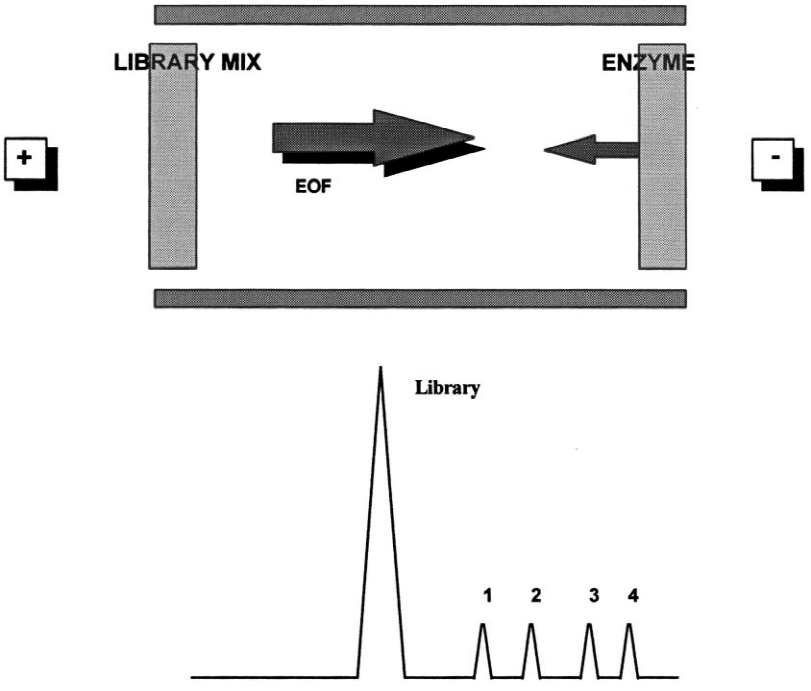

Fig. 4. Screening of combinatorial library using affinity CE.

drug within the body. The hydrophobicity of a compound can be expressed as its partition coefficient, *P*, between water and an immiscible, non-polar solvent. Octanol is a typical solvent used and log P_{ow} values are determined by a ''shake-flask technique''. Data from migration times obtained by MEKC [107] or MEEKC [108] has been correlated with log P_{ow} values. A highly hydrophobic compound will be strongly retained by the micelles or oil droplets used in MEKC and MEEKC, respectively and would have a high log P_{ow} value.

Binding constants between solutes and additives such as micelles, cyclodextrins, antibiotics, proteins, RNA, DNA can be determined [109] by CE. Generally the migration time of the compound is determined in the electrolyte containing no binding agent. The migration time is then determined in the electrolyte containing the binding agent. A change in migration time can be related to the binding constant associated between the test solute and the binding agent.

12. Microchip capillary electrophoresis and capillary arrays

There is a large volume of activity [110–112] in the area of microchip CE as this could offer great possibilities in the area of fast and inexpensive test equipment for clinical analysis, DNA and protein analysis. Generally entire CE systems are constructed [110–112] on glass or plastic chips. Gel-filled channels in the microchips can be used for DNA analysis. These devices contain [113] buffer and sample channels and have a connection to a detection system. Detection may be accomplished by a post-separation derivatisation, however appropriate use of buffer additives may also be used to generate derivatised species within the separation channel. It is anticipated that the current rate of progress and interest in this area will lead to major advances and to the widespread use of commercial systems in the mid-term future.

CE is a sequential technique, which allows analysis of one sample per analysis, which limits the sample throughput. One remedy to this problem is the use of capillary array instruments [33–35,114] which incorporate a number of capillaries. Alternative microarrays are possible [114] where the microchip has several separation and injection channels. This allows multiple samples to be analysed in a single analysis. Application of these technologies include molecular diagnostics, sequencing by hybridisation, cDNA expression profiling, comparative genome hybridisation and genetic linkage analysis and parallel DNA sequence analysis and genotyping. DNA sequencing by capillary array electrophoresis [31] is the focus of a recent paper.

A commercial capillary array instrument is routinely used [115] to profile serum proteins. Several capillaries are simultaneously employed to analyse protein standards and serum samples and relative standard deviations (RSDs) have been reported [115] of $<2\%$ for albumin and γ-globulins and 4–7% for α(1)-, α(2)-, and β-globulins.

13. Instrumentation developments

The increased uptake of CE in recent years has partially been due to advances in the instrumentation availability and performance. Readers particularly interested in the instrumentation associated with CE are referred to the monograph by Kok [2] which deals with this subject. Most notably the detector sensitivity has been improved through use of developments such as fibre optics, reduced noise capillary modifications and an off-capillary flow cell [116]. Limited improvements have been achieved with respect to injection precision but CE still gives poorer injection precision than HPLC unless an internal standard is used. Use of an internal standard is recommended for all quantitative analysis as it corrects for many factors including viscosity and surface tension differences between samples and standards. As CE becomes more routinely used industrial practitioners and instrument suppliers need to address the long-term aspects associated with the techniques. For example there is gradual deterioration of the buffer over the course of a number of injections due to electrolysis effects. This problem has been termed ''buffer depletion'' and instrumentation companies have development several solutions including a replenishment system which automatically empty and replace the buffer in the separation vials. Large volume buffer vials have also been

introduced which dilute the effect of any electrolysis products or evaporation effects. There is a healthy competition between the major CE instrument suppliers which encourages technological developments and the overall effect is to increase the advance of the CE technique.

The majority of CE is performed using UV absorbance detection often at low wavelengths such as 200nm to maximise sensitivity. Diode-array UV detectors are also frequently used. Indirect UV detection is used especially for non-chromophoric species (Section 6) such as metal ions and small anions.

Fluorescence and LIF are further detection options which can [117] offer more sensitive and selective detection compared to UV absorbance. Relatively few compounds possess natural appropriate fluorescence but several derivatisation schemes have been developed for non-fluorescent compounds. A recent review [118] has covered aspects of solute derivatisation in CE. Solute derivatisation is not normally preferred as it involves additional sample handling requirements. However in-capillary derivatisation has been developed [119] for CE. In this approach a volume of the derivatisation reagent solution is injected into the end of the capillary, sample solution is then injected, followed by another injection of the derivatisation reagent solution. The capillary is then left to stand for a short time for the solutions inside the capillary to mix together by diffusion and for the derivatisation reaction to occur. The voltage is then applied to resolve the derivatised species. This in-capillary derivatisation has been demonstrated for amino acids [119] using OPA (*o*-phthaladehyde) solutions. LIF detection offers the possibility of extremely high sensitivity and has been used especially for the analysis of DNA and derivatised carbohydrates. Specific DNA binding dyes are added to the buffer to produce fluorescent DNA complexes.

Electrochemical detection offers the possibility of sensitive and selective detection. Various detector designs are possible [120–122] but they generally involve insertion of microscale electrodes into the detection end of the capillary. In particular electrochemical detection has been used to measure [120–122] catecholamines and the contents of single nerve cells.

Various other detection systems have been used in CE and recent reviews have covered inductively coupled plasma MS [123] and chemiluminescence [124].

The hyphenation of CE and CEC to MS detectors is well-established [125] and interfaces are commercially available from many MS suppliers. Recent reviews specifically cover the interfacing of CE to specific MS detectors including electrospray [126] and ion-trap [127]. Electrospray is currently the most widely used ionisation technique with positive ion being the most frequent detection mode. The use of non-aqueous CE electrolytes is particularly attractive for CE–MS as sensitivity can be significantly improved. The use of MEEKC or MEKC buffers requires specific operating procedures, or interfaces, to avoid fouling of the MS source with the involatile surfactants.

Capillary isotachophoresis (cITP) is capillary based electroseparation method [128] with a particular capability to concentrate trace components in diluted samples. cITP can also be used as a first step in on-line combination with CE. Applications of cITP include [129] determination of pollutants such as organic acids, phenols, nitrophenols, aldehydes, quaternary ammonium compounds, triazine herbicides, pyrethroid insecticides and separation of inorganic anions in mineral waters, surface and well waters and sewage.

Typically voltages in the region of 5 to 30 kV are employed. Application of higher voltages allows faster analysis but can cause problems of containment of the voltage. A commercial 30 kV power supply was extensively modified [130] in order to provide electrical potentials up to 120 kV. A unique electrical shielding system was developed to prevent capillary breakdown and corona or spark discharges. Use of this high voltage generated [130] theoretical plate counts ranging from 2.7 to 6.1 million plates for peptides.

14. Capillary electrochromatography

CEC is a hybrid between CE and HPLC that has gained popularity in recent years. CEC uses an electrically driven flow to transport the solutes through a capillary packed with stationary phase material. Separation can be achieved by differential

partition between two phases, differential electromigration, or a combination of these two. Several reviews have been published [131–134] in recent years covering aspects such as the application range, packing procedure and packing material range [135,136] and CEC practical aspects.

Generally capillaries are packed with small (3 μm or below) particles which are generally coated with traditional HPLC reverse phase packing material such as ODS. The EOF generates a flat profile, which improves the separation efficiencies generated in CEC when compared to the laminar flow obtained in pumped LC separations. The EOF generates no back-pressure which allows the use of small particle sizes. CEC separations are normally conducted at high pH to ensure a fast EOF, which reduces analysis times. Use of high voltages and short capillaries can produce sub minute separations. For example five polycyclic aromatic hydrocarbons (PAHs) were separated [137] in less than 5 s by applying 28 kV across a 10 cm total length column which had a packed length of 6.5 cm.

Over 95% of all papers currently published on CEC have used [138] C_{18}, and to a much lesser extent, C_8 stationary phases. However, ion-exchange phases such as strong cation-exchange (SCX) and C_6/SCX mixed-mode phases have also [138] been used in CEC. The sulphonic acid groups on the SCX phase remains ionised at low pH and therefore continues to generate a substantial EOF compare to typical stationary phase material. CEC capillaries have also been filled with monolithic polymers [139] which can have manufacturing and operational advances over conventional packed capillaries. Gel CEC is also possible [140] where the capillary is filled with replaceable media. Generally CEC capillaries are packed with stationary phase material but internally coated capillaries have also been successfully [141,142] employed. The linking of CEC to MS detection has been reported [143].

Chiral separations are achieved in CEC [144] by using chiral stationary phases or by using conventional achiral stationary phases and adding chiral additives such as cyclodextrin to the mobile phase.

Much of the development in CEC has originated from pharmaceutical industry based investigators where CEC has been examined as an alternative to both CE and HPLC [145–147]. A range of other analyte types have been separated by CEC including nucleotides [148] tryptic digest mapping [149], free fatty acids and fatty acid phenacyl esters [150].

The majority of papers in the area of CEC have concentrated on the resolution of test mixtures of neutral compounds and separation of charged solutes can be problematic in CEC. Generally CEC is performed at high pH in order to obtain a fast EOF. However at high pH acidic compounds [151] have an electrophoretic mobility which resists the EOF which can result in no peaks being obtained. The main approach to this difficulty has been to operate at low pH where the acid is unionised but the EOF is slow and extensive analysis times are obtained. Optimal performance is obtained [151] using a sulphonic acid containing phase which generates a fast EOF at low pH. Basic compounds tail extensively on standard ODS type material due to silanol interactions. This situation can be improved [152] by addition of a silanol masking agent such as triethylamine or hexylamine to the electrolyte. Fig. 5 shows resolution [153] of a range of acidic, basic and neutral drug components in drug seizure samples.

The main technical problem [154] which arises in the operation of CEC is the formation of bubbles in the capillaries. These arise from Joule heating and variations in EOF velocity on passing from the stationary phase through the frit and into the open tube. A frit is used to retain the packing inside the capillary. However it is possible [155] to produce capillaries without a frit which reduces the air bubble formation. Application of an air pressure across the vials during separation also reduces [131–134] the possibility of the air bubble formation.

Unexplained focusing effects can occur in CEC which can lead to the production of highly efficient peaks with theoretical plate counts in the millions. This effect has been reported for both basic drugs [156] and both neutral and anionic compounds [157]. Prediction and control of this effect would greatly enhance the possibilities in CEC.

There are a wide range of views on the future possibilities and direction of CEC. A discussion paper [158] has been published which summarises the opinions of leading CEC investigators on the advantages and limitations of CEC. Advantages include high separation efficiencies, low UV detection wavelength possibilities, ability to use high

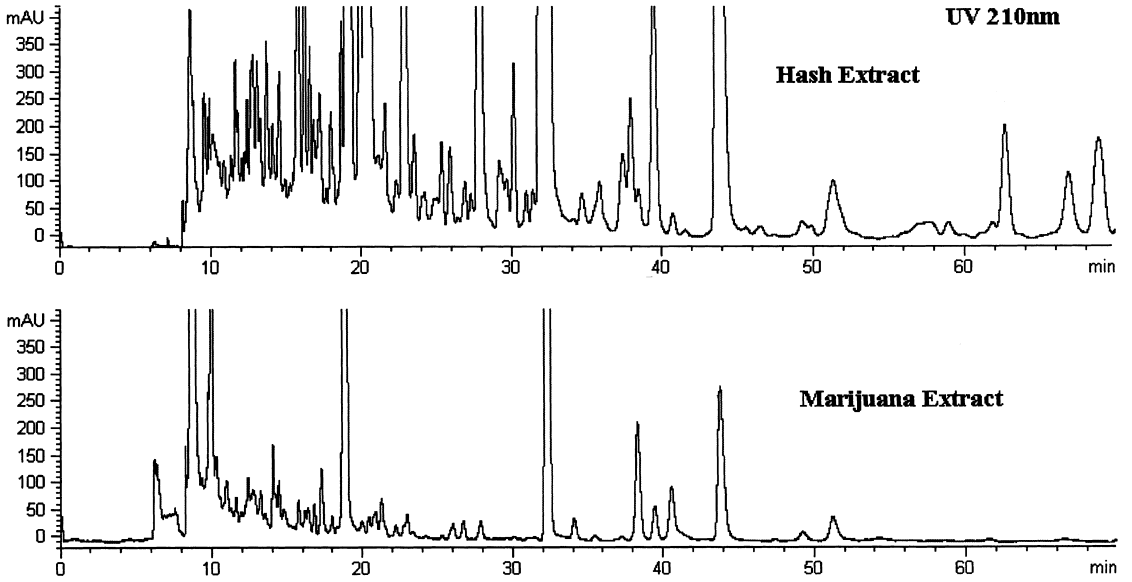

Fig. 5. Analysis of drug seizures by CEC. Capillary: Hypersil C_{18}, 3 μm, high sensitivity cell. Mobile phase: acetonitrile–25 m*M* phosphate, pH 2.57 (75:25). Temperature: 20°C. Injection: 32.0 s×5.0 kV. Reproduced with kind permission from Ref. [153].

organic solvent contents, and separation of neutral components. Disadvantages include formation of air bubbles, capillary fragility, analyte range and lack of analyst experience.

15. Miscellaneous application areas

A recent review [159] has covered use of CE for the analysis of compounds possessing carboxyl groups. Applications included analysis of acidic drugs or metabolites in complicated sample matrices such as plasma, serum and urine and automated system for diagnosis.

Fluorescent derivatised amino acids were one of the initial analyte type separated by CE. Pre-, post-, and on-column derivatization techniques and indirect UV detection methods have been used [160] for amino acid analysis by CE. Chiral resolution of amino acids is also readily achieved [160] in CE using a variety of approaches including cyclodextrin and crown ether additives.

The low injection and detection volumes employed in CE [161] make it an ideal tool for the study of single biological cells or the material secreted from cells. The use of native fluorescence detection or electrochemical detection allows sensitive and rapid determinations of bio-molecules such as catecholamines and proteins.

There are many [162] applications of CE in food including determinations of carbohydrates, amino acids, proteins, colourants and dyestuffs, flavonoids and vitamins. Many natural products such as humic substances, flavonoids, isoflavonoids, coumarins, alkaloids, steroids and herbal preparations, mycotoxins, heptapeptide toxins can be analysed [163] by CE.

The coupling of microdialysis sampling to CE allows [164] continuous monitoring of sample solution changes with time. For example [164] pharmacokinetics in animals can be monitored by microdialysis sampling with samples taken directly from living animals.

The application range for CE in pesticide analysis [165] is similar to that for pharmaceuticals. This include purity testing, assay of pesticide formulations, chiral separations, metabolite studies, and trace level determinations. The use of sensitive detection methods (e.g., LIF detection) and trace enrichment techniques allows [166] determination of pesticides at trace levels (low ppb) in environmental samples. CE has been used to measure a number of different

pollutants [167,168] including phenols, polynuclear hydrocarbons, amines, carbonyl compounds, surfactants, dyes and inorganic pollutants in water and soil samples.

Preparative CE is possible but small amounts are collected given the nl injection volumes injected into the capillary. However these small amounts can be collected into small volumes which maintains an acceptable concentration of the fraction. Generally wider bore capillaries (greater than 100 μm) are used to maximise the amounts collected. The collection of the fractions from repeated separations can be fully automated using standard commercial CE equipment. For example leucine enkephalin was purified [169] from a bovine pituitary using preparative CE. Preparative CE is also useful [170] for isolation of biologically active species such as enzymes as physiological conditions can be used for the separation and the active of the fraction remains intact. Preparative CE can be used [170] in a variety of modes include gel-electrophoresis, free solution and IEF.

16. Future directions

Undoubtably the future direction of CE will lie in improved instrumentation and enhanced method development possibilities.

The major instrumentation advance may be the evolution of a single platform instrument that is capable of performing capillary LC, CE and CEC. Details of a prototype instrument have recently been published [171], which describes an instrument that has these capabilities. This approach would have several benefits in relation to the advances of these emerging techniques. Use of a single instrument would reduce training requirements and would also be a cost-effective instrument as it would be capable of a wide range of applications. The difficulties associated with capillary LC such as the small volume handling and capillary coupling would be significantly reduced. Technological difficulties that currently occur in CEC would also be reduced as automated gradient CEC could be performed, and high pressure solvent rinsing and sample injection could be achieved. Combined pressure and voltage operation could also be performed to give faster and unique separations. The uptake of CE would benefit through increased instrument experience and availability. Instrument manufacturers may also invest heavily in development of a combined instrument, as it would have sales impact on both their CE and HPLC marketshare.

A further technological advance would be the introduction of a fixed loop injection device, which would improve the injection precision in CE and CEC, which is currently poorer than HPLC. Development of a fixed volume injector prototype was reported in 1987 [172] and hopefully a commercial device may evolve – possibly from the microchip CE research efforts.

External control of the level and direction of the EOF inside the capillary is possible [173] by applying voltages to the exterior of the capillary. Typically the capillaries are externally coated with a suitable conductive material. When the external voltage is applied it generates a charge on the internal capillary wall which controls the magnitude and direction of the EOF inside the capillary. In this way the EOF can be controlled and the selectivity and analysis time can be additionally varied through application of the external field.

A range of internally coated capillaries are commercially available which can enhance, reverse or suppress the EOF. Over recent years the stability and performance of coated capillaries has improved and they are in routine use especially for analysis of proteins and DNA. It is anticipated that the coating range and materials used to make capillaries will develop. Currently traditional fused-silica is the predominant capillary material used in CE. However, plastic CE capillaries such as polyether ether ketone (PEEK) [174] or PTFE [175] have been used which may have better properties. In addition the manufacture of specially purified or doped fused-silica may have benefits in the control and reproducibility of EOF levels. The format of capillary may also change as round capillaries scatter light which reduces sensitivity and detector linear range. Use of rectangular capillaries can improve optics and sample loading possibilities but can also produce [176] peak broadening effects such as temperature and EOF differences across the capillary. Undoubtably further ingenious capillary format designs will follow in due course.

Buffer additives can be used to coat dynamically the interior wall of the capillary to affect the EOF obtained. Commercial buffer systems are being available which uniformly coat the internal walls of capillaries with polyionic species which reduces capillary-to-capillary variability. This type of coating can also reduce the pH dependency of the EOF if the coating for example contains sulphonic acid groups which are fully ionised over a wide pH range. This extra reliability will prove important in routine applications of CE.

Buffer additives are now being specifically manufactured for use in CE. In particular highly purified chemically derivatised cyclodextrins have becoming commercialised for chiral CE, the most popular being the sulphated cyclodextrin which can be used for separations of both charged and neutral chiral species. Other examples of CE specific reagents include synthetic surfactants, aminated cyclodextrins and anionic crown ethers.

Molecular imprinted polymers are another future development [177] that will become important in CE and CEC. These polymers are synthesised to contain levels of a specific target compound. This compound is then washed out of the polymer to leave holes in the polymer which are an exact fit for the target compound. The polymer can therefore be used in CE and CEC to perform highly selective separations as the analyte will be well-retained in the polymer pores. If the compound used to generate the pores is a single enantiomer then the polymer can be used [177] to achieve chiral separations. Other uses of the molecular imprinted polymer include a sorbent for solid-phase extraction and affinity studies.

17. Conclusions

There has been a steady, though not spectacular, growth in CE during the 1990s. Routine methods have been established in a number of application areas including the analysis of pharmaceuticals, DNA, chiral compounds, proteins, peptides, clinical and forensic samples, metal ions and inorganic anions. Considerable research and commercial effort is currently focussed on the development of microchip CE that offers the possibility of hand-held disposable CE devices for clinical testing. Capillary array CE is already a commercial product, which allows simultaneous analysis of multiple samples, which increases sample throughput in areas such as serum protein analysis and DNA profiling. Recent methodology advances in CE have included the development of non-aqueous CE, novel capillary coatings and CE-specific buffer additives such as sulphated cyclodextrins.

Considerable interest and application for CEC have been developed in recent years and research is intense in this area. Particular attention is being paid to the development of novel packing materials, which would enhance the performance of the technique.

Possibly the most significant future advance will be the emergence of single platform instruments capable of performing capillary LC, CEC and CE. These instruments should solve the technical difficulties currently associated with these three techniques and lead to their combined advance.

In terms of commercial impact both CE and CEC are still relatively small compared to HPLC. However, HPLC is a mature technique where advances are only slight increments. CE and CEC methodology and instrumentation developments are still rapidly advancing which bodes well for the future of the techniques. Undoubtedly the miniaturisation possibilities associated with CE and CEC will serve their development well in the next millennium of separation science.

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