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Review

Cyclodextrins: a versatile tool in separation science

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Abstract

Cyclodextrins have been used extensively in separation science because they have been shown to discriminate between positional isomers, functional groups, homologues and enantiomers. This property makes them one of the most useful agents for a wide variety of separations. The main goal of this review is a discussion of somewhat more exotic applications of cyclodextrins to separation methods. Techniques examined in detail include gel electrophoresis, isotachophoresis, isoelectric focusing, preparative scale electrophoretic techniques, thin-layer chromatography, electrochemically modulated liquid chromatography, use of monolithic media in liquid chromatography, microdialysis, separation on hollow fibers, foam flotation enrichment, solid- and liquid-phase extractions, countercurrent chromatography, separation through liquid and composite membranes, and cyclodextrin applications in molecularly imprinted polymers. Since a lot of attention has been paid to use of cyclodextrins in capillary electrophoresis, liquid, gas and supercritical fluid chromatography, these techniques will be only briefly discussed. The second goal of this review is a discussion of a scaling-up the analytical separations to semi-preparative or preparative techniques. It was found that despite a need for large scale separations in the industry, development of these techniques has been somewhat lagging behind development of miniaturized analytical separations. It is hoped that the focus on areas outside more traditional separation applications might stimulate further research.

Keywords: Reviews; Cyclodextrin

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1. Introduction

The unusual properties of cyclodextrins originate in their unique structure. In general, cyclodextrins are fairly soluble in water. Despite a hydrophilic surface, cyclodextrins contain a hydrophobic cavity. It is the presence of this cavity that enables cyclodextrins to entrap hydrophobic molecules. Entrapment/inclusion occurs without the formation of formal chemical bonds. This intriguing property of cyclodextrins has inspired considerable basic research, applied science, and also use in a variety of products.

Separations represent a large area of analytical chemistry. Cyclodextrins are extensively used in separations due to their unique property to form inclusion compounds with other smaller hydrophobic molecules. In particular, the shape and size selectivity of cyclodextrins provides an important parameter for separations because of the impact stability constants of various magnitudes has on molecular discrimination.

Formation of inclusion complexes between ligand and a cyclodextrin is thought to be primarily a result of hydrophobic interactions between a ligand and the relatively hydrophobic cavity of cyclodextrin coupled with polar interactions between appropriate substituents on the ligand and the polar rim of the cyclodextrin. The native cyclodextrin rims are lined with primary hydroxyls on one side and secondary hydroxyl groups on other side of the cavity. However primary and secondary hydroxyl groups may be functionalized with hydrophobic (e.g., methyl, propyl) or hydrophilic groups (sulfate, phosphate, quaternary amine), to further enhance complex forming ability and selectivity towards certain analytes [1]. Because many derivatized and all non-derivatized cyclodextrins are soluble in water, they are often used in aqueous environments as solubilizers of hydrophobic compounds via inclusion complex formation. Under appropriate conditions, this ability also makes them potentially useful agents for various types of separations that were either previously performed under non-aqueous conditions or not at all.

Three major types of cyclodextrins are known, α-, β- and γ-CD. The α-CD contains six, β-CD seven, and γ-CD eight glucose units. A general trend is that hydrophobic organic compounds with a relatively small molecular volume form the strongest complexes with α-CD and the weakest with γ-CD [2]. Table 1 shows stability constants of several analytes with α-, β- and γ-CD. Experimental evidence also suggests that besides size, the charge density on a molecule also influences the strength of the complexation. Nonionized species generally seem to

Table 1
Dependence of the solute size and cyclodextrin cavity on stability constants of inclusion complexes [2]

	α-CD, cavity volume=174 Å^3	β-CD, cavity volume=262 Å^3	γ-CD, cavity volume=427 Å^3
1,3-Butanediol	16 200	12 023	NR
4-Methoxy cinnamic acid	10 300	658	NR
Adamantane carboxylic acid	130	330 000	24 000
Testosterone	5058	7540	16 500
Naphtalene	77	608	130
Anthracene	40	2300	1500
Phenantrene	60	1500	770

NR=Not reported.

Table 2
Binding constant of neutral and ionized forms of various solutes with β-CD [2]

	0	−1
Barbital	210	100
Benzoic acid	632	35
n-Decanoic acid	9440	6730
Hexobarbital	1280	380
	0	+1
Aminoadamantane	110 000	8430
4-Nitroaniline	300	100
Morpholine	17	7

have higher stability constants with non-derivatized cyclodextrins than their charged equivalents [2]. Table 2 shows examples of several compounds and stability constants with β-CD of their neutral and charged forms. These dependencies, however, are not always straightforward and can be more complex with functionalized cyclodextrins [3].

During the developmental stages on the use of cyclodextrins in separation methods, positional, geometrical isomers, structurally related compounds, and homologues were mainly separated [4]. Cyclodextrins were used as chemically bonded or sorbed ligands in stationary phases [5], or as solution additives (e.g., mobile phase, extraction solution, or buffer media) [4]. Currently, chiral separations seem to be one of the most significant areas of application in the use of cyclodextrins and their derivatives.

In some cases, there has been considerable time lag between initial reports of the application of cyclodextrins in a particular separation technique to its widespread acceptance. However, two general trends seem to be emerging in separation research and that is reflected in the application of cyclodextrins to separation science as well. The first trend is the micro scaling of existing separation technologies. These techniques include various capillary techniques such as CE, microbore-LC, microdialysis, etc. A second trend is the development of bulk scale separations, useful for large scale and industrial applications. Some examples include large-scale extractions, dialysis, foam flotation, membrane separation, and continuous flow electrophoresis. Table 3 shows a list of some of the techniques discussed in this review and their use in chemistry according to the amount of separated material. The impact of both these trends in cyclodextrin applications will be discussed along with a brief review of more classical separations.

2. Thin-layer chromatography

Thin-layer chromatography (TLC) is one of the oldest chromatographic techniques and has been used to study cyclodextrin inclusion complexes. The first to use cyclodextrins for a separation in TLC was Armstrong and co-workers [4,6] in 1980. Partitioning of a solute was thought to occur between a polar stationary phase (e.g., polyamide, alumina, silanized silica) and cyclodextrin dissolved in a mobile phase [4]. The presence of the CD in the mobile phase eliminated or reduced the need for organic solvents. Thus, a significant advantage of these aqueous cyclodextrin-containing phases was their safety profile (e.g., low toxicity, and flammability) [7]. In addition, the UV-transparency of the cyclodextrin facilitated analyte detection.

Table 3
Category division of separation techniques according to throughput

Analytical separations (less than 1 mg/h)	Semi-preparative separations (mg/h)	Preparative separations (more than 1 mg/h)
Capillary electrophoresis	Gel electrophoresis	Extractions
Microbore liquid chromatography	Foam flotation enrichment	Countercurrent chromatography
Gas chromatography	Membrane separations and hollow fibers separations	Large scale membrane separations
Supercritical fluid chromatography	Liquid chromatography	Recycling isotachophoresis
Thin-layer chromatography		Continuous flow preparative electrophoresis
Microdialysis		Large scale dialysis
Isotachophoresis		Preparative liquid chromatography
Membranes in sensors		Preparative scale gas chromatography

Table 4
Stability constants of inclusion complexes of some *o*-, *m*- and *p*-derivatives with α-CD [2]

	K_{stab} for *o*-isomer	K_{stab} for *m*-isomer	K_{stab} for *p*-isomer
Methylcinnamic acid	277	3070	13 600
Hydroxycinnamic acid	1110	1320	1990
Methoxybenzoic acid	31.6	855	1514
Xylene	22	40	72

Mobile phases containing α-CD were used to separate substituted isomeric benzoic acid derivatives [4], phenols and naphthols [6]. In general, interactions between *p*-isomer with α-CD were stronger that that of *m*-, which were in turn, stronger than with *o*-isomers; the trend was ascribed mainly to steric hindrance. Table 4 shows examples of some *o*-, *m*- and *p*-benzene derivatives and stability constants with α-CD. Additionally, separation of enantiomers was also achieved via TLC [8].

β-CD was used less frequently as a mobile phase additive in TLC than α-CD, probably as a result of its more limited solubility in water. However, a variety of strategies, including the addition of urea [9] and the development of water-soluble derivatives of β-CD [10] or β-CD polymers [11] have furthered successful TLC separations.

TLC separations utilizing cyclodextrins incorporated in a stationary phase, similar to those used in HPLC, have also been reported, although the lack of a commercial source for these plates has limited their use [13].

3. Gas chromatography

Gas chromatography (GC) is one of the most common chromatographic techniques. As in all chromatographic techniques, the extent to which a solute is retained on a GC column is reflective of the strength of its interaction with a stationary phase. Most commonly, cyclodextrins are immobilized as a thin film on a capillary wall or deposited on GC packing material.

Schlnek and co-workers first reported the use of cyclodextrin derivatives in GC separations in 1962 [12,13]. Acetate, propionate, butyrate, and valerate derivatives of β-CD, were investigated for their potential to separate homologues of fatty alcohols, fatty esters, methyl esters, olefins, aldehyde esters, and aldehydes. Cyclodextrin derivatives influenced the polarity of the stationary phase and conferred additional stability on the phase. Overall, the best resolution for the compounds studied was on the valerate modified β-CD; the poorest was on the acetate modified β-CD.

Smolkova-Keulemansova and co-workers [14–16] were one of the first to systematically investigate cyclodextrins for GC separations using solutions of underivatized cyclodextrins (α- and β-CD) deposited on a solid support. Separations of homologues and positional isomers were achieved. However, these phases showed poor efficiency due to stationary phase inhomogeneities (e.g., poor coating). Despite these disadvantages, characterization of the retention process, description of the structural, positional, and steric preference for formation of inclusion complexes was first detailed in these studies. Empirical rules of the solute–cyclodextrin complexation derived from GC data were in good agreement with results from TLC experiments, performed independently by Armstrong [4,14].

The utility of the native cyclodextrins in GC applications was hampered by their high crystallinity while their insolubility in most organic solvents made them difficult to formulate into GC stationary phases. However, some functionalized cyclodextrins form viscous oils suitable for GC stationary phase coatings and have been used either neat or diluted in a polysiloxane polymer as chiral stationary phases for GC. The commercial availability of these phases has enabled tremendous growth in this area in the last few years. They have been used to separate inorganic gases [17], light hydrocarbons [18] and enantiomers [19,20]. Multiple retention mechanisms, including interaction of solute with the cyclodextrin cavity, and/or with OH-groups or other groups appended onto the cyclodextrin molecule were post-

ulated. In the case of chiral separations on these phases, many of the separations are nothing less than remarkable. Cyclodextrin GC phases are particularly adept at separating chiral synthons, small chiral organic precursors used in stereospecific synthetic pathways [21–23].

Preparative scale GC was developed to separate enantiomers of anesthetic drugs [24,25]. Separations were achieved on 1 and 2 m packed GC columns, respectively, with 10 mm I.D. Chromosorb WAW (80–100 mesh) was coated with trifluoroacetyl γ-CD as a stationary phase (23%, w/w). Product purity depended on particle size, concentration of stationary phase, and separation conditions. Separations were performed in the overloaded elution mode. The GC column was connected to a fraction collector. The purity of individual fractions was checked via a sampling interface between the preparative column and a short, efficient analytical capillary GC. Enantiomeric purity, recovery and production rate was monitored *on-line* in 30s intervals. The throughput of this system was up to 60 mg/h.

Another approach for preparative-scale GC chiral separations is the application of simulated moving bed (SMB) chromatography. This approach has been used for the continuous production of the pure enantiomers of enflurane, a volatile inhalation anesthetic [26].

4. Supercritical fluid chromatography

Cyclodextrin phases have been used as stationary phases in supercritical fluid chromatography (SFC). The main advantage of SFC over other chromatographic techniques is that non-volatile, thermally labile compounds can be separated. Carbon dioxide is preferably used for these chiral separations, since a CO_2 molecule is small, and can be readily displaced from a cyclodextrin cavity by a solute molecule [27]. It has been found that use of other mobile phases such as hexane was disadvantageous. Another significant advantage of CO_2 as a mobile phase is the ease with which it can be removed which has important implications for scaling up a separation.

Although both packed and open tubular columns can be used, packed columns are most often employed. They seem to provide better resolution for a wider range of racemates. Moreover, optimization of chiral separations by the means of polar modifiers to the mobile phase is reported to be more beneficial in packed columns [27,28].

The most common cyclodextrin-containing stationary phases used in SFC incorporate the cyclodextrin to the silica support via a spacer arm [28]. Cyclodextrins and their derivatives are usually chemically immobilized onto the wall of a capillary to prevent leaching of the stationary phase. Development of copolymeric stationary phases containing dimethylsiloxane and β-CD [27] proved to be stable and efficient stationary phases for chiral separations. SFC capillaries were coated with these copolymers (film thickness, 0.25 μm). Permethylated β-CD has also been chemically linked onto the capillary wall.

The most common applications of SFC using cyclodextrins are chiral separations [27,28]. Chiral separations of diols, monoalcohols, ketones and carboxylic acids containing aromatic substituents have been reported. Various applications of SFC included chiral analysis in the perfume and food industry. For instance, volatile components of flowers were analyzed with SFC [29] on cyclodextrin phases. Although a significant advantage of SFC is facile mobile phase removal, thus far, there are no reports of preparative scale SFC separation utilizing cyclodextrin.

Chiral recognition is believed to occur by superposition of several influences. Whether or not ''docking'' of an analyte on the secondary rim of cyclodextrin cavity is an initial step in an inclusion-type process is not clear. However, successful chiral discrimination seems to require a tight fit between the analyte and a cyclodextrin molecule. Chiral resolution in SFC on stationary phases containing derivatized cyclodextrins, in general, seems to be less reproducible and efficient relative to phases containing native cyclodextrins.

5. Countercurrent chromatography

Recently, a successful use of cyclodextrin derivatives in countercurrent chromatography (CCC) was reported [30]. The main advantage of CCC is that it can be a large-scale separation technique, suitable for preparative separation of enantiomers. Sulfated β-

CD was used for the preparative chiral separation of 7-des-methyl-ormeloxifene. It was determined that solvent hydrophobicity and concentration of cyclodextrin in stationary phase influenced separation efficiency and resolution. Analytical CCC was used to optimize separation conditions.

6. Liquid chromatography and related techniques

As in TLC, addition of cyclodextrin into a LC mobile phase influences partitioning of the solute between the stationary phase and inclusion complexes formed in the mobile phase. Resolution and separation efficiency of these systems depend on the type and concentration of cyclodextrin used [31,32], type of the mobile phase [33], the temperature [34], and the type of the stationary phase [35,36]. Under some conditions, cyclodextrins can be absorbed on the surface of stationary phase and form a ''pseudo-stationary'' phase. For example, methylated β-CD was strongly absorbed on a hydrophobic stationary phase and enabled chiral resolution [37].

An interesting variant on this approach is electrochemically modulated LC [38]. It was used for the chiral separation of benzodiazepines on porous graphitic carbon with a β-CD mobile phase additive. The HPLC column was configured as an electrochemical cell. During the separation, a voltage was applied to the stationary phase. Under the influence of the applied voltage, native β-CD was electrosorbed on the surface of graphitic carbon. The electrosorption process influenced the amount of β-CD in the mobile phase, which was available for the complexation with solutes. For the benzodiazepines studied, enantiomeric separation was more efficient when positive voltage was used and the maximum amount of β-CD was electrosorbed on the silica surface. The same approach was also successful for the enantiomeric separation of hexobarbital, and mephenytoin.

The first chemically immobilized cyclodextrins used in LC were in the form of crosslinked cyclodextrin gels [39–41]. These initial results were followed by other developments in stationary phase technology. Initially, cyclodextrins were attached onto a solid support via ethylene diamine linkages [42,43]; however, later LC packing attached the cyclodextrins to silica gel via six to ten atom long spacers [44]. Currently, cyclodextrins are either chemically bonded onto a silica beads via several spacer arms [45–48], adsorbed onto silica as a cyclodextrin polymer [49,50] or grafted onto a linear polymer as a pendant group [51,52]. Currently research efforts seem to be aimed at developing more pH stable stationary phases. An example of this trend is in the development of monolithic media containing cyclodextrins [53].

Cyclodextrin stationary phases have been used in the reversed-phase [54], normal-phase [55], and polar organic [56] phase modes. Various types of compounds have been separated on these liquid chromatographic columns and have been previously extensively reviewed [44,57,58].

7. Isotachophoresis

Isotachophoresis (ITP) is not as widespread an electrophoretic separation technique as capillary electrophoresis; however, it provides several advantages over CE. Larger quantities of sample may be analyzed which renders ITP somewhat more amenable to scale-up. In addition, sample components can be preconcentrated from a sample matrix. Furthermore, isotachophoresis can be easily interfaced to other electrophoretic techniques, thereby enabling two-dimensional separations.

Isotachophoretic separations are performed in non-packed capillaries. Briefly, a sample plug is introduced between leading and terminating electrolytes. The leading electrolyte contains an ion that has the highest mobility of the system whereas the terminating electrolyte has an ion with the lowest mobility, with a common counterion. Upon application of an external electric field, sample constituents are separated into zones between the leading and terminating electrolytes. The separated sample components are preconcentrated or diluted according to the equations describing steady state condition [59]. The main influences on the final composition of the sample zones are the electrophoretic mobilities of the leading and terminating electrolytes and their concentration. Further details can be found in extensive monographs devoted to this subject [59].

The most common method to achieve separation of individual components in isotachophoresis is a variation in the pH of the leading and terminating electrolytes. Addition of cyclodextrins into the leading electrolyte, however, has been proven to be the most effective in achieving successful separations in isotachophoresis.

Tazaki et al. [60] reported, in 1982, the first application on the use of cyclodextrins in isotachophoresis. α-CD was added into the leading electrolyte together with 18-crown-6 for the separation of ammonium, alkali, and alkali earth metals. In the succeeding cyclodextrin applications in isotachophoresis, positional isomers of sulfonic acids and aromatic sulfonic acids were separated with β-CD [61]. Ionic surfactants incorporating *o*-, *m*- and *p*-isomers of disubstituted benzene rings were separated with α-CD [62].

A systematic study was done on the influence of α-, β- or γ-CD on the isotachophoretic separation of related penicillins [63], bile acids [64], substituted halogenobenzoic acids [65], and reaction intermediates of naftidrofuryl hydrogenoxalate [66]. Not surprisingly, it was determined that the relationship between the size of an analyte and the size of the cyclodextrin was an important factor in the separation. In subsequent studies, it was determined that the counterion, present in both leading and terminating electrolytes, also greatly influenced separation efficiency [67]. Counterions with strong complex forming abilities decreased the resolution and efficiency of the isotachophoretic process. Smolkova-Keulemansova and co-workers also reported the first successful chiral separation using isotachophoresis with cyclodextrins [68–70].

Enantiomeric enrichment on a preparative scale was achieved by the use of HP-γ-CD. Recycling isotachophoresis was used for the preparation of enantiomerically enriched fractions of barbiturates and their metabolites. Racemic mixtures of barbiturates and their metabolites were analyzed in the model mixtures as well as in plasma samples [71].

Dubrovcakova et al. developed a mathematical model describing isotachophoretic separation in the presence of neutral additives such as cyclodextrin [72]. Detailed analysis and computer modeling of the isotachophoretic separation process showed that total cyclodextrin concentration was not uniform in the isotachophoretic capillary. In the zones corresponding to the analyte with the highest cyclodextrin affinity, the total cyclodextrin concentration was higher than in the bulk solution. Fig. 1 shows a graphic representation of the concentration profile of the various components in an ITP capillary at steady state. It was noted that if the counterion also formed a complex with cyclodextrin, a competitive equilibrium was established within the sample zone, further perturbing the cyclodextrin distribution.

To further enhance applicability of isotachophoresis for chiral separations of complex mixtures, two-dimensional isotachophoresis was developed [73]. Two isotachophoretic units were coupled *on-line*. The enantiomeric mixture of pseudoephedrine and thiridazine derivatives was analyzed. In the first isotachophoretic compartment, enantiomers of pseudoephedrine derivatives were separated via use of diMe-β-CD. In the second ITP column, derivatives of thioridazine were separated with γ-CD. Complete separation and resolution was not achieved using a single column with the cyclodextrin mixture.

Two-dimensional isotachophoretic separations were also applied for the separation of organic and inorganic acids in feed additive preparations [74]. In the first ITP capillary, ions of analyzed acids were

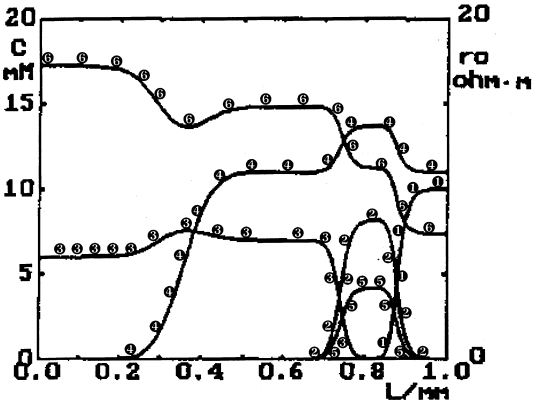

Fig. 1. Simulation of isotachophoretic migration in the presence of neutral cyclodextrin 1.35 s after application of electric field. Figure shows concentration courses of individual components along a capillary. 1=Leading anion, 2=total sample, 3=terminating anion, 4=total cyclodextrin, 5=uncomplexed sample, 6=specific resistance. *L*=Length coordinate of the capillary tube, *c*=concentration, ro=specific resistance. With permission from Ref. [72].

pre-separated with the leading electrolyte at pH 6.1. In the second ITP capillary, the pH of the leading electrolyte was 2.5; β-CD was used as an additive to facilitate the resolution of tartrate and fumarate.

Further exploitation of ITP occurred in the development of on-line coupling of ITP with CE. An isotachophoretic step was used to preconcentrate sample components and reduce a large excess of one of the analytes. Quantitation of the sample components was accomplished using either CE or ITP [75]. Tryptophan was used as a model analyte in a multicomponent sample containing other organic anions in urine. Upon the preconcentration and isotachophoretic sample clean-up, tryptophan enantiomers were resolved in a CE step using α-CD. Detection limits for the enantiomers were 1.5 ng/ml in the presence of 200-times molar excess of other anions.

8. Gel electrophoresis

For capillary gel electrophoresis, the most commonly used gel is polyacrylamide. Fourteen *N*-dansyl-DL amino acids were separated on polyacrylamide gels using cyclodextrins [76]. Isomers of nitrobenzoic, hydroxybenzoic acid, and optical isomers of dansyl-D,L-leucine, and *R,S*-1,1′-binaphthyl-2,2′-dihydrogenphosphate were separated using cyclodextrin in a polyacrylamide gel [77]. The effect of seven different organic solvents was studied. It was found that at low concentration (up to 10%) of organic solvent, a higher dielectric constant of the organic solvent corresponded to higher resolution and longer migration times. The resolution and efficiency of the separation was also found to be dependent upon the concentration of β-CD in the buffer and in the gel. Resolution increased with increasing field strength; however, high voltage resulted in gel failure.

In contrast to polyacrylamide gels, which are polymerized in the capillary, and are not renewable and removable, polyethylene glycol gels are polymer solutions that are pumped into the capillary column. They have been used along with cyclodextrins for enantiomeric separations [78,79]. The main advantage of polyethylene glycol gels is that they can be removed from the capillary or regenerated. Efficiency of the separation was found to be dependent on the molecular mass and concentration of polyethylene glycol, and the temperature of the separation. Terbutaline and enantiomers of 2-adrenergic sympathiocomimetics were employed for these studies.

Stalcup et al. were one of the first to use CE for optimization of chiral separation conditions prior to classical gel electrophoresis [80]. Buffer pH, composition and concentration of cyclodextrin necessary for a baseline enantiomeric resolution was determined and applied to classical gel electrophoresis. Milligram quantities of enantiomerically pure or enriched terbutaline were collected by classical gel electrophoresis using sulfated β-CD (7–10 sulfate groups/per molecule of β-CD) as a chiral selector. For the chiral separation, a horizontal distillation column filled with sulfated β-CD in an agarose gel was used. The negatively charged cyclodextrin was important for two reasons. Significant binding between the analyte and the cyclodextrin was thought to be critical to circumvent solute diffusivity problems associated with classical gel electrophoresis. In an addition, anionic cyclodextrin was necessary to transport the analyte through the electrophoretic bed. The gel was extruded, sliced and extracted, with the individual extracts analyzed by chiral CE to determine the enantiomeric distribution.

Alternatively, a continuous elution electrophoresis cell (Fig. 2) was used in which one end of the gel was continually washed with buffer. The eluent was pumped to an HPLC UV detector and subsequently collected in a fraction collector. In this configuration,

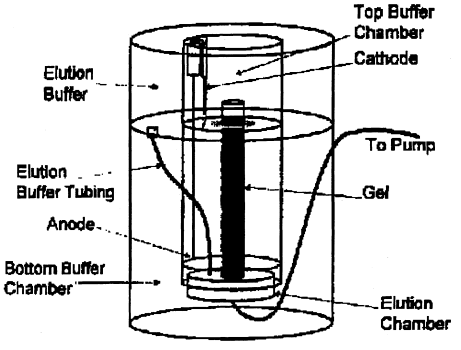

Fig. 2. Schematic figure of Bio-Rad Preparation Cell used in gel electrophoresis. With permission from Ref. [81].

the gel could be used repeatedly without noticeable deterioration [81].

9. Isoelectric focusing

Isoelectric focusing (IEF) is a technique that can be employed in a free solution or in a gel media. Special buffer systems are used. Upon the application of electric field, a pH gradient is established along the length of a separation cell (capillary or slab gel). The composition of the buffer system determines the steepness of the pH gradient [82].

A classical gel approach to chiral separations using cyclodextrins was an enantiomeric separation of dansyl derivatives of phenylalanine and tryptophan [83]. Separation was accomplished in a slab gel with a pH gradient between 3.0 and 4.0 in the presence of 7.0 *M* urea, 10% methanol and 60 m*M* β-CD. This separation exploited the isoelectric point (p*I*) differences between the enantiomers of phenylalanine and tryptophan (ΔpH=0.05 and 0.025 units, respectively) due to a lowering of the pK_a of the dansyl tertiary amino group upon the complexation with β-CD.

Recent work of Glukhovski and Vigh [83] described development of analytical and preparative scale IEF in free solution. The authors proposed a model describing the magnitude of change in the isoelectric point as a result of cyclodextrin complexation. The model could be used to predict the best pH gradient and cyclodextrin concentration necessary for efficient separations, if the stability constants for the individual enantiomers with the cyclodextrin are known. According to the model, a difference in the isoelectric point of two enantiomers bound onto cyclodextrin molecule can vary up to 0.1 pH units, if stability constants differ by only 10%. Analytical enantiomer separations of dansyl phenylalanine were performed with two IEF buffers, using hydroxypropyl-β-CD (HP-β-CD) as a chiral selector. To test the model, the separation was performed in a capillary with a pH gradient 3–5. As predicted by the model, both enantiomers were baseline separated in an analytical capillary. It was also noted that the p*I* did not change significantly in response to changes in HP-β-CD concentration. To increase resolution of enantiomers under IEF conditions, Bier's buffer (serine+propionic acid) in a pH interval 3.5–3.6 was employed. Enantiomers were separated by 3–4 cm in 40 cm long capillary, depending on the concentration of HP-β-CD.

10. Preparative scale continuous free flow electrophoresis, recycling isotachophoresis and isoelectric focusing

Preparative continuous free flow electrophoresis (CFFE), first reported in 1958 [84], translates the resolving power of electrophoresis into a continuous feed process. As in CE, the addition of cyclodextrins to the buffer can be used to accomplish enantiomeric separations. In preparative CFFE, employing continuous buffer and sample feed, sample and buffer are introduced at the top of a thin, rectangular electrophoresis chamber while an electric field is imposed perpendicular to the flow. Thus, the separations are accomplished in free solution. Differential interaction between the various solutes and cyclodextrin in the electric field produce a lateral displacement of the individual analytes between the two electrodes, based on the differences in electrophoretic mobilities and stability constants with cyclodextrin. Individual sample components are collected at various locations across the bottom of the chamber. A simplified figure of preparative scale electrophoretic instrument is shown in Fig. 3.

Methadone enantiomers were separated by CFFE and recycling isotachophoresis using HP-β-CD as a chiral discriminator [85,86]. Although baseline sepa-

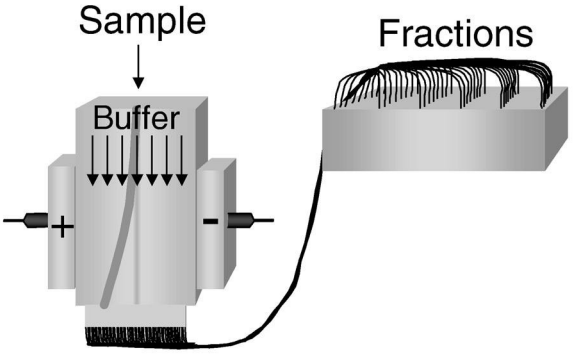

Fig. 3. Schematic representation of preparative scale electrophoretic unit.

ration of methadone enantiomers was achieved under the same conditions in CE, the resolution was lower (e.g., ~80% enrichment) with CFFE no doubt due to the larger volumes and the impact of Joule heat on analyte diffusion.

One strategy devised to improve the separation in CFFE was the development of continuous multistage processing and interval flow electrophoresis [85,86]. Effluent from each channel was continuously re-injected into the corresponding influent port. Fluid recycling was maintained until final separation was attained and resulted in a complete enantiomeric separation. Throughout this work, chiral CE using the same chiral discriminator determined enantiomeric distribution of the collected fractions.

Continuous flow isoelectric focusing was performed on a preparative scale electrophoresis instrument for dansyl phenylalanine using HP-β-CD as a chiral selector with Bier's buffers (serine+propionic acid) [83]. Baseline separation of the two enantiomers required a pH gradient interval of 3.5–3.6.

11. Capillary electrophoresis and electrokinetic micellar chromatography

As of September, 1999, a Chemical Abstracts search using the keywords ''cyclodextrins'' and ''capillary electrophoresis'' came up with 840 ''hits'' including approximately 40 review articles. Since many aspects of electrophoretic separations have been covered in past, only brief discussion and some review references will be provided.

Although separation of positional isomers and achiral compounds with cyclodextrin additives has been explored [87–89], as in other applications of cyclodextrins to separations, the most significant efforts in cyclodextrin use in capillary electrophoresis has been on chiral separations. A variety of approaches have been investigated. For instance, cyclodextrins have been used individually [90] or as mixtures of different types of cyclodextrins [91] as additives in the separation buffer. Cyclodextrins have also been covalently bound on the capillary wall and successful chiral separations were performed [92].

Cyclodextrins have been used with other types of additives, as well. For instance, electrokinetic micellar chromatography [93,94], developed by Terabe et al., exploits solute partitioning between micelles or cyclodextrins and the bulk buffer. In addition, a variety of cyclodextrin derivatives have been used. For instance, charged cyclodextrin derivatives enables direct separation of neutral compounds [95].

12. Foam flotation enrichment

Foam flotation enrichment techniques are established techniques used mainly in the mining industry for concentration of ores. A solution containing small particulate matter is purged with a gas. Slurries and fine particles present in the solution adhere to a hydrophobic surface of a collector (surfactant) and are carried to the surface by gas bubbles. They can be collected at the surface, resulting in preconcentration. Foam flotation can be also used in homogenous solutions. The principle remains the same. Material, which does not adhere to a collector, is separated from adhered species. In principle, flotation methods could be used for any compound if a suitable carrier is found. Since instrumentation requirements are simple, this technique could be used at low cost [96]. Fig. 4 shows a schematic of a foam flotation experiment.

Some derivatized cyclodextrins also form bubbles when purged with N_2 [96,97]. The foaming capa-

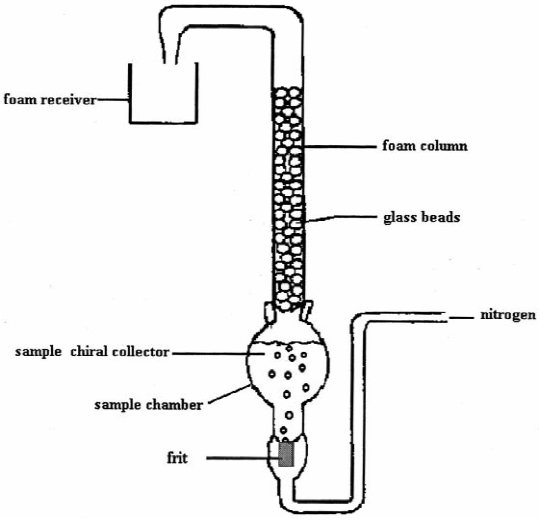

Fig. 4. Schematic representation of glass foaming device used for foam flotation experiment in Ref. [96] (with permission).

bility of cyclodextrins, coupled with their ability to preferentially form inclusion complexes with one isomer (positional or enantiomer) makes them a promising candidate as a collector in foam flotation. Indeed, enantioselective preconcentration of warfarin, as well as underivatized and derivatized amino acid racemates was achieved via use of derivatized cyclodextrin as a collector [96]. Foam exiting from a fractionation column was collected and components of the foam were quantitatively analyzed via HPLC on a Cyclobond column. It was found that the enantiomeric excess found in the collected foam depended on several factors including the temperature of the foam flotation column (4°C), the number of passes of the foam through the column, the foam ''dryness'', and the length of the column. For every system studied, there appeared to be an optimum concentration of the collector and the racemate. Different cyclodextrin derivatives sometimes produced the opposite enrichment. For instance, permethyl-β-CD enriched one warfarin isomer, while 2,6-di-*O*-methyl β-CD enriched the other isomer [96]. The use of cyclodextrin derivatives in a foam flotation experiment resulted in enantiomeric enrichment, in most cases, from 52 to 88%, depending on the analyte. Clearly, the approach is promising but more work needs to be done.

13. Liquid–liquid and liquid–solid extraction

Extraction is a very common wet chemistry technique. The separation of compounds is based on different affinities of a solute between two immiscible liquid phases (liquid–liquid extraction) or a solid and a liquid phase (solid-phase extraction). Since relatively large volumes and large sample amounts are used in extraction methods, extraction is primarily a preparative or a semi-preparative technique, particularly for sample clean-up.

A common difficulty encountered with using cyclodextrins in extraction is that the complex can either precipitate or the presence of an organic solvent can inhibit complexation with the cyclodextrin. Various derivatized cyclodextrins have been applied in liquid–liquid extraction processes since derivatization can partially prevent this limitation.

The first utilization of cyclodextrin (α-CD) in an extraction process was reported in 1969 [98]. Matsunaga et al. [99] reported the use of γ-CD for separation, preconcentration, and qualitative analysis of alkyl *p*-hydroxybenzoates. γ-CD formed an insoluble complex with a solute and the solute was back-extracted from the precipitate into a non-aqueous solvent. This technique was used for quantitation of *p*-hydroxybenzoate in soy sauce. Various solvents were investigated for the best release of solute from the complex. Higher releases were observed for diethyl ether, dioxane and ethanol, while lower recoveries were obtained with methanol, chloroform and dimethylsulfoxide. It was thought that the low recoveries from dimethylsulfoxide may be due to the solubility of the complexes while low-molecular-mass solvent molecules (e.g., methanol) may be either incapable of replacing a solute molecule or may participate in the formation of more stable ternary complexes.

Cyclodextrins have been evaluated for the selective extraction of polycyclic aromatic hydrocarbons (PAHs) [100] from oil. PAHs were extracted from non-aqueous solvents into an aqueous solution of γ-CD. As noted above, many common organic solvents formed insoluble complexes with γ-CD. Additionally, insoluble ternary complexes were formed between solvent molecules, solute, and cyclodextrin. The most useful organic solvents were solvents which were incapable of ternary complex formation and which were too small to efficiently fit cyclodextrin cavity. Quantitation of PAHs after selective extraction was followed by fluorescent spectroscopy. Large compounds such as benzo[*e*]pyrene, perylene, benzo[*ghi*]perylene, and coronen had extraction efficiencies varying from 30 to 98%, depending on the particular compound and the solvent system used.

Elliott et al. developed an interesting modification of this approach [101]. PAHs were extracted from micro-emulsion systems containing cyclodextrins. The three major cyclodextrins, α-, β- and γ-CD were investigated for this purpose. Micro-emulsions are organized media with at least three components: water, oil, and surfactant. Some PAHs were selectively precipitated from these systems by the addition of cyclodextrin. The composition change was followed by fluorescent spectroscopy. It was found that while β-CD was selective toward smaller PAHs,

such as naphthalene, γ-CD preferentially precipitated pyrene and benzo[*a*]pyrene.

More recent studies of the extractions of PAHs, from systems containing cyclodextrins and sodium dodecyl sulfate (SDS), revealed that SDS can influence partitioning, possibly through participation in ternary complexes [102]. It was suggested that pyrene forms stronger complexes with Me-γ-CD due to additional hydrophobic interactions arising from the presence of methyl groups on the rim of a cyclodextrin cavity. However, this extraction protocol was found inadequate for the separation of pyrene from anthracene.

Techniques developed for extraction of PAHs has been extended to the extraction of pollutants from soil [103]. Cyclodextrin derivatives (e.g., methyl, hydroxypropyl, and β-CD polymer) were used for desorption of priority pollutants such as pyrene and pentachlorophenol from soil particles. Branched β-cyclodextrins were used for the separation of mixed xylenes [104] and permethyl hydroxypropyl β-CD was used for cloud point extraction of aromatics from water [105]. Branched α-CD (glucosyl and maltosyl) were used for the selective extraction of xylene isomers and ethylbenzene [106].

In another interesting twist, lipophilic cyclodextrin derivatives were used to extract alkali metal cations from a water solution into benzene [107]. Hydroxyl groups at the 2,6-positions of the glucose residues in cyclodextrin were dipropylated while the hydroxyl groups at 3-positions were acetylated to provide complexation sites for alkali metals. No extraction was observed without the acetyl groups. The extraction equilibrium depended strongly on the initial concentration of cations and picrate anion in the aqueous solution. The order of stability constant was the same as observed for crown ethers (e.g., $Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$).

Extractions that utilize cyclodextrin have also found their way into solution of complex biological and biochemical problems. HP-β-CD, hydroxyethyl-β-CD (HE-β-CD) and hydroxypropyl-γ-CD (HP-γ-CD) were used in the sample preparation for the determination of progesterone and testosterone in human serum [108]. The method consisted of a traditional extraction of progesterone with hexane and testosterone with dichloromethane, respectively. It was followed by a back extraction into an aqueous cyclodextrin solution. Prior to GC–MS analysis, analytes were again extracted into hexane and dichloromethane, respectively. Increased concentration of derivatized cyclodextrin in an aqueous phase increased the amount of material extracted. Additionally, it was found that the degree of cyclodextrin derivatization slightly impacted extraction efficiency. Highest extraction efficiency was achieved using the β-CD with the smallest degree of substitution. However, this influence was slight and could be compensated by an increase in the cyclodextrin concentration.

A novel approach was described for the selective extraction of detergents from mixed detergent–lipid–protein mixtures [109]. Stability constants for surfactants were measured in range of 10^4–10^5 for α-CD and 10^3 for β-CD. Interactions of surfactants with cyclodextrin prevented formation of mixed micelles and droplets, forcing lipids to associate into bilayers. Recoveries for membrane proteins ranged from 70 to 90%. Recoveries for lipids depended on the lipid–protein ratio. For relatively small molar ratios of 50–150, recoveries were 60–90%; for higher molar ratios, recoveries dropped to 30–60%. Studies were performed with bacterial rhodopsin and recombinant rhodopsin. To separate vesicles containing lipid and protein from complexes of surfactant and cyclodextrin, three approaches were tested. Velocity sedimentation did not provide a complete separation between vesicles and complexes. Dialysis results were also less than satisfying because the dialysation membrane seemed to be modified by cyclodextrin, allowing protein molecules from vesicles to pass through the membrane. Ultimately, centrifugation through a sucrose step gradient proved to be the most effective for the separation because the cyclodextrin–surfactant complexes stayed in the top layers, while the vesicles migrated toward the bottom.

Presently, liquid extraction processes employing cyclodextrins are used in the food industry to modify the behavior of biological materials partitioning between two aqueous phases, one containing arabinogalactan and the other containing polyethylene glycol [110], for the isolation of mushroom flavors from mushrooms [111], and for the extraction of cholesterol from egg yolks [112], etc.

Process scale enrichment of the enantiomers of atropine and rulin via solid-phase extraction, using

β-CD as the solid and non-aqueous acetonitrile as the liquid phase, was developed by Shushi [113]. Enrichment of enantiomers was possible in solvents which did not form H-bonds and which did not complex with β-CD. Lowering the temperature and introducing additives such as acetic acid and triethylamine decreased the enrichment.

Cyclobond I solid-phase extraction cartridges were used in various application for pre-separation and pretreatment of samples. Applications include analysis of sulfonamides [114] and cocaine metabolites [115].

14. Dialysis, microdialysis and hollow fiber separations

Dialysis is a diffusion-based separation process in which analyte flux is controlled by the concentration gradient across a semi-permeable membrane. Typically, dialysis membranes are polymers with a specific molecular mass cut-off. Size discrimination is a primary factor in the separation. The most common dialysis set-up is one in which the sample is placed into a dialysis bag and the bag is placed into a flask filled with, most commonly, deionized water. Since a concentration gradient is created between the dialysis bag and the environment, analyte and/or impurities pass through the membrane. Sample from the dialysis bag is analyzed after equilibrium has been achieved.

Microdialysis is based on the same principle as dialysis; however, it is a non-equilibrium based process, used for on-line monitoring and quantitation of analytes in vivo. In a microdialysis probe, perfusion fluid constantly flows in and out of the probe, carrying an analyte away from the probe. Analytes cross a membrane from the environment outside the probe to the inside of the probe based on a concentration gradient across the membrane. Thus, the concentration of the analyte in the probe can always be related to the concentration of analyte in the sample being probed. Fig. 5 shows a schematic of a microdialysis probe. The size of a probe can be small enough to be placed inside a hypodermic needle.

The first report of the use of cyclodextrin in a dialysis separation involved the selective transport of benzene derivatives through a polyvinyl chloride

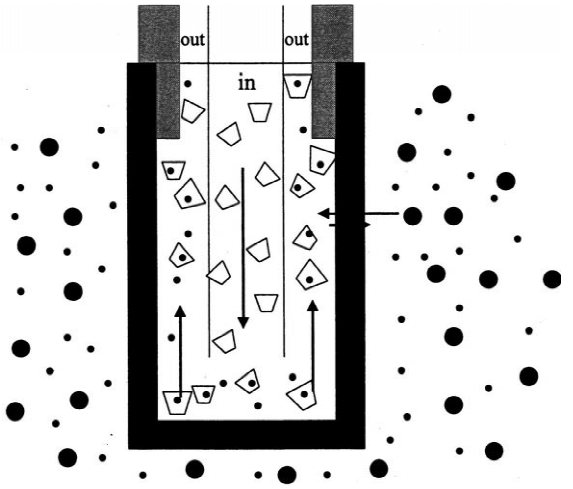

Fig. 5. Schematic representation of microdialysis probe.

membrane [116]. However, the most common use of dialysis in biological and biochemical applications is for desalting of biological samples and separation of low molecular mass components from biopolymers. One of the main difficulties in the analysis of low molecular mass compounds in a complex biological matrix is that complexation with proteins or other constituents of the sample can reduce the free concentration of the analyte. Cyclodextrin added into the perfusion fluid can interact with the analyte and increase analyte flux across the membrane.

The influence of the addition of β-CD to a perfusion fluid was studied by Khramov and Stenken [117]. The driving force of the separation was inclusion complexation between β-CD and the drug. Complex formation produced a decrease in the free concentration of the drug and increased the flux of the drug across the membrane. A schematic illustrating the various complex equilibria occurring in this set-up is shown in Fig. 6a. It was found that extraction efficiencies of two drugs, ibuprofen and antipyrine, depended on the flow-rate of the perfusion fluid, on the β-CD concentration, on pH, and on the type of membrane (e.g., polycarbonate/polyether, polyacrylonitrile, cellulose). Optimum concentration of β-CD in the perfusion fluid was found to be independent of the perfusion fluid flow-rate but somewhat dependent on the membrane used. Similar results were obtained in other microdialysis studies

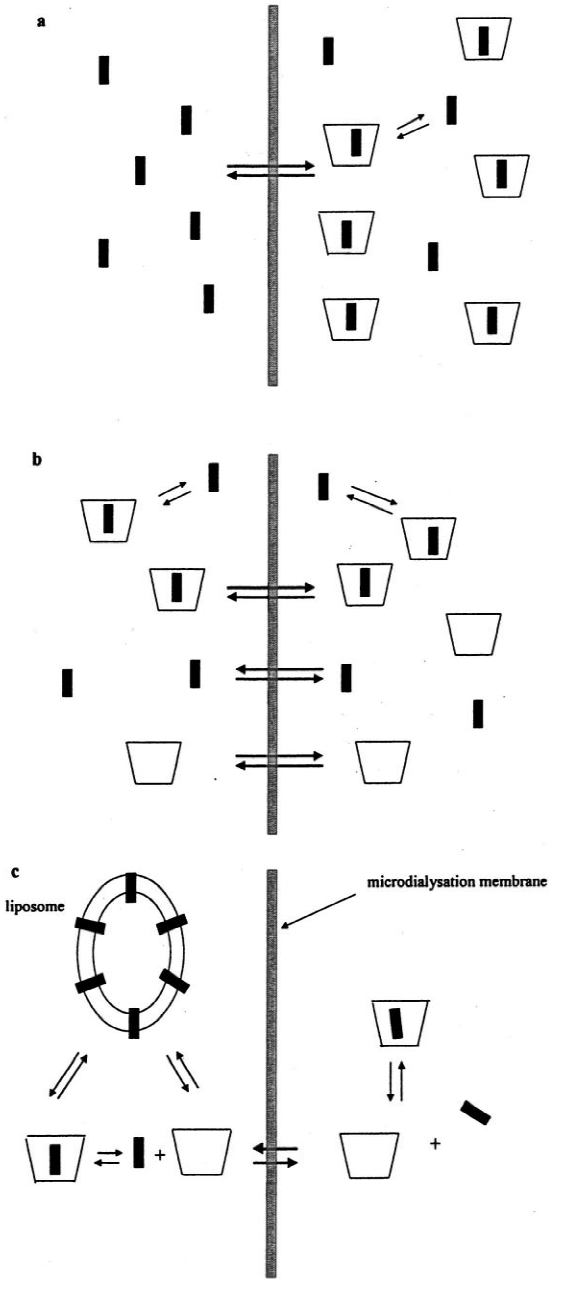

Fig. 6. Schematic representation of possible equilibrium processes occurring on a dialysation membrane as a result of cyclodextrin addition. (a) Cyclodextrin was added to perfusion fluid only to increase analyte flux through the membrane, (b) cyclodextrin was added to a sample and perfusion fluid to prevent nonspecific adsorption and increase stability of an analyte, (c) cyclodextrin was added to a sample to release an analyte from liposomes.

with tricyclic antidepressants using β-CD and HP-β-CD [118]. Different rates of diffusion of the drug and drug–cyclodextrin complex through a membrane, and different stability constants between a drug and β-CD and a drug and HP-β-CD contributed to variable extraction efficiency [119].

A microdialysis probe was also coupled to HPLC for the determination of peptide and non-peptide leukotrienes [120]. Determination of the concentration of leukotrienes is an important diagnostic tool for the detection and diagnosis of inflammatory processes. A significant problem encountered during analysis of leukotrienes is their instability and nonspecific adsorption onto various surfaces. In contrast to other dialysis work using cyclodextrins, in this study, cyclodextrin was added to both the perfusion fluid and the sample and served to prevent nonspecific analyte absorption and increase leukotriene stability. All three major underivatized cyclodextrins, α-, β- and γ-CD were investigated. Fig. 6b illustrates the various equilibrium processes occurring. Perfusion fluid was collected and injected onto an HPLC column (10 μl injection).

β-CD was also used to study drug release from liposomes [121]. HP-β-CD was added to a dialysis bag together with a solution of liposomes. The release of steroids, hydrocortisone, budesonide, and calcein were studied in this set-up. The exact mechanism of the separation is unclear; however, it is likely that the drugs of interest partition between the liposomes or the cyclodextrin and the bulk solution. The study did not delineate if transport across the dialysis membrane involved only free drug or the drug–cyclodextrin complex. Since leakage of encapsulated calcein from the liposomes was not affected by cyclodextrin addition, it is probable that the structure of lipid bilayers was not disturbed. A schematic representation of possible equilibrium processes is shown in Fig. 6c.

Incorporation of cyclodextrins into dialysis membranes was also investigated. Permeability of polyvinylalcohol films changed when oligomers of β-CD were added [122,123]. Selectivity of a membrane for water increased with increasing alcohol concentration, while the flux of water across the membrane decreased. This effect was more significant for mixtures of 2-propanol–water than for 1-propanol–water and ethanol–water. In general, permeation of

water and alcohols increased when membrane contained β-CD oligomers.

A continuous process for the separation of isomers was developed using a dual-stage hollow fiber membrane contactor system [124]. The wall of the fibers was formed with macroporous material containing immobilized organic phase. Fibers were intertwined so sufficient sample component transport could be achieved. An aqueous solution of cyclodextrins, serving as a discriminatory liquid membrane, flowed in the space between the fibers. An organic feed solution, containing the mixture of isomers to be separated, flowed through the hollow of the first stage, containing 300 aligned fibers. In this first stage, the solute was selectively extracted, by diffusing through the fiber walls into the cyclodextrin aqueous phase. In the second hollow fiber, the solute was back-extracted from the cyclodextrin liquid phase into a flowing organic strip solution in the interstitial volume between the hollow fibers. Isomers of nitroaniline, *p*- and *o*-, and stilbene, *cis* and *trans*, were investigated. Although all analytes were transported in the presence of pure water, the addition of cyclodextrin dramatically enhanced the transport of the preferred chemical species. A decrease in membrane selectivity as feed time increased was reported.

Although the selectivity and efficiency of a continuous feed process using hollow fibers is reported to be higher that that of liquid membranes (see following section), high purity isomers require a cascade of several modules. Continuous hollow fibers based separations have not yet been utilized for enantiomeric separations and it remains to be seen if this is a viable approach.

A similar process scale extraction was used for the fractionation of citrus oil to produce an oil stream enriched in oxygenated components [125]. The process was based on the preferential binding of oxygenated components by cyclodextrin. Low temperature citrus oil flowed on one side of a non-porous hydrophilic membrane. A continuous stream of an aqueous solution containing cyclodextrin flowed past the other side of the membrane. Oxygenated components of orange oil partitioned into the membrane and were preferentially collected, via inclusion complexation, by the cyclodextrins on the other side. The solution containing the inclusion complexes was subsequently heated and passed by a second membrane. The complexes dissociated at the higher temperature and the free components diffused through the second membrane back into an oil flow stream on the other side of the membrane, thereby enriching it in the oxygenated components. The cyclodextrin solution was recycled and fully recovered. Not surprisingly, the type of cyclodextrin and the process temperature influenced the enrichment process.

15. Liquid membranes and sensors

Development of various membranes and single Langmuir–Blodgett monolayers for separation and preconcentration of various compounds represents a new approach in the use of cyclodextrins. The principle is a variation of dialysis and microdialysis separation principle. However, in this approach, the analyte may be preconcentrated via specific interactions with one or more species contained within a membrane. In a sense, a membrane selective separation of isomers could be viewed as an efficient chromatographic process. The analyte does not cross the membrane into another media before detection.

Developments in the separation of isomers using membranes and Langmuir–Blodgett monolayers include the development of various sensors (e.g., spectroscopic, electrochemical or acoustic). Thin films, analogous to gas chromatographic stationary phases, containing cyclodextrins have been used on the surface of these sensors.

The ability of β-CD to enhance the fluorescence of pyrene was exploited in the development of an O_2 sensor [126] and fiber optic sensors for the quantitation of pyrene [127] and other polycyclic aromatic hydrocarbons. Modification of β-CD with dansylglycine with subsequent incorporation in a sol–gel film immobilized on a SiO_2 plates was used in a fiber optic sensor for the detection of non-fluorescent compounds [128]. Since dansyl can complex with β-CD, displacement of the fluorophore by the analyte resulted in fluorescence quenching.

Optical isomers of various chiral aromatics were separated on a thin membrane containing cyclodextrins and consequently detected using a quartz resonator sensor [129]. To selectively detect chiral

isomers of 2-substituted methylpropionates and anesthetics in vapor, membrane modified thickness-shear mode resonators were developed [130]. Chiral discrimination of these isomers was in excellent agreement with results from GC.

Selective binding of alkyl and aryl ammonium ions by lipophilic cyclodextrins was used for development of selective films on electrodes for quantitative potentiometric analysis [131–133]. Various derivatives of α-, β- and γ-CD were included into polymeric membranes (polyvinyl chloride films). Selectivities were highly dependent on the type and number of substituents appended onto the cyclodextrin. For instance, peroctylated cyclodextrin provided a high sensitivity towards alkyl ammonium ions [e.g., $(CH_3)_4N^+$, $(CH_3CH_2)_4N^+$] with minimal interference from group Ia and IIa cations; peroctylated α-CD was selective to dopamine hydrochloride, while partially octylated α-CD was selective to ephedrine and amphetamine.

Langmuir–Blodget films were prepared from amphiphilic derivatives of α-, β- and γ-CD [134]. UV–Vis spectroscopy revealed that the films discriminated between azobenzene derivatives (e.g., *o*-isomers were excluded while *m*- and *p*-isomers were preferably bound). This investigation led to the development of a fiber optic sensor for analysis of aromatics in the gas phase.

A slightly different approach in the separation and permeability of thin layers was in the development of single Langmuir–Blodget monolayers for gas separation [135]. The basic support material consisted of poly[1-trimethylsilyl)-1-propyne] and a single monolayer of calix[6]arene. Pores of the basic support were measured to be about 10 Å and were sufficient for non-selective permeation of gases. When α- or β-CD was added into the basic polymer matrix, they created additional molecular pores through which a gas could be transported. However, both cyclodextrins also formed complexes with the polymer and the selectivity of resultant layer dramatically improved for He and Ne. Further improvement was obtained with the addition of phenoxide.

In a liquid membrane separation, an analyte passes from one liquid media to another. While dialysation membrane serves as discrimination media usually based on size exclusion, liquid membrane separations are based on preferential inclusion. Armstrong and Jin [136] first reported enrichment of the bulk solution with enantiomers and various other types of isomers using aqueous liquid membranes containing cyclodextrins. They used aqueous liquid membranes containing α-, β- or γ-CD. Liquid membranes were of several configurations. In all configurations, however, cyclodextrins were contained in aqueous solutions. In the case of an aqueous membrane, a small volume of water containing cyclodextrin was placed between two ether solutions, one pure, and the other containing the isomers to be separated. Transport efficiency of the membrane was determined by the *off-line* quantitative analysis of isomers on either side of the membrane via HPLC. Positional isomers of nitroaniline, nitrotoluene, bromobenzoic acid, and aspirin, and enantiomers of benzylnornicotine, mephenytoin, disopyramide, stilbene and others were used. It was found that the extent of discrimination was proportional to the membrane thickness [137–139].

16. Molecularly imprinted polymers

The approach of molecular imprinting is elegant and simple. Spatial memory of the molecule of interest (template) is created in an extensively cross-linked polymer or copolymer. Imprinted polymers can be used the same way as stationary phases in various techniques, with higher selectivity and specificity resulting from the imprint. Although the slow kinetics may be disadvantageous for chromatographic media, other potential applications include extraction and sample clean-up.

The copolymerization of cyclodextrin with monomers and a template molecule can further improve selectivity and binding. In addition, cyclodextrin-containing molecularly imprinted polymers (MIPs) may have enhanced absorption capacity relative to analogous silica-based materials. Polymerization of the monomer, cyclodextrin, and template has been done in both aqueous and non-aqueous environments. The general approach in the synthesis of MIPs is to use non-aqueous solvents for the synthesis. The main recognition elements for templates used in organic solvents are polar and electrostatic interactions. However, it is known that these interactions are weaker in water and the resultant MIPs

generally are reported to have diminished recognition if aqueous solvents are used in the separation.

Hishiya et al. carried out a comprehensive characterization of MIPs containing cyclodextrins [140]. β-CD was crosslinked with toluene 2,4-diisocyanate in the presence of various steroids and dimethyl sulfoxide. The ability of the resulting MIPs to bind template molecules was examined in free solution. GC monitored depletion of the steroid from the solution upon addition of MIP. Analysis suggested that binding of any steroid onto an MIP was a reversible process. Based on the systematic study of cholesterol, stigmasterol, testosterone, 4-cholesten-3-one, progesterone, and pregnenolone, it was suggested that three β-CD molecules are mutually oriented to form a complex with three parts of the sterol molecule. The schematic picture of this binding can be seen in Fig. 7. Binding activity of the MIP was highest toward cholesterol, stigmasterol and 4-cholesten-3-on. All these molecules contained bulky alkyl residues on a C-14 position, likely enhancing binding affinity. As suggested by the authors, the discrimination of the MIP could be further enhanced in a chromatographic process, in which the number of theoretical plates can be increased.

Sreenivasan obtained similar results [141]. In this study, the MIP was prepared by copolymerization of β-CD, 2-hydroxyethyl methacrylate and testosterone or cholesterol, respectively, in chloroform. Cholesterol was preferentially bound in both MIPs.

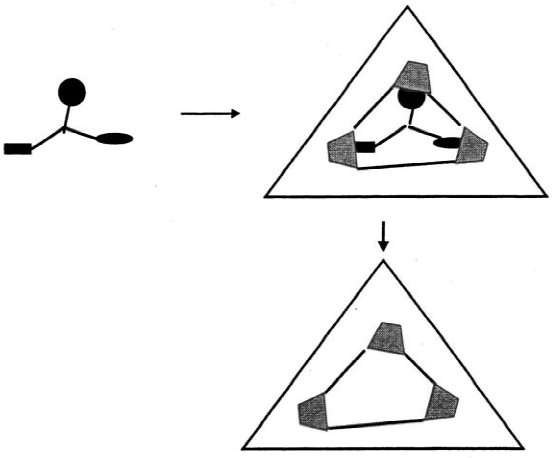

Fig. 7. Schematic representation of binding sites in MIPs for selective binding of steroids. For details see text.

Acryloyl-6-*O*-α-D-glucosyl β-CD was polymerized with short peptide templates in water [142]. The resultant MIPs exhibited good selectivity.

The first approach to design MIPs with both hydrophobic and polar selectivity elements was for D-phenylalanine [143,144]. The polymerization of MIPs was performed in water, using D-phenylalanine as a template, acryloyl chloride β-CD for hydrophobicity, and 2,2′-dimethylpropane sulfonic acid for electrostatic recognition. The proposed structure of the recognition site in MIPs is shown in Fig. 8. In this study, four MIPs were synthesized and used as HPLC packings, containing all or missing one of the components. In addition to routine HPLC experiments, chromatographic displacement studies and fluorescent studies were used to estimate enantioselectivity of the four MIPs. Although β-CD was shown to have inherent preferential binding for L-phenylalanine, molecular imprinting with D-phenylalanine reversed this selectivity. If 2,2′-dimethylpropane sulfonic acid was not incorporated, D-isomer selectivity was lost. The effect of the

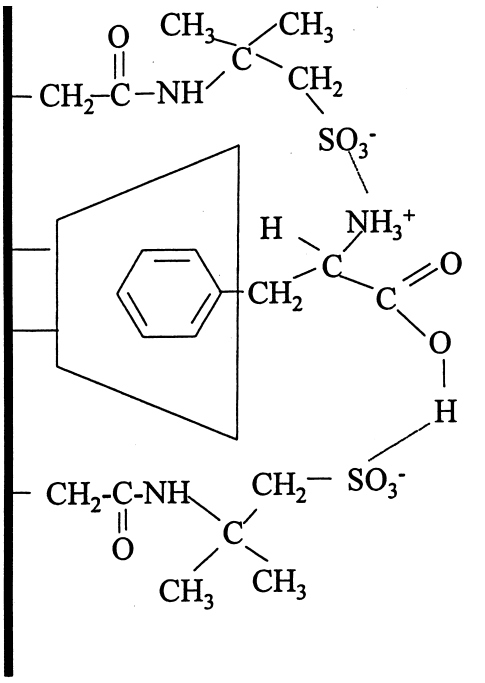

Fig. 8. Molecular imprinting in aqueous environment – taking advantage of hydrophobic and ionic interactions in the recognition process.

separation efficiency and selectivity of the polymer used as a chromatographic packing depended on chromatographic conditions used. For example, in the presence of a highly organic mobile phase, the elution order for the D- and L-isomer was either reversed, or the isomers were not retained on the column.

17. Conclusions and future research

Cyclodextrins are a versatile tool in the separations of positional isomers and enantiomers. Additionally, they are used to increase stability of an analyte, prevent non-specific absorption and promote analyte detection. They have been successfully used in all areas of separation science and they continue to inspire creative applications. Specific derivatization of the cyclodextrin rim often enhances its recognition ability. Thus, systematic development of cyclodextrin molecules with enhanced specificity towards particular analytes is feasible. Because derivatization of cyclodextrins can not only enhance but also diminish specificity of cyclodextrin towards some analytes, understanding the influence of hydrogen bonding, polar, and ionic interactions with the cyclodextrin and pendant groups on the recognition process are important for further enhancement of separations.

Miniaturization of existing techniques, adaptation of developments made in seemingly diverse areas as well as development of large-scale separations are necessary to extend cyclodextrin application to their full potential. Further developments of Langmuir–Blodget monolayers, solid and liquid membranes, hollow fibers, and advancement in sensor manufacturing will enable miniaturization of existing techniques. Large-scale separations, vital for industrial processes, will likely involve development of chiral separations in continuous flow electrophoresis and development of membrane based separations for complete separation of isomers.

18. Abbreviations

CCC	Countercurrent chromatography
CD	Cyclodextrin
CE	Capillary electrophoresis
CFFE	Continuous free flow electrophoresis
diMe-β-CD	Dimethyl beta cyclodextrin
GC	Gas chromatography
HPLC	High-performance liquid chromatography
HP-γ-CD	Hydroxypropyl gamma cyclodextrin
HP-β-CD	Hydroxypropyl beta cyclodextrin
IEF	Isoelectric focusing
ITP	Isotachophoresis
LC	Liquid chromatography
Me-γ-CD	Methyl gamma cyclodextrin
m-	*Meta*
MIP	Molecularly imprinted polymer
o-	*Ortho*
p-	*Para*
PAH	Polycyclic aromatic hydrocarbon
SDS	Sodium dodecyl sulfate
SFC	Supercritical fluid chromatography
SMB	Simulated moving bed
TLC	Thin-layer chromatography
UV	Ultraviolet

References

[1] J. Szejtli, T. Osa, Comprehensive Supramolecular Chemistry, Cyclodextrins, Vol. 3, Pergamon, Elsevier, Oxford, 1996.
[2] K.A. Connors, J. Pharm. Sci. 84 (1995) 843.
[3] D. Duchene, New Trends in Cyclodextrins and Derivatives, Edition de Sante, Paris, 1991.
[4] W.L. Hinze, D.W. Armstrong, Anal. Lett. 13 (A12) (1980) 1093.
[5] T.J. Ward, D.W. Armstrong, J. Liq. Chromatogr. 9 (2&3) (1986) 407.
[6] D.W. Armstrong, J. Liq. Chromatogr. 3 (6) (1980) 895.
[7] W.G. Burkert, C.N. Owensby, W.L. Hinze, J. Liq. Chromatogr. 4 (6) (1981) 1065.
[8] S.M. Han, D.W. Armstrong, Chem. Anal. 108 (1990) 81.
[9] W.L. Hinze, D.Y. Pharr, Z.S. Fu, W.G. Burkert, Anal. Chem. 61 (1989) 422.
[10] J.S. Pagington, Chem. Br. 23 (1987) 457.
[11] T. Cserhati, J. Szjetli, E. Fenyvesi, J. Chromatogr. 439 (1988) 393.
[12] D.M. Sand, H. Schlenk, Anal. Chem. 33 (1961) 1624.
[13] H. Schlenk, J.L. Gellerman, D.M. Sand, Anal. Chem. 34 (1962) 1529.
[14] J. Mraz, L. Feltl, E. Smolkova-Keulemansova, J. Chromatogr. 286 (1986) 17.
[15] E. Keulemansova-Smolkova, S. Krysl, J. Chromatogr. 84 (1980) 347.
[16] E. Keulemansova-Smolkova, H. Kralova, S. Krysl, L. Feltl, J. Chomatogr. 241 (1982) 3.
[17] G.L. Reid, W.T. Wall, D.W. Armstrong, J. Chromatogr. 633 (1993) 143.

[18] G.L. Reid, C.A. Monge, W.T. Wall, D.W. Armstrong, J. Chromatogr. 633 (1993) 135.
[19] M. Jung, S. Mayer, V. Schurig, LC·GC 12 (6) (1994) 458.
[20] V. Schurig, J. Chromatogr. A 666 (1–2) (1994) 111.
[21] D.W. Armstrong, J.T. Lee, L.W. Chang, Tetrahedron: Asymmetry 9 (12) (1998) 2043.
[22] E. Francotte, Chim. Nouv. 14 (53) (1996) 1541.
[23] A. Geerlof, J. Bert, A. Van Tol, J.A. Jongejan, J.A. Duine, J. Chromatogr. 648 (1) (1993) 119.
[24] D.U. Staerk, A. Shitangkoon, G. Vigh, J. Chromatogr. A 677 (1) (1994) 133.
[25] D.U. Staerk, A. Shitangkoon, G. Vigh, J. Chromatogr. A 663 (1) (1994) 79.
[26] M. Juza, O. Di Giovanni, G. Biressi, V. Schurig, M. Mazzotti, M. Morbidelli, J. Chromatogr. A 813 (1998) 333.
[27] P. Macaudiere, M. Caude, R. Rosset, A. Tambute, J. Chromatogr. 405 (1987) 135.
[28] P. Macaudiere, M. Caude, R. Rosset, A. Tambute, J. Chromatogr. Sci. 27 (1989) 383.
[29] A. Kenichi, Fragrance J. 27 (2) (1999) 28.
[30] J. Breinholt, S.V. Lehmann, A.R. Varming, Chirality, in press.
[31] D. Sybilska, M. Asztemborska, A. Bielejewska, J. Kowalczyk, H. Dodziuk, K. Duszczyk, H. Lamparczyk, P. Zarzycki, Chromatographia 35 (1993) 637.
[32] H. Lamparczyk, P.K. Zarzycki, J. Nowakowska, J. Chromatogr. A 668 (1994) 413.
[33] A. Walhagen, L.E. Edholm, Chromatographia 32 (1991) 215.
[34] H. Lamparczyk, P.K. Zarzycki, R.J. Ochocka, M. Asztemborska, D. Sybilska, Chromatographia 31 (1991) 157.
[35] M. Gazdak, G. Szepesi, L. Huszar, J. Chromatogr. 351 (1986) 128.
[36] M. Gazdak, G. Szepesi, L. Huszar L, J. Chromatogr. 371 (1986) 227.
[37] J. Zukowski, D. Sybilska, J. Bojarski, J. Szjetli, J. Chromatogr. 436 (1988) 381.
[38] S. Wang, M.D. Porter, J. Chromatogr. A 828 (1998) 157.
[39] A. Harada, M. Furue, S.I. Nozakura, J. Polym. Sci. 16 (1978) 189.
[40] N. Widdenhot, Starke 21 (1969) 164.
[41] J.L. Hoffman, Anal. Biochem. 33 (1970) 209.
[42] Y. Kawaguchi, M. Tanaka, M. Nakae, K. Funazo, T. Shono, Anal. Chem. 55 (1983) 1852.
[43] K. Fujimura, T. Veda, T. Ando, Anal. Chem. 55 (1983) 336.
[44] M. Waksmundzka-Hajnos, J. Chromatogr. B 717 (1998) 93.
[45] M. Tanaka, J. Okazaki, H. Ikeda, T. Shono, J. Chromatogr. 370 (1986) 293.
[46] M. Tanaka, Y. Kawaguchi, M. Nakae, K. Funazo, T. Shono, J. Chromatogr. 299 (1984) 341.
[47] K. Fujimura, K. Suzuki, K. Hagashi, S. Masuda, Anal. Chem. 60 (1990) 2198.
[48] Y. Kuroda, T. Koto, H. Ogoshi, Bull. Chem. Soc. Jpn. 66 (1993) 1116.
[49] N. Thuaud, B. Sebille, A. Deratani, B. Popping, C. Pellet, Chromatographia 36 (1993) 373.
[50] N. Thuaud, B. Sebille, A. Deratani, G. Lelievre, J. Chromatogr. 555 (1991) 53.
[51] N. Thuaud, B. Sebille, J. Chromatogr. A 685 (1994) 15.
[52] Y. Mizobuchi, M. Tanaka, T. Shono, J. Chromatogr. 208 (1981) 35.
[53] E. Muller, D. Lubda, G. Wieland, K. Cabrera, K. Czerny, E. Dicks, PCT Int. Appl. 22p. 1998 Patent.
[54] C. Pham-Huy, B. Radenen, A. Sahui-Gnassi, J.R. Claude, J. Chromatogr. B 665 (1995) 125.
[55] S.C. Chang, G.L. Reid, S. Chen, C.D. Chang, D.W. Armstrong, Trends Anal. Chem. 12 (1993) 144.
[56] D.W. Armstrong, S. Chen, C. Chang, S. Chang, J. Liq. Chromatogr. 15 (1992) 545.
[57] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[58] J.D. Duncan, J. Liq. Chromatogr. 13 (14) (1990) 2737.
[59] F.M. Everts, J.L. Beckers, Th.P.E.M. Verheggen, Isotachophoresis Theory, Instrumentation, and Applications, Elsevier, Amsterdam, 1984.
[60] M. Tazaki, M. Tagaki, U. Keihei, Chem. Lett. 5 (1982) 639.
[61] N. Kuramoto, Chem. Express 1 (6) (1986) 343.
[62] M. Tazaki, T. Hayashita, Y. Fujino, M. Takagi, Bull. Chem. Soc. Jpn. 59 (11) (1986) 3459.
[63] I. Jelinek, J. Snopek, E. Smolkova-Keulemansova, J. Chromatogr. 405 (1987) 379.
[64] J. Snopek, E. Smolkova-Keulemansova, I. Jelinek, J. Dohnal, J. Klinot, E. Klinotova, J. Chromatogr. 450 (1988) 373.
[65] J. Snopek, I. Jelinek, E. Smolkova-Keulemnasova, J. Chromatogr. 411 (1987) 153.
[66] I. Jelinek, J. Snopek, E. Smolkova-Keulemnasova, J. Chromatogr. 435 (1988) 496.
[67] I. Jelinek, J. Snopek, E. Smolkova-Keulemansova, J. Chromatogr. 557 (1–2) (1991) 215.
[68] J. Snopek, I. Jelinek, E. Smolkova-Keulemnsova, J. Chromatogr. 435 (3) (1988) 211.
[69] I. Jelinek, J. Snopek, E. Smolkova-Keulemansova, J. Chromatogr. 439 (2) (1988) 386.
[70] I. Jelinek, J. Dohnal, J. Snopek, E. Smolkova-Keulemansova, J. Chromatogr. 464 (1) (1989) 113.
[71] S. Zaugg, J. Caslavska, R. Theurillat, W. Thorman, J. Chromatogr. A 838 (1–2) (1999) 237.
[72] E. Dubrovcakova, B. Gas, J. Vacik, E. Smolkova-Keulemansova, J. Chromatogr. 623 (1992) 337.
[73] J. Snopek, I. Jelinek, E. Smolkova-Keulemansova, J. Chromatogr. 472 (1989) 808.
[74] P. Blatny, F. Kvasnicka, E. Kenndler, J. Chromatogr. A 737 (2) (1996) 255.
[75] M. Dankova, D. Kaniansky, S. Fanali, F. Ivanyi, J. Chromatogr. A 838 (1–2) (1999) 31.
[76] H. Nishizawa, K. Nakajima, M. Kobayashi, Y. Abe, Anal. Sci. 7 (6) (1991) 959.
[77] J.M. Lin, T. Nakagama, H. Okazawa, X.Z. Wu, T. Hobo, Fresenius J. Anal. Chem. 354 (1996) 451.
[78] T. De Boer, E.K. Theo, J. Pharm. Biomed. Anal. 17 (6–7) (1998) 1047.
[79] T. De Boer, R. Bijma, K. Ensing, J. Pharm. Biomed. Anal. 19 (3–4) (1999) 529.
[80] A.M. Stalcup, K.H. Gahm, S.R. Gratz, R.M. C Sutton, Anal. Chem. 70 (1998) 144.
[81] R.M.C. Sutton, S.R. Gratz, A.M. Stalcup, Analyst 123 (1998) 1477.
[82] P.G. Righeti, C. Ettori, P. Chafey, J.P. Wahrmann, Electrophoresis 11 (1) (1990) 1.
[83] P. Glukhovski, G. Vigh, Anal. Chem. 71 (17) (1999) 3814.

[84] M.C. Roman, P.R. Brown, Anal. Chem. 68 (1994) 86A.
[85] M. Lanz, J. Caslavska, W. Thormann, Electrophoresis 19 (7) (1998) 1081.
[86] P. Hoffmann, H. Wagner, G. Weber, M. Lanz, J. Caslavska, W. Thormann, Anal. Chem. 71 (9) (1999) 1840.
[87] S. Fanali, J. Chromatogr. A 792 (1997) 227.
[88] F. Bressolle, M. Audran, T.N. Pham, J.J. Vallon, J. Chromatogr. B 687 (1996) 303.
[89] J.H.T. Luong, A.L. Nguyen, J. Chromatogr. A 792 (1+2) (1997) 431.
[90] K. Verleysen, P. Sandra, Electrophoresis 19 (16–17) (1998) 2798.
[91] I.S. Lurie, J. Chromatogr. A 792 (1+2) (1997) 297.
[92] M. Jung, S. Mayer, V. Schurig, LC·GC 12 (6) (1994) 458.
[93] S. Terabe, Trends Anal. Chem. 8 (4) (1989) 129.
[94] K. Otsuka, S. Terabe, Chromatogr. Sci. Ser. 64 (1993) 617.
[95] G. Vigh, A.D. Sokolowski, Electrophoresis 18 (12–13) (1997) 2305.
[96] D.W. Armstrong, E.Y. Zhou, S. Chen, K. Le, Y. Tang, Anal. Chem. 66 (1994) 4278.
[97] D.W. Armstrong, E.Y. Zhou, S. Chen, K. Le, Y. Tang, Anal. Chem. 70 (1998) 3717.
[98] US Pat. 3 456 028 (Chem. Abstr. 71, 91044x 1969); US Pat. 3 456 055.
[99] K. Matsunaga, M. Imanaka, T. Ishida, T. Oda, Anal. Chem. 56 (1984) 1980.
[100] L.A. Blyshak, T.M. Rossi, G. Patonay, I.M. Warner, Anal. Chem. 60 (1988) 2127.
[101] N.B. Elliott, A.J. Prenni, T.T. Ndou, I.M. Warner, J. Colloid Interface Sci. 156 (1993) 359.
[102] J. Andreaus, J. Draxler, R. Marr, A. Hermetter, J. Colloid Interface Sci. 193 (1997) 8.
[103] E. Fenyvesi, J. Szeman, J. Szjetli, J. Incl. Phenom. Mol. Recognit. Chem. 25 (1–3) (1996) 229.
[104] I. Uemasu, H. Takahashi, Sekiyu Gakkaishi 36 (3) (1993) 210.
[105] D. Warner-Schmid, S. Hoshi, D.W. Armstrong, Sep. Sci. Technol. 28 (4) (1993) 1009.
[106] I. Uemasu, J. Incl. Phenom. Mol. Recognit. 13 (1992) 1.
[107] K. Kobayashi, G. Wenz, W. Knoll, J. Incl. Phenom. Mol. Recognit. Chem. 28 (4) (1997) 349.
[108] L.M. Thienpont, V.I. De Brabandere, D. Stöckl, A.P. De Leenheer, Anal. Chem. 66 (1994) 4116.
[109] W.J. DeGrip, J. VanOostrum, P.H.M. Bovee-Geurts, Biochem. J. 330 (1998) 667.
[110] G.N. Richards, M. Manley-Harris, US Pat., 5882520 A 990316.
[111] Y. Ohira, N. Ishikawa, Y. Takeuchi, M. Takeyama, Jpn. Pat., JP 11009218 A2 990119.
[112] D. Oakenfull, in: G.A. Spiller (Ed.), Handbook of Lipids in Human Nutrition, CRC Press, Boca Raton, FL, 1996, p. 221.
[113] C. Shushi, J. Chin. Chem. Soc. (Taipei) 44 (6) (1997) 629.
[114] V.P. Agarwal, J. Liq. Chromatogr. 14 (4) (1991) 699.
[115] H. Sherma, J.E. Bernado, M.H. Higgs, J. Liq. Chromatogr. 11 (15) (1988) 3135.
[116] J. Anzai, Y. Kobayashi, A. Ueno, T. Osa, J. Chem. Soc. Chem. Commun. 15 (1985) 1023.
[117] A.N. Khramov, J.A. Stenken, Anal. Chem. 71 (1999) 1257.
[118] A.N. Khramov, J.A. Stenken, Analyst 124 (1999) 107.
[119] C.A. Ventura, G. Puglisi, M. Zappala, G. Mazzone, Int. J. Pharm. 160 (2) (1998) 163.
[120] S. Kjellstorm, J. Emneus, T. Laurell, L. Heintz, G. Marko-Varga, J. Chromatogr. A 823 (1998) 489.
[121] P. Savolainen-Saarinen, T. Järvinen, H. Taipale, A. Urtti, Int. J. Pharm. 159 (1997) 27.
[122] A. Yamasaki, K. Ogasawara, T. Iwatsubo, T. Masuoka, K. Mizoguchi, Bussihitsu Kogaku Kogyo Gijutsu Kenkyusho Hokoku 3 (1) (1995) 19.
[123] A. Yamasaki, K. Ogasawara, K. Mizoguchi, J. Appl. Polym. Sci. 54 (7) (1994) 867.
[124] D.K. Mandal, A.K. Guha, K.K. Sirkar, J. Membr. Sci. 144 (1998) 13.
[125] D.J. Brose, M.B. Chidlaw, D.T. Friesen, E.D. laChapelle, P. Van Eikeren, Biotechnol. Prog. 11 (2) (1995) 214.
[126] M. Li, M. Pacholski, F.V. Bright, Appl. Spectrosc. 49 (12) (1995) 1809.
[127] A. Sadana, J.P. Alarie, T. Vo-Dinh, Talanta 42 (10) (1995) 1567.
[128] R.A. Dunbar, U. Narang, P.N. Prasad, F.V. Bright, Proc. SPIE-Int. Soc. Opt. Eng. 1796 (1993) 210.
[129] J. Ide, T. Nakamoto, T. Morizumi, Sens. Actuators A A49 (1–2) (1995) 73.
[130] K. Bodenhöfer, A. Hierlemann, F. Göpel, M. Juza, B. Gross, V. Schurig, Chim. OGGI/Chem. Today May (1998) 56.
[131] P.M. Kelly, R. Kataky, D. Parker, A.F. Patti, J. Chem. Soc., Perkin Trans. 2 (11) (1995) 1955.
[132] R. Kataky, D. Parker, P.M. Kelly, Scan. J. Clin. Lab. Invest. 55 (5) (1995) 409.
[133] R. Kataki, P.S. Bates, D. Parker, Analyst 117 (8) (1992) 1313.
[134] M. Matsumoto, M. Tanaka, R. Azumi, H. Tachibana, T. Nakamura, Y. Kawabata, T. Miyasaka, H. Tagaki, H. Nakahara, H. Fukuda, Thin Solid Films 210/211 (1992) 803.
[135] L. Zhang, R.A. Hendel, P.G. Cozzi, S.L. Regen, J. Am. Chem. Soc. 121 (1999) 1621.
[136] D.W. Armstrong, H.L. Jin, Anal. Chem. 59 (1987) 2237.
[137] D.K. Mandal, A.K. Guha, K.K. Sirkar, J. Membr. Sci. 144 (1–2) (1998) 13.
[138] I.K. Lednev, M.C. Petty, Adv. Mater. 8 (1996) 615.
[139] H.Y. Aboul-Enein, R.I. Stefan, Crit. Rev. Anal. Chem. 28 (3) (1998) 59.
[140] T. Hishiya, M. Shibata, M. Kakazu, H. Asanuma, M. Komiyama, Macromolecules 32 (1999) 2265.
[141] K. Sreenivasan, J. Appl. Polym. Sci. 70 (1998) 15.
[142] H. Asanuma, H. Kajiya, T. Hishiya, M. Komiyama, Chem. Lett. 7 (1999) 665.
[143] S.A. Piletsky, H.S. Anderson, I.A. Nicholls, Macromolecules 32 (1999) 633.
[144] S.A. Piletsky, H.S. Anderson, I.A. Nicholls, J. Mol. Recognit. 11 (1998) 94.