field-portable GC-MS instrumentation currently in use by the OPCW inspectorate. Use of GC with one or more spectrometric technique such as mass spectrometry is required to confirm the presence of chemical warfare agents. For this reason many analyses are carried out by GC-MS under electron impact or chemical ionization conditions. For analyses involving low levels of chemical warfare agents in the presence of high levels of interfering chemical background, GC-MS-MS is receiving increased attention.

Atmospheric pressure ionization (e.g. electrospray (ESI), ionspray and atmospheric pressure CI) techniques may be used for the direct mass spectrometric analysis of the hydrolysis products of chemical warfare agents in aqueous samples. Examples of LC-ESI-MS methods for these analyses are relatively few, and only recently has this technique been demonstrated for the analysis of the nerve agents as well. These new LC-ESI-MS methods complement existing GC-MS methods for the analysis of chemical warfare agents and their hydrolysis products and will likely replace some GC-MS methods currently in use for the analysis of contaminated aqueous samples.

See also: **II/Chromatography: Gas:** Column Technology; Detectors: Mass Spectrometry; Detectors: Selective. **Chromatography: Liquid:** Detectors: Mass Spectrometry.

Further Reading

Bonierbale E, Debordes L and Coppet L (1997) Application of capillary column gas chromatography to the study of hydrolysis of the nerve agent VX in rat plasma. *Journal of Chromatography B* 688: 255–264.

Compton JAF (1988) *Military Chemical and Biological Agents*. Caldwell, NJ: The Telford Press.

D'Agostino PA (1995) Chemical warfare agents – analysis and characterization. In: Townshend A and Worsfold PJ (eds.), *Encyclopedia of Analytical Science*, pp. 599–608. London: Academic Press.

D'Agostino PA, Hancock JR and Provost LR (1999) Packed capillary liquid chromatography-electrospray-mass spectrometry analysis of organophosphorus chemical warfare agents. *Journal of Chromatography A* 840: 289–294.

Gander TJ (1996) *Jane's NBC Protective Equipment*. London: Butler and Tanner Inc.

Ivarsson U, Nilsson H and Santesson J (1992) *A Briefing Book on Chemical Weapons*. Ljungforeytagen Oregro.

Kaipainen A, Kostiainen O and Riekkola M-L (1992) Identification of chemical warfare agents in air samples using capillary column gas chromatography with three simultaneous detectors. *Journal of Microcolumn Separations* 4: 245–251.

Kientz ChE (1998) Review: Chromatography and mass spectrometry of chemical warfare agents, toxins and related compounds: state of the art and future prospects. *Journal of Chromatography* 814: 1–23.

Methodology and Instrumentation for Sampling and Analysis in the Verification of Chemical Disarmament (1977–1994) Helsinki: The Ministry of Foreign Affairs of Finland.

Somani SM (1992) *Chemical Warfare Agents*. New York: Academic Press.

Witkiewicz Z, Mazurek M and Szulc J (1990) Review: Chromatographic analysis of chemical warfare agents. *Journal of Chromatography* 503: 293–357.

CHIRAL SEPARATIONS

Amino Acids and Derivatives

See **III / AMINO ACIDS AND DERIVATIVES: CHIRAL SEPARATIONS**

Capillary Electrophoresis

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Background

Since its commercial inception in 1988 capillary electrophoresis (CE) has increasingly offered the separation scientist a very wide range of application (biopolymers to cations), with ultra-high efficiency. It is generally cost-effective, easy in use, with low sample and buffer requirements and has the capability for automation. For these reasons CE has expanded considerably and is now used as an orthogonal and complementary technique alongside well-established analytical procedures. Of all its

application fields, the separation of compounds with one or more chiral centres has been particularly successful. Analytical chiral separations increased in importance throughout the 1980s: considerable interest was generated in the resolution of stereoisomers of food additives, agrochemicals, petrochemicals and pharmaceuticals. During this time the analyst was presented with numerous procedures to examine chiral compounds. Chromatography and, particularly, high performance liquid chromatography (HPLC) with immobilized chiral stationary phases used as column packings was the most successful for separation of enantiomeric mixtures, albeit at considerable cost.

However, chiral separations by chromatography do not give a unique solution to all difficulties relating to the resolution of chiral compounds. Thus, with CE analysts have another tool at their disposal, with all the benefits of the technique to be exploited. Two major processes of separation are undertaken in enantiomeric separations. The commonest procedure is where the enantiomers are resolved directly as diastereoisomers in the conditions of a chiral environment present in the capillary. The transient diastereoisomers are formed *in situ* and resolved on the basis of their different physicochemical properties. Alternatively, conventional CE separations follow after off-line diastereomeric derivatives are produced through reaction of the analyte with optically active reagents, in a procedure which is commonly referred to as an indirect method. An example is the resolution of the diastereomeric pairs of D, L-tryptophan with (+)-diacetyl-L-tartaric anhydride which are resolved on a polyacrylamide-coated, conventional silica capillary. Certain precautions are necessary within this procedure; primarily, a reagent of very high optical purity (100%) is required for specific reaction with the individual enantiomers. Variability in purity of a reagent sample would result in diastereomeric interference and errors in the determination of the analytes. Incomplete reaction and differences in the speed of reaction are also problems which must be addressed when carrying out the derivatization.

Over the last 10 years, a number of CE modes have been examined for separation of stereoisomers. Of these, capillary zone electrophoresis (CZE) in conjunction with chiral additives in the buffer phase is the most frequently used for charged chiral compounds. For neutral chiral analytes (and charged compounds), micellar electrokinetic chromatography (MEKC) has been applied through a chiral surfactant or a chiral additive and achiral surfactant, as additives to the buffer in a conventional silica capillary, when operated at the critical micelle concentration. In contrast to these applications where the selector acts in free solution, some success with constraining the chiral selector within the capillary has been achieved. Two approaches have been considered – attaching the selector to the capillary wall or trapping it within a gel (in capillary gel electrophoresis), although in practice, limited applications have been reported so far. The most recent proposal for optical separations is through electrochromatography, where a chiral stationary phase is packed into the silica capillary and the mobile phase moves under electroosmotic flow (EOF).

Chiral Selector in the Buffer Phase

Ligand Exchange

CE enantiomeric resolution was first established, through a procedure named ligand exchange. This is based on metal chelate complexation with copper or zinc at the centre of the complex. It was directed towards amino acids, which as dansyl-labelled derivatives were resolved through initially interacting copper (Cu(II)) and L-histidine in the run buffer and then forming a ternary metal complex with an enantiomer of a chiral amino acid. In an enantiomeric mixture the metal complexes can have differential stability, and therefore mobility differences between the enantiomers result. In the case above, a neutral pH buffer was used, which gives a positively charged metal–histidine interaction and the enantiomer forming the higher stability complex migrates faster. One drawback of this procedure is the requirement that the amino acid in the metal–amino acid interaction has to be of very high purity to stop enantiomeric impurities being introduced into the assay. Following the initial research, the same group impressively resolved 14 racemic amino acids by this procedure where the complex was Cu(II)–aspartame. At present the procedure is limited to amino acids, with some extension to peptides and additional separations, and most applications appeared in the early years of CE.

Cyclodextrins

The interest in cyclodextrins (CDs) in the chemical, cosmetic, food and drug industries has grown considerably. In the pharmaceutical field incorporation within the CD has led to improvements in bioavailability, pharmacokinetics and stability. However, to the analyst, their major influence has been in enantiomer stereoselectivity, which originates from the chirality of the glucose units and involves stereochemical interaction through hydrophobic inclusion, hydrogen bonding and often steric repulsion, when the CD is used as an additive in an aqueous CE buffer. The CDs, produced enzymatically from starch, are

comprised of 6–8 D-(+)-glycopyranose units as α-, β- and γ-CD. The molecules are shaped as truncated cones, with relatively hydrophilic exteriors, due to secondary C2 and C3 hydroxy groups at the top and primary C6 hydroxy groups at the bottom of the cone. The rigid structure presents an internal hydrophobic cavity, which will accommodate ring-structured enantiomers. Therefore, for chiral recognition of enantiomers, the most favourable chemical structure is where the hydrophobic centre, such as a cycloalkane or aromatic group (which generally interacts most closely with β-CD), gives a close cavity fit. But one of the enantiomers gives a poorer steric fit, which results in differential binding constants between the enantiomers. Typically, a small molecule with a single aromatic ring will include with a tight fit into the β-CD cavity. Additionally, hydrogen-bonding sites are required for interaction with the secondary hydroxyls on the rim of the cavity and at least one repulsive interaction with the CD. Generally, for interaction with the CDs the chiral molecule's hydrophobic centre should be close to the chiral centre (**Figure 1**).

CDs are generally reasonably straightforward to work with in CE. They are commercially available, ultraviolet transparent and, during method development, the concentration and the buffer conditions, such as pH, can easily be changed, without the need for long equilibration times. One area of slight difficulty is solubility which, although adequate in water or organic solvent for α and γ (14.5 and 23.2 g per 100 mL in water), is only 1.85 g per 100 mL with the commonly used β-CD. Other general properties are

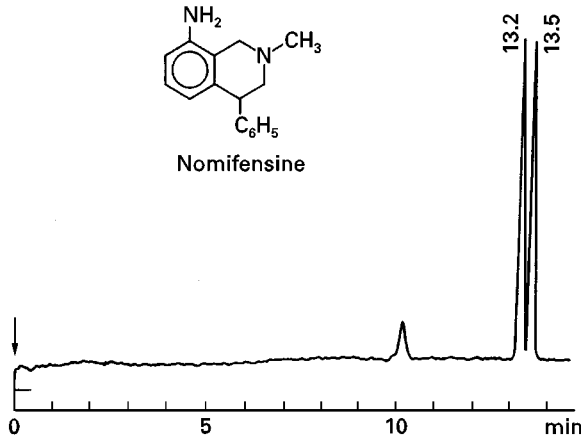

Figure 1 Capillary-zone electrophoresis resolution with β-cyclodextrin (β-CD) of the drug nomifensine maleate (65 mg L^{-1}) which has the hydrophobic group close to the chiral centre. The buffer was 20 mmol L^{-1} sodium tetraborate (pH 12.5) containing 20 mmol L^{-1} β-CD. The capillary was 37 cm (30 cm to detector) × 50 μm i.d. The applied voltage was 20 kV, detection wavelength 284 nm and temperature 30°C.

that CDs are neutral molecules and that the difference in electrophoretic mobility in mixtures has to occur from the charge on the chiral analytes. In method development it is important to optimize the enantiomeric separation, and there are three main areas of interest, two of which revolve around solvation effects, influenced through varying the pH and the organic additive concentrations. The CD concentration is also important: a theoretical model, has been described which indicates a maximum concentration for enantiomeric resolution. Other researchers have confirmed this and extended discussions to chiral separation models which include the function of pH and organic additives in the buffer. Other parameters shown to have an influence on resolution include buffer concentration and internal diameter of the capillary.

It was clear, however, in method development and in stereoselective HPLC assays with chiral additives that the solubility of the native CDs was a limiting factor, particularly with β-CD. As a result a large number of CD derivatives have been synthesized and tested and are now commercially available for use in CE. These range from the electrophoretically neutral methylated β-CD and hydroxypropyl β-CD to charged species, such as methylamino β-CD and sulfobutylated β-CD. In the former case solubility is improved manifold and, in the latter case, in addition to improved solubility the charge on the CD gives electrophoretic mobility to uncharged and neutral chiral compounds. Apart from these advantages the derivatized CDs often have different enantioselectivities over the native CDs (**Figure 2**). The first enantioseparations with CDs were carried out in 1985 as an extension to isotachophoresis, where the CD was added to the leading buffer. However Fanali is credited with one of the first uses of β-CD for the resolution of ephedrine alkaloids by CZE.

With a better understanding of the implications of chiral compounds in everything from the chemical to food and pharmaceutical industries, stereospecific synthesis has developed to a point where very few racemic compounds will be used in the future. However, the analyst still has a role to play in checking the enantiopurity of the synthetic product. Detection levels are regularly at $< 0.5\%$ m/m and the order of migration can often dictate whether low enantiomer levels are detected. Most favourable conditions generally exist when the minor enantiomer precedes the major component. One option to control the migration order is to operate with anionic, cationic or neutral coated silica capillaries. A typical example is shown in **Figure 3** of a cationic coating, a polyamine on the surface and α-CD, which gives a limit of quantitation of $< 0.05\%$ m/m for D-tryptophan, in

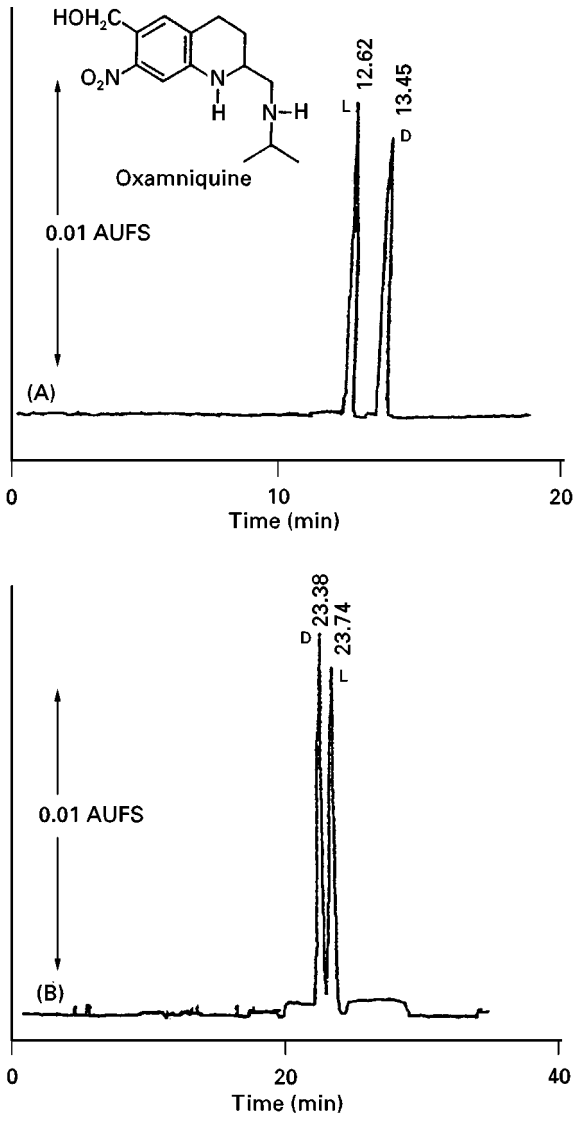

Figure 2 The resolution of oxamniquine by capillary-zone electrophoresis with neutral cyclodextrins. Resolution was at pH 2.25 with hydroxypropyl β-CD, but there was no resolution with β-CD at this pH. The β-CD gives resolution at pH 12, but with reversed migration order. (A) 50 mmol L^{-1} disodium hydrogen phosphate (pH 12) with 25 mmol L^{-1} β-CD; applied voltage 15 kV; (B) 50 mmol L^{-1} sodium dihydrogen phosphate (pH 2.25) containing 40 mmol L^{-1} hydroxypropyl β-CD; applied voltage 20 kV. The temperature was 30°C and detection wavelength 246 nm.

the presence of the L-enantiomer. During these runs the polarity is reversed over the conventional direction of operation. The coated capillaries mentioned above are commercially available and have been shown to be stable if used within the suggested limits. The anionic (sulfonic acid) coated capillaries are of particular interest in chiral and conventional separations as they give a consistent EOF over the range pH 3–9 and therefore are more controlled in operation.

Crown Ethers

Crown ethers act in a similar manner of enantiomer inclusion as CDs and contain a central cavity, although the mechanism is based on ionic and hydrogen bonding. They are macrocyclic polyethers, soluble in both aqueous and organic solvents, which form stable complexes with enantiomers, which have a primary amine or alkylamine functionality. For resolution, differential stability of the host–guest complex is required. This is reliant on the spatial arrangement between the amine and its hydrogen bonds with the ether oxygens. 18-Crown-6-tetracarboxylic acid is the easiest to obtain commercially and has been used to resolve a number of primary amines and racemic amino acids on a silica capillary, by addition of the crown ether into the buffer phase at low concentration, under CZE conditions. By this means a few chiral separations have been achieved which have not been fully successful by other means, such as chiral peptides. Crown ethers have also been used in combination with CDs for complex mixtures. In general, however, crown ethers have not been

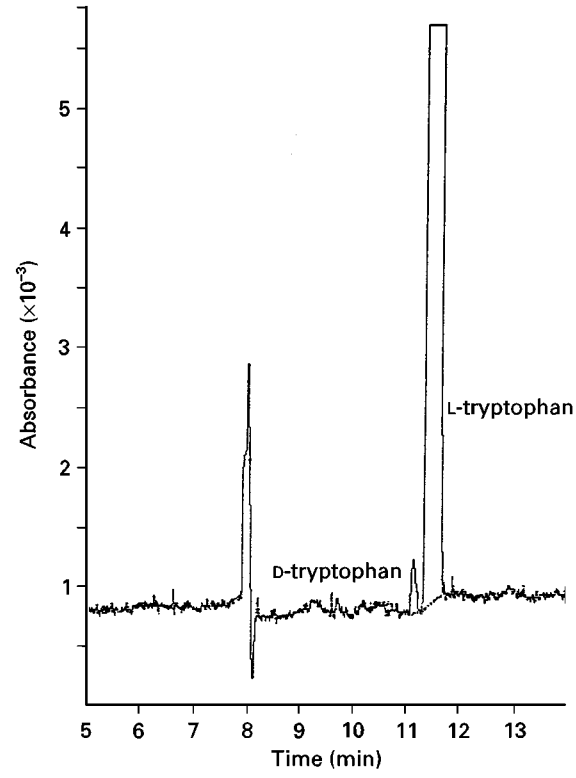

Figure 3 Detection of D-tryptophan at 0.05% m/m in L-tryptophan (LOD ($3 \times s/n$) 0.01% m/m) with a polyamine coated capillary (eCAP™ with polyamine regeneration solution) to give a positive charge on the capillary. Capillary was 37 cm (30 cm to detector) × 50 μm fused silica and buffer 40 mmol L^{-1} tris-phosphoric acid containing 75 mmol L^{-1} α-CD. The applied voltage was −10 kV, detection wavelength 241 nm, injection time 2 s and operating temperature 30°C.

extensively used because they need to be applied under controlled conditions as they are highly toxic and they are limited to amines and alkylamines. However, in the applications reported they give highly efficient resolutions of certain chiral compounds.

Other Chiral Additives

Apart from the CDs, linear oligosaccharides are potential chiral discriminators in CE, and maltodextrin mixtures, corn syrups and pure malto-oligosaccharides have all been used to resolve non-steroidal anti-inflammatory drugs (NSAIDs). Polysaccharides have also been discussed in these reports, with applications of heparin, a naturally occurring mammalian mucopolysaccharide (anticoagulant), in the resolution of chiral drugs. The molecule is chiral, highly anionic and helical and has been used in the chiral discrimination of antimalarials and antihistaminics. In **Figure 4**, oxamniquine is resolved with a large resolution factor with 2% w/v heparin. Thus heparin and some of the other polysaccharides with their high separation efficiencies appeared to be good alternatives to CDs. There is one drawback: because of their heterogeneous character, batch-to-batch and different source material can result in changes in the resolution and therefore method robustness would be questionable for regular assay for industry.

Proteins provide another alternative based on successes in HPLC and particularly separations with α_1-acid glycoprotein (AGP) and bovine serum albumin (BSA) for chiral drug separations. BSA has been cross-linked with glutaraldehyde to form a polymer matrix. However, BSA has a high ultraviolet background and as such needs to be used in indirect detection mode or where the gel is only partially loaded on to the capillary and stops just before the detection window. AGP, ovosomucoid (egg white) and fungal cellulose have all been used, with some success. The major problem with the fungal cellulose is absorption on to the capillary wall if used with uncoated capillaries, although adding materials such as polyethylene glycol or methyl cellulose into the electrolyte does alleviate wall interaction.

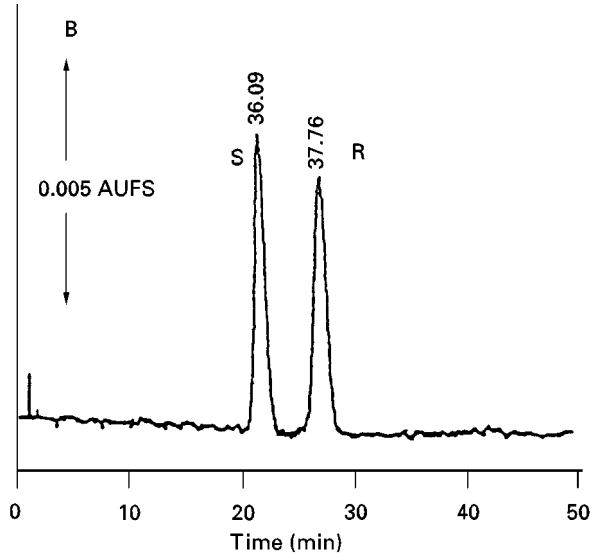

Figure 4 The resolution of oxamniquine by capillary-zone electrophoresis with heparin in the electrolyte solution. The electrolyte was 50 mmol L^{-1} sodium dihydrogen phosphate (pH 3.0) containing 2% m/v heparin. The capillary was 71 cm (50 cm to detector) $\times$ 50 μm i.d., the applied voltage 20 kV, temperature 30°C and the detection wavelength 246 nm.

Micellar Electrokinetic Capillary Chromatography

Neutral compounds present a problem in CE since they have no differential mobility because of their lack of charge and are carried without separation by the EOF. One solution is to operate with surfactants, which form micelles above a certain concentration, and then differentiate between the compounds on the basis of both electrophoretic migration and micellar partitioning. This technique has been so successful that it is now one of the commonest procedures used in CE. Initially the emphasis was on achiral compounds, but it was quickly extended to chiral compounds by the introduction of either chiral surfactants or chiral additives, such as CDs. Surfactants are long chain molecules, characterized by a hydrophobic tail pointing inwards and a hydrophilic head pointing outwards into the aqueous buffer. Micelles are amphiphilic aggregates of surfactant molecules which form above a surfactant concentration, known as the critical micelle concentration (CMC) (**Table 1**). There are four classes of surfactants – anionic,

Table 1 The critical micelle concentrations of chiral surfactants and achiral surfactants

Surfactant	*Critical micelle concentration (mmol L^{-1})*
Chiral	
Sodium cholate	14
Sodium taurocholate	13
Sodium deoxycholate	5
Sodium taurodeoxycholate	4
Sodium *N*-dodecanoyl-L-valinate	6 (40°C)
Achiral	
Sodium dodecyl sulfate (SDS)	8
Sodium decane sulfate	40
Tetradecyltrimethylammonium bromide (TTAB)	4
Cetyltrimethylammonium bromide (CTAB)	1

cationic, zwitterionic and nonionic – which can be both synthetic and naturally occurring. The synthetic group includes the anionic sodium dodecyl sulfate (SDS) and cationic cetyltrimethylammonium bromide (CTAB).

A slightly different process, microemulsion electrokinetic chromatography, has recently been developed. In this process a combination of oil–water–surfactant and co-surfactant such as an alkyl chain alcohol is used as a pseudo-stationary phase.

Chiral Surfactants

These can be synthetic optically active amino acid derivatives and natural surfactants, such as glycyrrhic acid, β-escin, bile salts and digitonin. Digitonin is nonionic, but has been used as a mixed micelle with SDS to give the electrophoretic mobility, to resolve chiral amino acids. These materials are generally water-soluble and can be added easily at micelle concentrations to the electrolytes in MEKC, although viscosity increases should be noted. Initially enantiomeric separations were directed towards neutral chiral compounds such as benzoin, and warfarin, a heart drug, which were selectively resolved with materials such as sodium *N*-dodecanoyl-L-valinate (SDVal) and *N*-dodecanoyl-L-glutamate (SDGlu). But it was soon realized that charged chiral compounds could also be resolved. Sodium taurocholate (STC) or the taurodeoxycholate (STDC) has been used under acidic conditions for the resolution of dansylated-DL-amino acids and for carboline derivatives and 2,2′-dihydroxy-1,1′-dinaphthyl used under neutral conditions for the drug diltiazem (**Figure 5**).

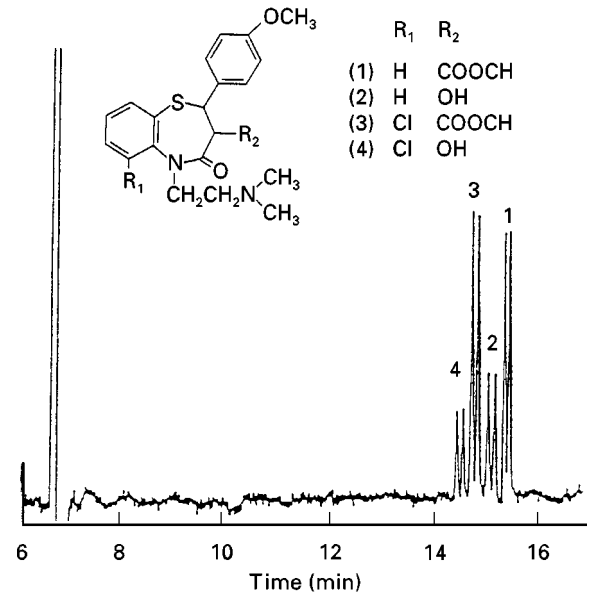

Figure 5 The use of bile salts to resolve the enantiomers of diltiazem and related substances by chiral micellar electrokinetic chromatography (MEKC). The electrolyte was 20 mmol L^{-1} disodium hydrogen phosphate–sodium tetraborate (pH 7.0) containing 50 mmol L^{-1} sodium taurodeoxycholate. The capillary was 65 cm (50 cm to detector) $\times$ 50 μm i.d. Applied voltage was 20 kV, detection wavelength was 210 nm and temperature was 25°C.

Chiral Selector with an Achiral Surfactant

This mode can give added selectivity to resolution of both neutral and charged chiral molecules, as the solutes are distributed between chiral selector such as a CD, the aqueous phase and the micelle (**Figure 6**). As a result, the chiral analytes will move in and out of the micelle/CD, with the CD moving with the EOF and the micelle (depending on polarity) moving according to charge. Racemic amino acids, β-blocker drugs, Trogers base and the drug terbutaline have all been resolved with this procedure. In the case of terbutaline either 5 mmol L^{-1} di-*O*-methyl-β-cyclodextrin or 15 mmol L^{-1} β-cyclodextrin was successfully used with 15 mmol L^{-1} SDS. Other approaches have been to carry out competetive separations with CD-MEKC, where an additive, such as D-camphor-10-sulfonate, is introduced into the buffer and competes for inclusion with the chiral analytes. With this procedure an improved resolution of Trogers base was obtained. Overall, this form of separation of chiral analytes has become quite popular, particularly as there are many derivatized CDs to choose from, and many examples have been published. Of the native CDs, γ-CD has shown the greatest success, which is the opposite of the case with the conventional addition of CDs to the buffer phase. In this case the larger inner diameter of the cavity of the γ-CD allows not only the chiral molecules to be included but also the surfactant.

Capillary Electrochromatography (CEC)

There is much current interest in electrochromatography, which is a hybrid technique between HPLC and CE, operating with packed capillaries. It is based on partitioning the analytes between the stationary phase (in packed capillaries) and the mobile phase (electrolyte) under CE conditions with a high applied voltage. The procedure benefits from the large range of HPLC stationary phases and some specific phases which are beginning to be developed for CEC. It links the high efficiency of CE with the range of separations possible with reversed-phase HPLC. For transport of the analytes in CEC, EOF is still present, but now the effective EOF is mainly generated from the packed bed of stationary phase rather than at the walls of the capillary. This results from the electrical double layer

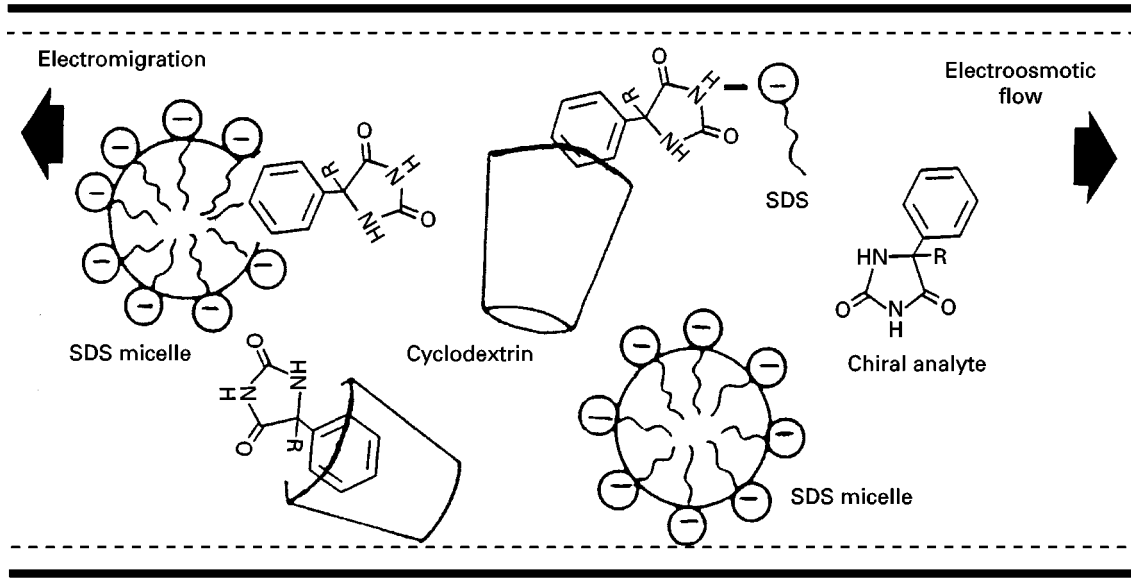

Figure 6 Schematic of the chiral analyte 5-alkyl-5-phenyl hydantoin distributed between the cyclodextrin, the aqueous phase and the micelle. SDS, Sodium dodecyl sulfate.

on the surface of the particles in contact with the mobile phase and produces a ζ potential. The potential of CEC was first demonstrated in 1987, showing the presence of the partitioning effects and, for charged analytes, the continued effect of electromigration. In terms of the separation conditions in CEC and HPLC, the stationary and mobile phases are very similar, but the separation efficiency can be much improved. Many applications of electrochromatography are currently being studied and the range of capillary packing materials continues to expand.

Chiral stationary phases are being examined by a number of researchers. In one of the first reports of chiral separations by CEC 5 μm AGP, immobilized on to silica, was packed into a 50 μm internal diameter capillary. Cationic and neutral enantiomers were resolved by this method, which included benzoin and the drugs cyclophosphamide and hexobarbital. This early work did not illustrate large improvements in peak efficiency over other procedures as the capillary packing methods required improvement, but it did illustrate the potential.

CDs have also been used as immobilized silica-based materials and the neutral CD hydroxypropyl-β-CD silica was used in resolution of the basic drug mianserin. In method design with the CDs, the choice of the background electrolyte has played an important part and pH, organic modifier and differences in field strength have all been considered. The direction of flow past the detector was shown to be dependent on the background electrolyte used. For β-cyclodextrin and a phosphate buffer the direction of flow is towards the cathode, but with triethylammonium acetate the flow is reversed. This can be beneficial in controlling the order of retention in CEC. The pH change in the electroosmotic mobility can be used to obtain a suitable overall retention time. Organic modifiers such as methanol and acetonitrile in the electrolyte have an effect on both the retention and enantioselectivity. The results from studies with CD-bonded silica phases indicate that a range of parameters and additives can affect the retention and enantioresolution in CEC, as they do with chiral stationary phases in HPLC.

Other stationary phases which have been shown to give enantioseparations are the cellulose and amylose derivatives which have been coated on to silica. These stationary phases are known as Chiralcel and Chiralpak phases (Daicel, Japan), respectively. These materials have been successful in HPLC for enantioselectivity with a wide range of chiral compounds and have recently been utilized as packing materials for CEC (**Figure 7**). Generally in CEC good resolution has been achieved for a number of chiral compounds resolved by HPLC, with improvements in separation efficiencies in many cases.

Future Developments

Capillary electrophoresis has had a major impact on the resolution of chiral compounds by the methods discussed above. The method is now regularly used to assay enantiomeric compounds in many fields with generally better efficiency than in HPLC and with

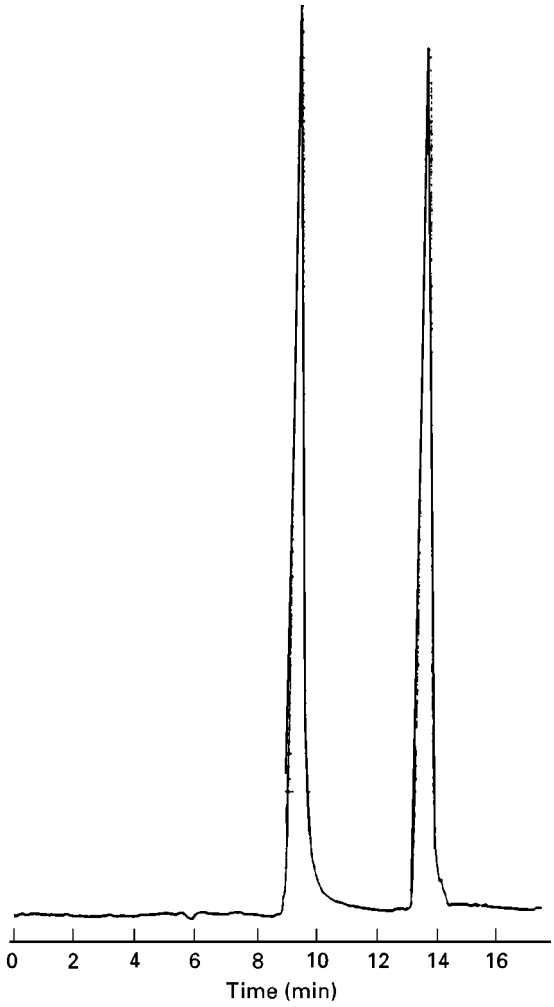

Figure 7 Electrochromatographic resolution of methylphenyl barbitone by a Chiralcel OJ (Daicel, Japan) chiral stationary phase. The cellulose ester derivative commercially coated on to silica was packed into a 30 cm (21 cm packed and 22 cm to detection window) × 75 μm i.d. fused silica capillary. The mobile phase was acetonitrile –water (70 : 30 v/v), applied voltage 15 kV and temperature 25°C.

shorter overall migration times. Many different areas are showing considerable research interest, such as electrochromatography, where in the future commercially available columns may prevail over in house methods of packing capillaries with chiral stationary phases. Generally, commercial interest in the area of capillary packings is likely to increase as more 'tailor-made' phases are introduced.

Further Reading

Chankvetadze B, Endresz G and Blaschke B (1996) Charged cyclodextrin derivatives as chiral selectors in capillary electrophoresis. *Chemical Society Reviews* 141–153.

Clark BJ (1994) Separations of enantiomeric compounds by chiral selectors in the mobile or solvent phase. In: Subramaninan G (ed) *A Practical Approach to Chiral Separations*, p. 311. Weinheim, Germany: VCH.

Kuhn R and Hoffstetter-Khun S (1993) *Capillary Electrophoresis: Principles and Practice*. Berlin: Springer-Verlag.

Lelievre F, Yan C and Zare RN (1996) Capillary electrochromatography: operating characteristics and enantiomeric separations. *Journal of Chromatography A* 723: 145–156.

Nishi H and Terabe S (1995) Optical resolution of drugs by capillary electrophoretic techniques. *Journal of Chromatography A* 694: 245–276.

Rabel SR and Stobaugh JF (1993) Applications of capillary electrophoresis in pharmaceutical analysis. *Pharmaceutical Research* 10: 171–175.

Schmitt T and Engelhardt H (1995) Optimisation of enantiomeric separations in capillary electrophoresis by reversal of the migration order and using different derivatized cyclodextrins. *Journal of Chromatography A* 697: 561–570.

Wren SAC (1993) Theoretical aspects of chiral separation in capillary electrophoresis. *Journal of Chromatography A* 603: 235–241; 609: 363–367; 636: 57–62.

Cellulose and Cellulose Derived Phases

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Introduction

The properties of cellulose derivatives for the separation of enantiomers were recognized in 1966 by Lütringhaus and Peters. The full potential of microcrystalline cellulose triacetate (CTA) was definitely established by Hesse and Hagel in 1973. Meanwhile, microcrystalline cellulose tribenzoate became commercially available. Another milestone in the development of cellulose- and amylose-based phases was the work of Okamoto and co-workers who in 1984 introduced polysaccharide derivatives coated on a macroporous silica. Currently such materials are certainly the most universally applicable type of commercially available chiral stationary phases. Then, in 1988 Francotte and co-workers introduced cellulose esters of aromatic acids in the form of almost spherical, partially crystalline particles with a relatively