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REVIEW ARTICLE

Application of crown ethers as stationary phase in the chromatographic methods

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Abstract Recently, much attention has been paid to chromatographic characteristics and applications of crown ethers. These compounds were employed as chiral stationary phase for resolution of various racemic compounds in high performance chromatography and capillary electrochromatography techniques. Crown ethers also used in gas chromatography as the stationary phase. Recently, it has been found that, crown ethers also may be useful in cation chromatographic separation in ion chromatography for the determination of alkali and alkaline-earth cations, ammonium, and amines. In this paper we have an overview on these applications of crown ethers.

Keywords Crown ethers · Chromatography · Chiral stationary phases · Solid phase microextraction

Introduction

Since the discovery of crown ethers [1], many investigation has been made on the complexation of these compounds with metal cations [2–6]. Due to this ability, these compounds have been used extensively in extraction analysis studies of various metal ions [7–9]. By polymerization or immobilization of crown ethers on inert support materials they have been used as stationary phases for chromatographic separations. Polymeric crown ethers possess special features such as high resistance to chemicals, to temperature, to radiolysis and also to polar solvents. Chromatographic techniques with chiral stationary phases (CSPs) having crown ether selectors have been extensively developed to achieve good enantiomer separation of amino compounds for analytical and preparative purposes. Crown ether is a widely used stationary phase in capillary gas chromatography (GC) because of its high coating efficiency and unique selectivity in separation of polar compounds having similar boiling points.

The development of simple and robust capillary electrochromatography (CEC) column technologies plays an important role for popularization of CEC. During the last several years, various approaches for the preparation of enantioselective columns based on crown ethers have been reported. Currently, the monolithic column technology (continuous beds) represents the most advanced approach for the preparation of CEC columns. Preconcentration method based SPME crown ethers have been used in the preparation of the fibers.

Crown ethers are macrocyclic polyether compounds that are capable of selectively forming complexes with a variety of different cationic species. The ability of a crown ether molecule to complex with a cation is dependent upon the size of the hole formed by macrocyclic structure and, as a result, crown ethers of different sizes exhibit significantly different specificities for the complexation of cations, and then may be useful in ion chromatographic separation quite different in chromatographic behavior from conventional "ionic" cation exchangers. In this review we discussed on the application of crown ethers as the stationary phase in the chromatographic methods.

Crown ether chiral stationary phases developments

One of the most frequently used methods for the determination of enantiomeric compositions and enantio separations of mixtures of enantiomers is chromatography on

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CSPs. Over the past few decades there has been great interest in designing new CSPs [10–14].

CSPs based on chiral crown ethers, were first developed by Sogah and Cram in 1970s [15], but these CSPs are not commercially available, presumably because of their low chromatographic performance. In 1987 Shinbo and co-workers reported the separation of amino acids using a CSP in which a hydrophobic chiral crown ether was dynamically coated on an ODS stationary phase [16, 17]. This type of CSP was commercialized as CROWNPAK CR, which is the first commercialized crown ether-based CSP, and it has been proven useful for the resolution of chiral primary amines [18]. However, solvents that can be used as a mobile phase on this CSP are limited because of the dynamic coating process. In view of the better complexation ability of crown ethers in organic solvents than in aqueous media, the use of a normal mobile phase would be advantageous for the separation of chiral amino compounds. Therefore, it is desirable to develop CSPs that can be used with a normal mobile phase eluent. Indeed, since 1998, chemically bonded type CSPs containing chiral crown ethers have been developed actively [19–24]. Machida and co-workers reported this type of CSP using (+)-18-crown-6-2,3,11,12-tetracarboxylic acid as a chiral selector bound by an amide linkage to silica gel [19]. Hyun and co-workers also developed CSPs using the same crown ether as a selector, but a different bonding process that Machida's CSP [20–22]. Bradshow's group reported CSPs using selectors having a metacyclophane framework containing a pyridine moiety [24].

Recently, chiral stationary phases-1(CSP-**1**) and chiral stationary phases-2 (CSP-**2**) were developed (Fig. 1) for ammonium salts where the chiral pseudo-18-crown-6 ethers as selector are covalently bound to silica gel [25–27].

CSP-**1** was commercialized as SUMICHIRAL OA-8000 in 2000 and it was one of the earliest CSPs with a covalently bound chiral crown ether selector [25, 26].

In order to respond to the increasing demand to separate chiral amino compounds including lipophilic examples, it is desirable to develop crown ether CSPs for which a normal mobile phase can be used as the eluent. In addition, it is also desirable to develop CSPs that provide short retention times but large differences in the separation of enantiomers. Therefore, Hirose designed CSP-**2**. He report the preparation of CSP-**2** and its use for enantiomer separation of chiral amino compounds such as amines, amino alcohols, and amino acids [27].

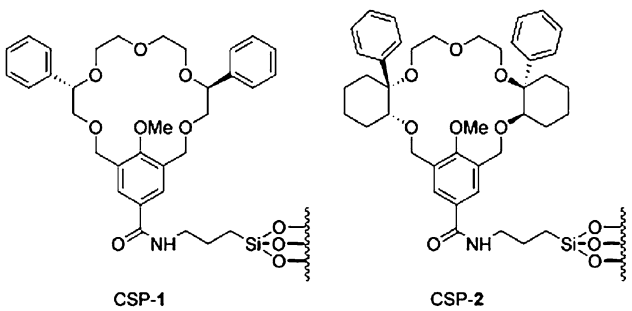

Fig. 1 Structure of CSP-**1** and CSP-**2**

Hyun found that the variation in the structure of α-amino acids effect on their resolution. In particular, the length of the alkyl group at the chiral center of α-amino acid homologues seems to be an important factor in control of their resolution on CSP. A crown ether-based chiral stationary phase (CSP **3**, Fig. 2) developed in Hyun laboratory by bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid to 3-aminopropylsilica gel was very successful in the resolution of various racemic primary amino compounds including α-amino acids, β-amino acids, α-amino acid derivatives [28] racemic amines [22] racemic amino alcohols [22] and racemic fluoroquinolone antibacterials [20, 29, 30]. However, CSP **3** intrinsically contains unreacted residual aminopropyl groups on the surface of silica gel because the process of bonding (+)-(18-crown-6)-2,3,11,12-tetracarboxylic anhydride to aminopropylsilica gel cannot be completed. The unreacted residual aminopropyl groups of CSP **3** can be protonated under the acidic mobile phase condition and the resulting primary ammonium ions were expected to compete with the primary ammonium ions of analytes for the complexation inside the cavity of the crown ether ring of the CSP. Protection of the unreacted residual aminopropyl groups of CSP **3** with acetyl or butyryl group actually improved the retention (k) and the resolution factors (RS) for the resolution of α-amino acids, but diminished the separation factors (α) slightly [31]. In order to avoid the problems related to the unreacted residual aminopropyl groups on CSP **3**, a new residual aminopropyl group-free CSP (CSP **4**, Fig. 2) was prepared by bonding *N*,*N*′-triethoxysilylpropyl syn-diamide of (+)-(18-crown-6)-2, 3,11,12-tetracarboxylic acid to silica gel directly [32].

CSP **4** has been successfully applied to the resolution of various α-amino acids, amines and amino alcohols [32]. The chiral recognition efficiency of CSP **4** was found generally superior to that of CSP **3** in terms of the separation and the resolution factors as expected. In one effort the effect of analyte lipophilicity on the resolution of α-amino acids on this CSP based on chiral crown ether has been examined by the chromatographic resolution trends for a homologous series of five α-amino acids with an alkyl group of different length at the chiral center. The retention factors k_1 and k_2 for the two enantiomers and the separation factors (α) were found to depend on the lipophilicity of the α-amino acid. In general, the retention factors increased as the organic modifier content in the mobile phase increased and the degree of

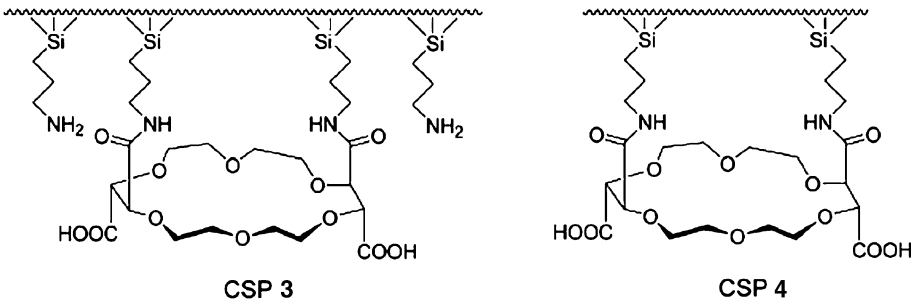

Fig. 2 Structure of two types of crown ether-based chiral stationary phases

the enhancement of retention factors being dependent on the analyte lipophilicity. The separation factors also increased as the analyte lipophilicity and the organic modifier content in the mobile phase increased [33]. Using of CSP **4** developed in Hyun laboratory and was successfully applied to the resolution of various racemic primary amino compounds [28, 29]. CSP **4** was also employed in resolving aromatic halogen substituted phenylalanines, phenylglycine homologues and other primary amino acids. The covalent immobilization of a chiral crown ether ligand on a silica substrate allows the use of methanol-rich mobile phases. Retention generally increases with increasing methanol. The addition of triethylamine also generally increases retention. Highest enantioselectivities were observed in the presence of high methanol, high triethylamine concentrations. However, the enhanced selectivity came at the expense of greatly increased retention times [34].

As an effort to extend the use of CSP **4** further, Hyun wish to first report the resolution of *N*-(3,5-dinitrobenzoyl)-α–amino acids on CSP **4**. *N*-(3,5-dinitrobenzoyl)-α-amino acids do not contain any primary or secondary amino group, but contain a free carboxylic acid group. In this instance, the resolution of *N*-(3,5-dinitrobenzoyl)- α-amino acids on this CSP is quite unusual and surprising (0.54–2.81) [35]. However, CSP **4** has not been applied to the resolution of *β*-amino acids yet while CSP **3** has been found useful for the resolution of *β*-amino acids [36]. Optically active *β*-amino acids have attracted considerable attention due to their potent pharmacological activities and their usefulness as building blocks of many natural products [37]. In this instance, analytical methods for the exact determination of the enantiomeric composition of *β*-amino acids are essential and the liquid chromatographic chiral separations on CSPs might be the desired analytical method. After that, he applied CSP **4** to the resolution of various *β*-amino acids and compared the chromatographic resolution results with those on CSP **3**. Comparison of the chromatographic resolution results on CSP **4** with those on CSP **3** is expected to elucidate the characteristics of CSP **4** in the resolution of *β*-amino acids. Results showed that the CSP **4** is also quite applicable for use in analytical enantioselective chromatography [38].

Machida et al. [39] modified (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid with *S*-1-(1-naphthyl)ethylamine as a π-donor. A CSP based on this material was used to enantioselectively separation of the secondary amine, *N*-3,5-dinitrobenzoyl-1-(α-naphthyl)ethylamine. The separation was explained by π–π interaction between the 3,5-dinitrobenzoyl function of the analyte (π-acceptor) and the *S*-1-(1-naphthyl)ethylamine moiety (π-donor),because a CSP based on (1)-(18-crown-6)-2,3,11,12-tetracarboxylic acid without the added π donor could not resolve the secondary amine.

In the another approach liquid chromatography columns based on the (+) and (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid CSPs used for the separation of secondary amine enantiomers. The method employs both the ability of crown ethers to complex with amine groups and the steric and electrostatic interactions afforded by the carboxylate functionalities [40].

Hyun and co-workers developed another effective crown ether CSP (Fig. 3). The chiral crown ether moiety of this CSP bears some similarity to the commercial CROWNPAK CR, but differs in that it is covalently bonded to silica gel. Consequently, covalently linked CSP is useful in a variety of mobile phases. This was very effective in resolving various natural and unnatural α-amino acids [41].

However, it has not been utilized for separating the enantiomers of other chiral compounds. After that they extend the use of this CSP to the enantioseparation of investigational racemic fluoroquinolone antibacterials containing a primary amino group [42]. However, it has not

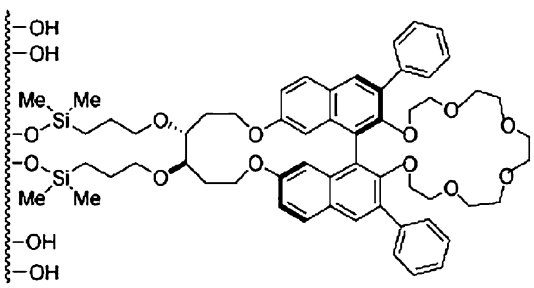

Fig. 3 Structure of a crown ether CSP covalently bonded to silica gel

been utilized in resolving other racemic compounds containing a primary amino group, and they wish extend the use of this CSP to the resolution of racemic amines, amino alcohols and the related compounds including pharmaceutically important compounds such as amphetamine analogues, mexiletine, norepinephrine and norephedrine. The resolution was quite successful and the resolution behaviors were quite dependent on the type of mobile phase additives and column temperature [43]. Hyun wish to extend the use of this CPS to the resolution of tocainide and its analogues. The resolution was quite good, the separation (α) and resolution factors (*Rs*) being 1.84–15.32 and 1.34–13.78, respectively. Especially, the result for the resolution of tocainide on the CSP turns out to be the best one among other reported previously. The chromatographic resolution behaviors were demonstrated to be dependent on the content and the type of organic and acidic modifiers and the ammonium acetate concentration in aqueous mobile phase [44].

As an effort to improve the chiral recognition efficiency of CSP (Fig. 3) even more, Hyun directed his attention to the residual silanol groups of the CSP. The residual silanol groups of CSPs were believed to provide non-enantioselective hydrogen bonding sites and consequently they were usually end capped by treating CSPs with hexamethyldisilazane (HMDS) or trimethylchlorosilane (TMCS) to increase enantioselectivity, sometimes by decreasing retention for the first enantiomer and increasing the retention for the second enantiomer [45]. In addition, he noted that the increasing in the lipophilicity of CSPs based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid improves the separation (α) and the resolution factors (*RS*) quite much even though the reason is not clear [46, 47]. To take the advantage of both the residual silanol group protection and the improved lipophilicity of the stationary phase, Hyun prepared CSP (Fig. 4) by treating CSP (Fig. 3) with *n*-octyltriethoxysilane. The *n*-octyl groups of CSP (Fig. 4) are expected to play a role of protecting the residual silanol groups on the silica gel surface and a role of increasing the lipophilicity of the stationary phase [48].

Machida and co-workers also developed CSPs using the same crown ether (Fig. 1) as a selector, but with a different bonding process from that of Hyun's CSP [19, 39]. The separation of racemic primary amino compounds on CSPs based on a neutral crown ether selector has been usually performed under highly acidic conditions with an aqueous mobile phase containing acid additives such as sulfuric acid, perchloric acid, trifluoroacetic acid, and acetic acid. In this instance, CSPs may not be ensured durable enough for use over a long period of time. On the other hand, an acidic crown ether such as a chiral phenolic pseudo-18-crown-6 is expected to form stable salt-complex with a neutral amine in the absence of acid additives, [49] because the phenolic

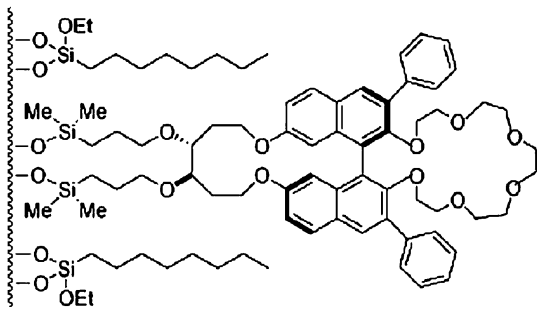

Fig. 4 Structure of a crown ether CSP covalently bonded to silica gel via *n*-octyltriethoxysilane

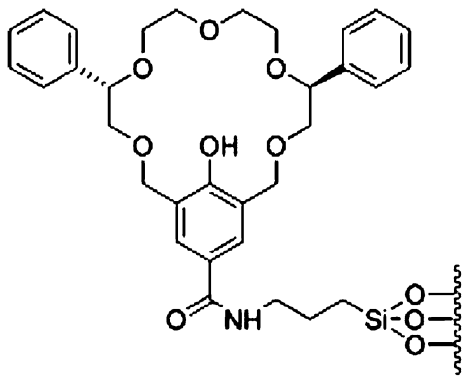

Fig. 5 Structure of a OH type crown ether CSP (CSP-3)

OH group in the binding site serves as an acid derivative to form an ammonium phenolate, called 'saltex,' in which an ammonium ion is stabilized by coordination of crown ether oxygen atoms [50]. A highly enantiomer-selective complexations between the OH type crown ethers and primary amines was reported [26, 51–56]. Consequently, high enantiomer separation on CSP having a chiral phenolic pseudo-18-crown-6 as a selector is expected. Therefore, OH type CSP-**3** (Fig. 5) having a phenolic pseudo-18-crown-6 as a selector was designed. After that CSP-**3** used for enantiomer separation of chiral amino compounds such as amines, amino alcohols and amino acids. Amines and amino alcohols were separated well on CSP-**3** when a non-acidic additive was used in a mobile phase [57].

Recently a chiral monoaza-15-crown-5 ether derivative was prepared from L-leucinol and used as a chiral stationary phase. This chiral stationary phase was employed in separating the enantiomers of the sodium and potassium salts of amino acids. The sodium and potassium salt of the D-enantiomers of all amino acids (PhyAlaNa, PhyAlaK and PhyGlyNa, PhyGlyK, and TrpNa, TrpK) show higher selectivity than the L-enantiomers for this chiral stationary phase [58].

Also a chiral stationary phase (CSP) based on (−)-(18-crown-6)-2,3,11,12-tetracarboxylic acid was evaluated for

the direct resolution of the enantiomers of dipeptides and tripeptides. The type and concentration of the acid and the methanol content were optimized with regard to retention time and resolution using Ala-Phe as model peptide. A mobile phase consisting of 10 mM sulfuric acid in 70 % aqueous methanol was applied to the separation of a set of 16 structurally diverse dipeptides and tripeptides. Generally, the configuration of the amino acid at the N-terminus determined the enantiomer elution order. With a few exceptions the LL- and LD-enantiomers interacted stronger with the CSP compared to the corresponding DD- or LD-enantiomers. The experimental conditions also allowed the simultaneous separation of all four stereoisomers of Ala-Phe. Addition of ammonium sulfate generally reduced retention times and enantiomer resolution. Addition of triethylamine as modifier led to an overall increase of the retention times while the resolution did not show a general trend, increasing in the case of Ala–Ala but decreasing in the case of Ala-Phe [59]. Liquid chromatographic two chiral stationary phases (CSPs) based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid were successfully applied in the resolution of aryl α-amino ketones, including cathinone, the (S) enantiomer of which is a psychoactive alkaloid found in the leaves of the khat plant. The chromatographic resolution behaviors were found to be dependent on the type, and the content, of organic and acidic modifiers in aqueous mobile phase. In addition, one of the two CSPs was demonstrated to be quite useful in the determination of the enantiomeric purity of cathinone [60].

A direct, isocratic high-performance liquid chromatographic method is described for the enantiomeric resolution of a number of phenylalkylamines, namely, racemic cathinone, amphetamine, norephedrine, and norphenylephrine, without sample derivatization. The separations were achieved on an S-18-crown-6-ether chiral stationary phase known as CR(+). The chromatographic parameters alpha (separation factor) and Rs (resolution factor) lay within a narrow range for all compounds used in this study except for cathinone, which resulted in high alpha and Rs values. The recognition mechanism for this column involves the interaction of the crown structure with a charged primary ammonium ion. The stereochemical structures of the compounds in this study contribute to the results obtained for the chromatographic parameters, especially in cathinone's case [61].

In the other work, a method for the enantiomeric separation and direct detection of trans-2-aminocyclohexanol by high performance liquid chromatography (HPLC) with evaporative light scattering detection (ELSD) using a crown ether column has been developed. The influence of mobile phase composition on the separation was investigated in detail. It was found that enantiomeric separation could be achieved when a strong chaotropic counterion, such as TFA, is used as the mobile phase modifier. Organic modifiers, such as methanol, influence the retention times, but have no effect on the separation factor [62].

With UHPLC, it is possible to use long capillary columns or very small particles to provide high efficiencies, even at high linear velocities [63]. Both cyclodextrins (CD) and crown ethers have proven to be effective chiral selectors [64]. While many chiral separations have been accomplished using CD-type stationary phases [65], Park et al. [66] reported that one drawback in utilizing CDs was the low binding constants for most guest molecules. Willner et al. [67] and Park et al. [66] reported that diaza-18-crown-6-capped β-CD exhibited high binding constants for several guest molecules due to cooperative functioning of the β-CD and the crown ether, they can be bonded onto silica to use as CSPs in LC [68, 69]. Crown ether-capped β-CD bonded phases gave better enantioselectivity in CEC for a number of analytes than either β-CD bonded phases or crown ether bonded phases alone under the same separation conditions [70]. After that, two novel types of crown ether-capped β-CD bonded stationary phases, 8-amino-quinoline-2-ylmethyl- and 8-aminoquinoline-7-ylmethyl-diaza-18-crown-6-capped [3-(2-*O*-b-cyclo-dextrin)-2-hydroxypropoxy] propylsilyl silica particles (AQ2D18C6-CD-HPS and AQ7D18C6-CD HPS), were prepared and used as new stationary phases in UHPLC for chiral separations. High column efficiency (up to 400,000 plates m^{-1}) was achieved for chiral separations. The selectivities of these bonded stationary phases were examined by application to positional isomers of *o*,*m*,*p*-nitroaniline and enantiomers of selected chiral compounds[71].

Crown ethers as stationary phase in GC

Crown ethers are useful as chromatographic stationary phases because of the good selectivity resulting from the cavity structure and the strong electronegative effect of heteroatoms on the crown ether ring [72]. Although the synthesis of crown ethers is expensive, the amount of crown ether for making a capillary column is small, only 3 mg of it is sufficient to prepare a typical 20-m capillary column [73]. Crown ethers are used as a stationary phase for capillary gas chromatography. There have been some articles concerning such use of crown ethers as stationary phases in capillary GC [74–76]. The use of small crown ethers is limited because of coating difficultly, poor column efficiency, and column bleeding at high temperature. Polymerization of the crown ethers may alleviate some of these problems. In 1985, Fine [75] bonded vinyl crown ethers onto the inner wall of capillary column, but his results were not satisfactory. Lee [76] synthesized a crown ether substituted polysiloxane with a polymer spacing of three, which showed a unique selectivity for nitrogen-

containing polycyclic aromatic compounds. Then w-undecyleneoxymethyl-18-crown-6 is substituted onto a polysiloxane backbone to yield a stationary phase for capillary GC.

Then a crown ether, *n*-undecyloxymethyC18-crown-6 poly- siloxane (PSO-11-18C6) is prepared and coated on a fused silica capillary column. Chromatographic characteristics, including column efficiency, allowable temperature range, thermal stability, polarity, and selectivity, are studied. The new stationary phase is comparable to Carbowax-2OM in polarity and selectivity and has an operational temperature range of 70–300 °C. Selectivity is superior to Carbowax-20 M for *n*-alcohols and esters, and some aromatic compounds separate well on the crown ether column [77].

The solution of compound, mono-6-(1-benzo-aza-15-crown-5)-2,3,6-permethyl-β-cyclodextrin, in the moderately polar polysiloxane OV-1701 was coated onto fused silica capillary column. The chromatographic characteristics including column efficiency, polarity and selectivity were studied. Excellent selectivity for the separation of enantiomers and positional isomers was obtained. The results show that the combined effect between the special caves of [3-cyclodextrin and crown ether plays a significant role in the separation [78]. Two new kinds of crown ethers: 3,5-dibutyl-unsymmetrydibenzo-14-crown-4-dihydroxy (cis-, and trans-) with the OH-terminal silicone oil in different proportion were coated on glass capillary columns, and immobilized by condensation using a coupling agent of alkyltrimethoxysilane. Chromatographic characteristics, including column efficiency, polarity, selectivity, phase transition temperature and thermal stability were studied. The columns were compared with PEG-20 M in terms of polarity and selectivity. The immobilization and retention mechanisms are also discussed [79].

A new type of capillary gas chromatographic stationary phase containing OH-dibenzo-14-crown-4 (OH-DB14C4) was fabricated by the sol–gel process and coated onto the inner walls of a fused silica capillary. Multiple preparation steps in conventional column technology were avoided. The column demonstrates high column efficiency, outstanding thermal stability (to 330 °C) and a significant ability of deactivation. Compared with a sol–gel OH-terminated silicone oil (OH-TSO) column, the sol–gel OH-DB14C4 column has unique selectivity for the separation of positional isomers of aromatic compounds. In comparison with the dibenzo-propyl-15-crown-5 polysiloxane (PSO-DB-3-15C5) stationary phase, the sol–gel OH-DB14C4 has a high column capacity in separating small-molecular-mass compounds, such as low-molecular-mass alcohols, short-chain fatty acids, and volatile amines. The possible mechanism involved in sol–gel coating with OH-DB14C4 is also discussed [80].

Cholesteric liquid crystal crown ether CH-B-15-C-5 has different phase states under a range of different temperatures, and a large variety of physical properties under different operating conditions in the same temperature range. When the temperature is decreased from 182 to 165 °C, the compound forms cholesteric liquid crystal. If it is decreased to 125 °C, it remains anisotropic liquid crystal; then if the temperature decreases below 125 °C it forms anisotropic solid crystal. But, in the course of heating from below 125–182 °C it cannot form cholesteric liquid crystal. Therefore, the stationary phases of CH-B-15-C-5 have three gas chromatographic functions: gas–solid chromatography (GSC), general gas–liquid chromatography (GLC) and crown ether liquid crystal gas–liquid chromatography (CL-GLC). Xiaotian separated organic compounds, such as, *n*-alkanes, alcohols, ethyl asters of C1–C8 carboxylic acid, halogen derivatives, etc., and position-isomers of the replacement aromatic hydrocarbons, such as the *o*,*m*, *p*-xylenes, -dichlorobenzenes, -nitrochlorobenzenes, and 1,3,5- and 1,2,4-trimethylbenzene, etc. Good separation results were achieved using crown ether liquid crystal stationary phase under the different chromatographic conditions. The chiral resolutions of optical isomers of ethyl lactate and isoamyl alcohol in gas chromatography (GC) with a new cholesteric liquid crystal crown ether (CH-B-15-C-5) stationary phase was also discussed [81].

Side-chain liquid crystalline polysiloxanes have been developed and used as capillary gas chromatographic stationary phases since 1982 by Finkelmann [82]. These kinds of stationary phase possess unique separation properties, especially for geometrical isomer separation. On the other hand, side-chain crown ether polysiloxanes have been used as stationary phases for capillary gas chromatography since 1988. These stationary phases exhibit some excellent selectivity. Both side-chain liquid crystalline siloxane and side-chain crown ether polysiloxane were synthesized and used as capillary gas chromatographic stationary phases. It was demonstrated that these stationary phases have unique selectivity and thermostability and are suitable for the separation of a variety of isomeric compounds. In 1989 Perceca [83] synthesized some side-chain liquid crystalline polysiloxanes containing crown ether. Then a novel side-chain liquid crystalline polysiloxane containing crown ether was developed, which was used as a stationary phase for capillary gas chromatography. This phase can be easily coated on the fused-silica tubing and possesses a high efficiency and moderate polarity. It exhibits the retention properties of both liquid crystal and crown ether stationary phases and is suitable for the separation of a variety of isomeric compounds [84].

Ion chromatography (IC)

Extensive studies about use of crown ethers in liquid chromatography of cationic species, especially, alkali and

alkaline-earth metal ions, have been made so far. In most cases polymeric crown ether resins [85–87] and silica gels on which crown ether moieties are immobilized through covalent bonding [88–90] or which are coated with polymeric crown ether [91, 92] have been applied to the stationary phases for the ion chromatography. The crown ether stationary phases are quite different in chromatographic behavior from conventional "ionic" cation exchangers. For instance, the elution order of alkali metal ions in the "ionic" cation exchangers follows the ionic size ($Li^+ < Na^+ < K^+ < Rb^+ < Cs^+$ in the retention time), not being varied from one cation exchanger to another. In contrast, the retention behavior of the crown ether stationary phases depends on the type of the immobilized crown ether moiety. This variable elution order on the crown ether stationary phases is attractive for chromatographic analyses of the cationic species. Hence, various cation-specific stationary phases may be designed for liquid chromatography of ionic species by selecting the immobilized crown ether moiety. The crown ether immobilized stationary phases, however, might not be easily available for most analytical chemists due to some difficulty with the syntheses.

During synthesis and purification of crown ether derivatives incorporating long aliphatic chain it has been found that the highly lipophilic crown ethers are retained strongly on octa-decylsilanized silica (ODS) by powerful hydrophobic interaction. Some of them could not be eluted out from ODS columns even by using pure methanol as the mobile phase. This incident induced the test ODS coated with highly lipophilic crown ethers for usefulness as stationary phases for liquid chromatography of ionic species. For convenience it has been attempted in situ coating of lipophilic crown ethers on ODS by passing coating solutions through commercially available, high-performance ODS-packed columns. Kimura communicated the preliminary results concerning the use of crown ether coated ODS for chromatography [93]. After that he wish to report in detail the liquid chromatography of alkali and alkaline-earth metal ions on ODS columns modified with lipophilic crown ethers and their analogues [94].

IC is one of the most widely used techniques for the determination of alkali and alkaline-earth cations, ammonium, and amines. However, samples that contain very different concentration ratios of cations are difficult to quantify by IC, especially if they elute next to each other. Many environmental and industrial samples contain either very low levels of ammonium in the presence of high concentration of sodium or very low levels of sodium in the presence of high concentrations of ammonium. It is difficult to separate these cations on a standard sulfonate or carboxylate-based cation exchangers because they have similar selectivities toward sodium and ammonium. In one approach crown ethers were added to the mobile phase [95]. This approach resolves sodium and ammonium; however, since crown ethers are toxic, this present hazard to the analysts and the toxic waste is expensive and difficult to dispose. Other approaches incorporated the crown ether onto the stationary phases, producing a trifunctional [96] or bifunctional [97] cation exchanger. A nice separation of sodium and ammonium and some amines can be achieved with these columns. After that an alternative approach to enhancing the cation selectivity was developed. Two separate packings, one carrying a conventional cation-exchange phase and the other carrying a crown ether phase are used. This new approach has advantages in controlling cation selectivity compared to previous methods. Independent carboxylate cation-exchange and 18-crown-6 ether packings enhance cation selectivity in ion chromatography. Columns packed with each material may be used in series, or the packings may be combined in one column. Both systems improve the resolution between sodium and ammonium and allow the separation of amines from alkali and alkaline-earth cations. This system eliminates the need for toxic crown ether mobile phase additives. The ability to independently control the cation-exchange and complexation contributions to resolution and retention is more flexible than fixed-ratio bifunctional media and column. In the two-column configuration, system peaks are present for cations that are retained on the CE column. The system peaks generally do not interfere with the analysis, and are eliminated when a suppressor is used [98].

Capillary electrochromatography (CEC)

CEC, which combines the desirable features of both HPLC and CE, has become increasingly popular. CEC is a powerful separation method which affords higher theoretical plate number and superior efficiency due to its plug like flow profile, compared with micro HPLC which uses the same packing material. In CEC, as in HPLC, the solutes of a given sample mixture can be preferentially separated based on differences in distribution ratios between the mobile phase and the stationary phase. Compounds possessing a charge can also be affected by the applied voltage, leading to differential migration caused by electrophoresis, as in CE. Therefore both charged and uncharged species can be separated according to their differential migration through the column based on the solute's interaction between the two phases or a combination of such interactions and the inherent electrophoretic mobilities of the solutes [99]. Capillaries packed with a typical stationary phase for HPLC have been used in various CEC studies [99], while monolithic stationary phases, which are ungranular polymeric separation media, are increasingly attracting attention for

CEC because of their simple preparation method, wide variety of functionalization and good stability [100–109]. Thus far, enantiomeric separation by CEC has been performed with various columns such as capillaries packed with CSPs for HPLC [110–113], capillaries with the inner surface coated with CSPs [114–116], capillaries filled with molecularly imprinted polymer as monolithic CSPs for CEC [117] and capillaries filled with monolithic CSPs for CEC (not molecularly imprinted polymer) [118–120]. It was also reported enantiomeric separation methods of CEC with monolithic CSPs immobilizing β-CDs as chiral selectors [121–124] with reference to the concept of enantiomeric separation by capillary gel electrophoresis [125].

Use of macrocyclic polyethers having oxygen atoms as donor atoms (crown ethers) is also effective. One of the fundamental characteristics of crown ethers is selective complexation ability. They bind the cationic portion of alkali and alkaline earth salts and that of ammonium salts into their cavity. The formation of host–guest complexes is based on ion–dipole interactions between the cation and the donor oxygen atoms in the cyclic polyether [126].

(+)-18-Crown-6 tetracarboxylic acid (18C6H4) shown in Fig. 6, which is one of chiral crown ethers, was synthesized for the first time by Behr et al. [127] and used as buffer additive in CE by Kuhn and coworkers [128] for the enantiomeric separations of racemic amino acids. Recently, 18C6H4 seems to be the first choice for enantiomeric separations of primary amino compounds in CE in spite of its high cost [129, 130]. Koide report the successful enantiomeric separations of primary amino compounds by CEC with monolithic chiral stationary phases of 18C6H4 derivative-bonded negatively charged poly acrylamide gels. High efficiencies of more than 100,000 plates m^{-1} were obtained for some compounds. Good within- and between-run reproducibilities of retention time and separation factor were obtained [131].

Koide developed another enantiomeric separation method by capillary electrochromatography with chiral crown ether-bonded negatively charged polyacrylamide gels. Two kinds of chiral crown ether derivatives, (+)-tetraallyl 18-crown-6 carboxylate and (+)-18-crown-6 tetracarboxylic acid 2-allyl ester were synthesized and allowed to covalently bind to a negatively charged polyacrylamide gel, a so-called monolithic stationary phase, respectively. The gel was placed in fused-silica tubing, the walls of which had been activated with a bifunctional reagent to make the resulting gel bind covalently to the inner surface. Enantiomeric separations of 12 primary amino compounds were achieved using these columns and mobile phases of 200 mM triethanolamine-300 mM boric acid buffers with high efficiencies of up to 135,000 plates m^{-1}. Both the within- and between-run reproducibilities of retention time and separation factor were good. The reproducibilities of retention time and separation factor for three different columns prepared from a different batch of monomers were acceptable. The gel-filled capillaries were stable for at least 13 months with intermittent use for 3 months followed by storage at room temperature for 10 months. The result of the optical purity test of alanine-2-naphthylamide is also described [131]. After that two novel types of crown ether capped β-cyclodextrin (β-CD) bonded silica, namely, 4′-aminobenzo-*X*-crown-*Y* ($X = 15$, 18 and $Y = 5$, 6, resp.) capped [3-(2-*O*-β-cyclodextrin)-2-hydroxypropoxy] propylsilyl-appended silica, have been prepared and used as stationary phases in CEC to separate chiral compounds. The two stationary phases have a chiral selector with two recognition sites: crown ether and β-CD. They exhibit excellent enantio selectivity in CEC for a wide range of compounds. After inclusion of metal ions (Na^+ or K^+) from the running buffer into the crown ether units, the stationary phases become positively charged and can provide extra electrostatic interaction with ionizable solutes and enhance the dipolar interaction with polar neutral solutes. This enhances the host–guest interaction with the solute and improves chiral recognition and enantioselectivity. Due to the cooperation of the anchored β-CD and the crown ether, this kind of crown ether capped β-CD bonded phase shows better enantioselectivity than either β-CD- or crown ether bonded phases only. These new types of stationary phases have good potential for fast chiral separation with CEC [70].

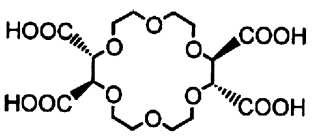

Fig. 6 (+)-18-Crown-6 tetracarboxylic acid

Conclusion

Crown ether-based CSPs have been known to be quite useful for the liquid chromatographic resolution of racemic compounds. Crown ethers also used in gas chromatography and Ion chromatography as the stationary phase. The development of new chiral stationary phase used for CEC is another important issue in this field. We expect that use of these compounds in this field will be more developed in the future.

References

1. Pedersen, C.J.: Cyclic polyethers and their complexes with metal salts. J. Am. Chem. Soc. **89**, 7017–7036 (1967)
2. Rounaghi, G.H., Mohammad Zade Kakhki, R.: Thermodynamic study of complex formation between dibenzo-18-crown-6 and

UO_2^{2+} cation in different non-aqueous binary solutions. J. Incl. Phenom. Macrocycl. Chem. **63**, 117–122 (2009)
3. Rounaghi, G.H., Zavar, M.H., Mohammad Zade Kakhki, R.: Thermodynamic behaviour of complexation process between DB18C6 with K+, Ag+, NH4+ and Hg2+ cations in ethylacetate-dimethylformamide binary media. Russ J Coord Chem **34**, 167–171 (2008)
4. Rounaghi, G.H., Mohajeri, M., Soruri, F., Mohamadzadeh Kakhki, R.: Solvent influence upon complex formation between dibenzo-18-crown-6 with the Y3+ metal cation in pure and binary mixed organic solvents. J. Chem. Eng. Data **56**, 2836–2840 (2011)
5. Razghandi, F., Rounaghi, Gh., Mohammadzadeh Kakhki, R.: Complexation study of dibenzo-18-crown-6 with UO_2^{2+} cation in binary mixed non-aqueous solutions. J. Incl. Phenom. Macrocycl. Chem. (2011). doi:10.1007/s10847-011-0053-0
6. Rounaghi, G.H., Mohajeri, M., Atashi, Z., Mohamadzadeh kakhki, R.: Conductometric study of complexation reaction between 15-crown-5 and Cr^{3+}, Mn^{2+} and Zn^{2+} metal cations in pure and binary mixed organic solvents. J. Incl. Phenom. Macrocycl. Chem. (2011). doi:10.1007/s10847-011-0081-9
7. Mohammad Zadeh Kakhki, R., Rounaghi, G.H.: Competitive bulk liquid membrane transport of heavy metal cations using the 18-crown-6 ligand as an ionophore. J. Chem. Eng. Data **56**, 3169–3174 (2011)
8. Mohapatra, P.K., Lakshmi, D.S., Bhattacharyya, A., Manchanda, V.K.: Evaluation of polymer inclusion membranes containing crown ethers for selective cesium separation from nuclear waste solution. J. Hazard. Mater. **169**, 472–479 (2009)
9. Shamsipur, M., Davarkhah, R., Khanchi, A.R.: Facilitated transport of uranium(VI) across a bulk liquid membrane containing thenoyltrifluoroacetone in the presence of crown ethers as synergistic agents. Sep. Purif. Technol. **71**, 63–69 (2010)
10. Pirkle, W.H., Pochapsky, T.C.: Considerations of chiral recognition relevant to the liquid chromatographic separation of enantiomers. Chem. Rev. **89**, 347–362 (1989)
11. Berthod, A., Chang, S.C., Armstrong, D.W.: Empirical procedure that uses molecular structure to predict enantioselectivity of chiral stationary phases. Anal. Chem. **64**, 395–404 (1992)
12. Allenmark, S.G.: Chromatographic Enantioseparations: Methods and Applications. Ellis Horwood, New York (1993)
13. Subramanian, G.: A Practical Approach to Chiral Separations by Liquid Chromatography. VHC, Weinheim (1994)
14. Zief, M., Crane, L.J.: Chromatographic Chiral Separations. Marcel Dekker, New York (1998)
15. Sogah, G.D.Y., Cram, D.J.: Chromatographic optical resolution through chiral complexation of amino ester salts by a host covalently bound to silica gel. J. Am. Chem. Soc. **97**, 1259–1261 (1975)
16. Shinbo, T., Yamaguchi, T., Nishimura, K., Sugiura, M.: Chromatographic separation of racemic amino acids by use of chiral crown ether-coated reversed-phase packings. J. Chromatogr. A **405**, 145–153 (1987)
17. Shinbo, T., Yamaguchi, T., Yanagishita, H., Kitamoto, D., Sakaki, K., Sugiura, M.: Improved crown ether-based chiral stationary phase. J. Chromatogr. A **625**, 101–108 (1992)
18. Lee, W., Hong, C.Y.: Direct liquid chromatographic enantiomer separation of new fluoroquinolones including gemifloxacin. J. Chromatogr. A **879**, 113–120 (2000)
19. Machida, Y., Nishi, H., Nakamura, K., Nakai, H., Sato, T.: Enantiomer separation of amino compounds by a novel chiral stationary phase derived from crown ether. J. Chromatogr. A **805**, 85–92 (1998)
20. Hyun, M.H., Jin, J.S., Lee, W.: A new hplc chiral stationary phase for the direct resolution of racemic quinolone antibacterials containing a primary amino group. Bull. Korean Chem. Soc. **19**, 819–821 (1998)
21. Hyun, M.H., Jin, J.S., Lee, W.: Liquid chromatographic resolution of racemic amino acids and their derivatives on a new chiral stationary phase based on crown ether. J. Chromatogr. A **822**, 155–161 (1998)
22. Hyun, M.H., Jin, J.S., Koo, H.J., Lee, W.: Liquid chromatographic resolution of racemic amines and amino alcohols on a chiral stationary phase derived from crown ether. J. Chromatogr. A **837**, 75–82 (1999)
23. Hyun, M.H., Han, S.C., Lipshutz, B.H., Shin, Y.J., Welch, C.: Liquid chromatographic resolution of racemic amines, amino alcohols and related compounds on a chiral crown ether stationary phase. J. Chromatogr. A **959**, 75–83 (2002)
24. Kontos, Z., Huszthy, P., Bradshaw, J.S., Izatt, R.M.: Enantioseparation of racemic organic ammonium perchlorates by a silica gel bound optically active di-tert-butylpyridino-18-crown-6 ligand. Tetrahedr. Asymmetry **10**, 2087–2099 (1999)
25. Hirose, K., Nakamura, T., Nishioka, R., Ueshige, T., Tobe, Y.: Preparation and evaluation of novel chiral stationary phases covalently bound with chiral pseudo-18-crown-6 ethers. Tetrahedron Lett. **44**, 1549–1551 (2003)
26. Hirose, K., Yongzhu, J., Nakamura, T., Nishioka, R., Ueshige, T., Tobe, Y.: Chiral stationary phase covalently bound with a chiral pseudo-18-crown-6 ether for enantiomer separation of amino compounds using a normal mobile phase. Chirality **17**, 142–148 (2005)
27. Hirose, K., Yongzhu, J., Nakamura, T., Nishioka, R., Ueshige, T., Tobe, Y.: Preparation and evaluation of a chiral stationary phase covalently bound with chiral pseudo-1 8-crown-6 ether having 1-phenyl-1,2-cyclohexanediol as a chiral unit. J. Chromatogr. A **1078**, 35–41 (2005)
28. Hyun, M.H., Min, H.J., Cho, Y.: Resolution of tocainide and its analogues on liquid chromatographic chiral stationary phases based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. J. Bull. Korean Chem. Soc. **24**, 911–915 (2003)
29. Hyun, M.H., Han, S.C., Jin, J.S., Lee, W.: Separation of the stereoisomers of racemic fluoroquinolone antibacterial agents on a crown-ether-based chiral HPLC stationary phase. Chromatographia **52**, 473–476 (2000)
30. Hyun, M.H., Han, S.C., Cho, Y.J., Jin, J.S., Lee, W.: Liquid chromatographic resolution of gemifloxacin mesylate on a chiral stationary phase derived from crown ether. Biomed. Chromatogr. **16**, 356–360 (2002)
31. Hyun, M.H., Kim, Y.H., Cho, Y.J.: Bull. Korean Chem. Soc. **25**, 400–402 (2004)
32. Hyun, M.H., Cho, Y.J.: Preparation and application of a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid without extra free aminopropyl groups on silica surface. J. Sep. Sci. **28**, 31–38 (2005)
33. Hyun, M.H., Jin, J.S., Han, SCh., Cho, Y.J.: The effect of analyte lipophilicity on the resolution of α-amino acids on a HPLC chiral stationary phase based on crown ether. Microchem. J. **70**, 205–209 (2001)
34. Jin, J.S., Stalcup, A.M., Hyun, M.H.: Impact of triethylamine as a mobile phase additive on the resolution of racemic amino acids on an (1)-18-crown-6-tetracarboxylic acid-derived chiral stationary phase. J. Chromatogr. A **933**, 83–90 (2001)
35. Hyun, M.H., Tan, G., Ying Xue, J.: Unusual resolution of *N*-(3,5-dinitrobenzoyl)-α-amino acids on a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. J. Chromatogr. A **1097**, 188–191 (2005)
36. Berkecz, R., Sztojkov-Ivanov, A., Ilisz, I., Forro, E., Fulop, F., Hyun, M.H., Peter, A.: High-performance liquid chromatographic enantioseparation of *β*-amino acid stereoisomers on a (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid-based chiral stationary phase. J. Chromatogr. **A1125**, 138–143 (2006)

37. Juaristi, E., Soloshonok, V.A.: Enantioselective Synthesis of α-Amino Acids, 2nd edn. Wiley, New York (2005)
38. Hyun, M.H., Choi, H.J., Kang, B.S., Tan, G., Cho, Y.J.: Resolution of β-amino acids on a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid without extra free aminopropyl groups on silica surface: The effect of ammonium ion mobile phase modifier on the resolution behaviors. Bull. Korean Chem. Soc. **27**, 1775–1779 (2006)
39. Machida, Y., Nishi, H., Nakamura, K.: Separation of the enantiomers of amino and amide compounds on novel chiral stationary phases derived from a crown ether. Chromatographia **49**, 621–627 (1999)
40. Steffeck, R.J., Zelechonok, Y., Gahm, K.H.: Enantioselective separation of racemic secondary amines on a chiral crown ether-based liquid chromatography stationary phase. J. Chromatogr. A **947**, 301–305 (2002)
41. Hyun, M.H., Han, S.C., Lipshutz, B.H., Shin, Y.-J., Welch, C.J.: New chiral crown ether stationary phase for the liquid chromatographic resolution of α-amino acid enantiomers. J. Chromatogr. A **910**, 359–365 (2001)
42. Myung, H.H., Han, SCh.: Liquid chromatographic separation of the enantiomers of fluoroquinolone antibacterials on a chiral stationary phase based on a chiral crown ether. J. Biochem. Biophys. Methods **54**, 235–243 (2002)
43. Hyun, M.H., Han, SCh., Bruce, L., Shin, H., Christopher, Y.-J., Welch, J.: Liquid chromatographic resolution of racemic amines, amino alcohols and related compounds on a chiral crown ether stationary phase. J. Chromatogr. A **959**, 75–83 (2002)
44. Hyun, M.H., Min, H.J., Cho, Y.J.: Enantiomeric separation of tocainide and its analogues on an optically active crown ether-based stationary phase by liquid chromatography. J. Chromatogr. A **996**, 233–237 (2003)
45. Pirkle, W.H., Readnour, R.S.: The influence of end-capping on the enantioselectivity of a chiral phase. Chromatographia **31**, 129–132 (1991)
46. Hyun, M.H., Kim, D.H.: Spacer length effect of a chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid. Chirality **16**, 294–301 (2004)
47. Hyun, M.H., Song, Y., Cho, Y.J., Kim, D.H.: Preparation of a new doubly tethered chiral stationary phase based on (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid and its application. J. Chromatogr. A **1108**, 208–217 (2006)
48. Hyun, M.H., Han, SCh., Choi, H.J., Kang, B.S., Hyun, J.H.: Effect of the residual silanol group protection on the liquid chromatographic resolution of racemic primary amino compounds on a chiral stationary phase based on optically active (3,3′-diphenyl-1,1′-binaphthyl)-20-crown-6. J. Chromatogr. A **1138**, 169–174 (2007)
49. Naemura, K., Takeuchi, S., Asada, M., Ueno, K., Hirose, K., Tobe, Y., Kaneda, T., Sakata, Y.: Synthesis of azophenolic crown ethers of C_s symmetry incorporating *cis*-1-phenylcyclohexane-1,2-diol residues as a steric barrier and diastereotopic face selectivity in complexation of amines by their diastereotopic faces. J. Chem. Soc. Perkin Trans. **1**, 1429–1435 (1995)
50. Kaneda, T., Hirose, K., Misumi, S.: Chiral azophenolic acerands: color indicators to judge the absolute configuration of chiral amines. J. Am. Chem. Soc. **111**, 742–743 (1989)
51. Naemura, K., Nishikawa, Y., Fuji, J., Hirose, K., Tobe, Y.: Preparation of homochiral phenolic crown ethers containing para-substituted phenol moiety and chiral subunits derived from (S)-1-phenylethane-1,2-diol: their chiral recognition behaviour in complexation with neutral amines. Tetrahedron Asymmetry **8**, 873–882 (1997)
52. Naemura, K., Nishioka, K., Ogasahara, K., Nishikawa, Y., Hirose, K., Tobe, Y.: Preparation and temperature-dependent enantioselectivities of homochiral phenolic crown ethers having aryl chiral barriers: thermodynamic parameters for enantioselective complexation with chiral amines. Tetrahedron Asymmetry **9**, 563–574 (1998)
53. Wang, H., Tian, X., Yang, D., Pan, Y., Wu, Q., He, Ch.: Synthesis and enantiomeric recognition ability of 22-crown-6 ethers derived from rosin acid and BINOL. Tetrahedron Asymmetry **22**, 381–386 (2011)
54. Hirose, E.K., Yachi, Y., Tobe, Y.: Novel chiral recognition beyond the limitation due to the law of mass action: highly enantioselective chiral sensing based on non-linear response in phase transition. Chem. Commun. **47**, 6617–6619 (2011)
55. Hirose, K., Fujiwara, A., Matsunaga, K., Aoki, N., Tobe, Y.: Chiral recognition of secondary amines by using chiral crown ether and podand. Tetrahedron Lett. **43**, 8539–8542 (2002)
56. Hirose, K., Fujiwara, A., Matsunaga, K., Aoki, N., Tobe, Y.: Preparation of phenolic chiral crown ethers and podands and their enantiomer recognition ability toward secondary amines. Tetrahedron Asymmetry **14**, 555–566 (2003)
57. Yongzhu, J., Hirose, K., Nakamuraa, T., Nishiok, R., Ueshige, T., Tobe, Y.: Preparation and evaluation of a chiral stationary phase covalently bound with a chiral pseudo-18-crown-6 ether having a phenolic hydroxy group for enantiomer separation of amino compounds. J. Chromatogr. A **1129**, 201–207 (2006)
58. Seyhan, S., Turgut, Y., Merdivan, M., Hos_go ren, H.: Chiral separation of amino acids using a chiral crown ether by impregnation on a polymeric support and monoamine modified silica gel. Tetrahedron Asymmetry **17**, 1700–1704 (2006)
59. Kartozia, I., D'Orazio, G., Chankvetadze, B., Fanali, S.: Evaluation of cyclodextrins modified with dichloro-, dimethyl-, and chloromethylphenylcarbamate groups as chiral stationary phases for capillary electrochromatography. J. Capill. Electrophor. **9**, 31–38 (2005)
60. Hyun, M.H., Tan, G., Cho, Y.J.: J. Liq. Chromatogr. Relat. Technol. **27**, 1671–1680 (2004)
61. Aboul-Enein, H.Y., Serignese, V.: Direct chiral resolution of phenylalkylamines using a crown ether chiral stationary phase. Biomed. Chromatogr. **11**, 7–10 (1997)
62. Chen, S., Yuan, H., Grinberg, N., Dovletoglou, A., Bicker, G.: J. Liq. Chromatogr. Technol **26**, 425–442 (2003)
63. Barder, T.J., Wohlman, P.J., Thrall, C., DuBois, P.D.: Fast chromatography and nonporous silica. LC GC **15**, 918–926 (1997)
64. Verleysen, K., Sandra, P.: Enantiomeric separation of some amino acids and derivatives by capillary electrophoresis with 18-crown-6-tetracarboxylic acid as chiral selector. J. Microcol. Sep. **11**, 37–43 (1999)
65. Schurig, V., Wistuba, D.: Recent innovations in enantiomer separation by electrochromatography utilizing modified cyclodextrins as stationary phases. Electrophoresis **20**, 2313–2328 (1999)
66. Park, J.W., Lee, S.Y., Park, K.K.: Molecular recognition of organic ammonium ions by diaza-crown ether-modified β-cyclodextrin in aqueous media. Chem. Lett. **6**, 594 (2000)
67. Willner, I., Goren, Z.: Diaza-crown ether capped cyclodextrin. A receptor with two recognition sites. J. Chem. Soc. Chem. Commun. **23**, 1469–1470 (1983)
68. Gong, Y., Xue, G., Bradshaw, J.S., Lee, M.L., Lee, H.K.: Synthesis of crown ether-capped 3-(2-O-β-cyclodextrin)-2-hydroxypropylsilyl silica particles for use as chiral stationary phases in chromatography. J. Heterocycl. Chem. **38**, 1317–1321 (2001)
69. Gong, Y., Xue, G., Xiang, Y., Bradshaw, J.S., Lee, M.L., Lee, H.K.: Synthesis of cyclam-capped -cyclodextrin-bonded silica particles for use as chiral stationary phases in capillary electrochromatography. Tetrahedron Lett. **43**, 2463–2466 (2002)

70. Gong, Y., Lee, H.K.: Enantiomeric separation in capillary electrochromatography by using crown ether-capped beta-cyclodextrin bonded silica particles as chiral stationary phase. Helv. Chim. Acta **85**, 3283–3293 (2002)
71. Gong, Y., Xiang, Y., Yue, B., Xue, G., Bradshaw, J.S., Lee, H.K., Lee, M.L.: Application of diaza-18-crown-6-capped β-cyclodextrin bonded silica particles as chiral stationary phases for ultrahigh pressure capillary liquid chromatography. J. Chromatogr. A **1002**, 63–70 (2003)
72. Zeng, Z., Qiu, W., Xing, H., Huang, Z.: Sol-gel-derived crown ether stationary phase for capillary gas chromatography. Anal Sci Capill Gas Chromatogr **16**, 851–856 (2000)
73. Blomberg, L.G.: Stationary phases for capillary gas chromatography. TrAC Trends Anal. Chem. **6**, 41–45 (1987)
74. Yong-Hao, J., Ruo-Nong, F., Zai-Fu, H.: Use of crown ethers in gas chromatography. J. Chromatogr. **469**, 153–159 (1989)
75. Fine, D.D., Gearhart, H.L., Mottola, H.A.: Preparation and gas chromatographic characterization of some crown-ether stationary phases. Talanta **32**, 751–756 (1985)
76. Rouse, C.A., Fintinson, A.C., Tarbet, B.J., Pixton, C., Djordjevie, N.M., Markides, K.E., Bradshaw, J.S., Lee, M.L.: Anal. Chem. **60**, 901 (1988)
77. Wu, C.-Y., Wang, Ch-M, Zeng, Zh-R, Lu, X.-R.: Anal. Chem. **62**, 968–971 (1990)
78. Zeng, Z.R., Liu, M.: Chromatographia **48**, 817–822 (1998)
79. Zeng, Z.-R., Wu, C.-Y., Yan, H., Huang, Z.-F., Wang, Y.-T.: Preparation and characteristics of two new GC stationary phases-dihydroxy crown ether containing polysiloxane. Chromatographia **34**, 85–90 (1992)
80. Zeng, Zh, Qiu, W., Xing, H., Huang, Z.: Sol-gel-derived crown ether stationary phase for capillary gas chromatography. J. Anal. Sci. **16**, 851–854 (2000)
81. Qing, X., Zhou, Z., Xie, M., Zeng, Y., Tang, Y.: Gas chromatographic characteristics and application studies of the stationary phase of CH-B-15-C-5. Talanta **46**, 45–51 (1998)
82. Finkelmann, H., et al.: Polynuclear Aromatic Hydrocarbons: Physical and Biological Chemistry, p. 275. Battelle Press, Columbus (1982)
83. Percec, V., Rodenhouse, R.: Liquid crystal polymers containing macroheterocyclic ligands. 2. Side chain liquid crystal polysiloxanes and polymethacrylates containing 4-(.omega.-alkan-1-yloxy)-4′-(4′-carboxybenzo-15-crown-5)biphenyl side groups. Macromolecules **22**, 4408–4412 (1989)
84. Fu, R., Jing, P., Gu, J.: Anal. Chem. **65**, 2141–2144 (1993)
85. Blasius, E., Janzen, K.-P., Adrian, W., Klautke, G., Lorschelder, R., Maurer, PA., Nguyen, V. B., Nguyen-Tlen, T., Schoiten, G., Stockem-er, J.: Fresenius' J. Anal. Chem. 284, 337–360
86. Blasius, E., Janzen, K.-P., Klein, W., Klotz, H., Nguyen, V.B., Nguyen-Tien, T., Pfeiffer, R., Scholten, G., Simon, H., Stockemer, H., Tous-saint, A.: Preparation, characterization and application of ion exchangers with cyclic polyether anchor groups. J. Chromatogr. **201**, 147–166 (1980)
87. Kimura, K., Harino, H., Hayata, E., Shono, T.: Liquid chromatography of alkali and alkaline-earth metal ions using octadecylsilanized silica columns modified in situ with lipophilic crown ethers. Anal. Chem. **58**, 22–2233 (1986)
88. Nakajlma, M., Kimura, K., Shono, T.: Liquid chromatography of alkali and alkaline earth metal salts on poly(benzo-15-crown-5)- and bis(benzo-15-crown-5)-modified silicas. Anal. Chem. **55**, 463–467 (1983)
89. Nakajima, M., Kimura, K., Shono, T.: Ion-chromatographic behavior of silica gels modified by poly- and bis(crown ether)s of benzo-18-crown-6. Bull. Chem. Soc. Japan **56**, 3052–3056 (1983)
90. NakaJima, M., Kimura, K., Hayata, E., Shono, T.: Ion chromatography on poly(crown ether)-modified silica possessing high affinity for sodium. J. Liq. Chromatogr. **7**, 2115–2125 (1984)
91. Igawa, M., Saito, K., Tsukamoto, J., Tanaka, M.: Ion chromatographic separation of anions on silica-coated polyamide crown resin. Anal. Chem. **53**, 1942–1944 (1981)
92. Igawa, M., Salto, K., Tanaka, M., Yamabe, T.: Separation mechanism of anion chromatography on silica gel coated with polyamide crown resin and its application. BunsekiKagaku **32**, E137–E141 (1983)
93. Kimura, K., Hayata, E., Shono, T.: Convenient, efficient crown ether-containing stationary phases for chromatographic separation of al-kali metal ions: dynamic coating of highly lipophilic crown ethers on octadecylsilanized silica. J. Chem. Soc. Chem. Commun. 271–272 (1984)
94. Kimura, K., Harino, H., Hayata, E., Shono, T.: Liquid chromatography of alkali and alkaline-earth metal ions using octadecylsilanized silica columns modified in situ with lipophilic crown ethers. Anal. Chem. **58**, 2233–2237 (1986)
95. Laubli, M.W., Kampus, B.: Selectivity enhancement on a poly(butadiene-maleic acid)-coated cation phase induced by ethylene oxide-based complexing agents. J. Chromatogr. A **706**, 103–107 (1995)
96. Rey, M.A., Pohl, C.A., Jagodzinski, J.J., Kaiser, E.Q., Riviello, J.M.: A new approach to dealing with high-to-low concentration ratios of sodium and ammonium ions in ion chromatography. J. Chromatogr. A **804**, 201–209 (1998)
97. Saari-Nordhaus, R. Pham, H. Anderson Jr. J.M. Presented at the Pittsburg Conference 1999, paper, p 2292
98. Saari-Nordhaus, R., Anderson Jr, J.M.: Alternative approach to enhancing cation selectivity in ion chromatography. J. Chromatogr. A **1039**, 123–127 (2004)
99. Colon, L.A., Guo, Y., Fermier, A.: Capillary electrochromatography. Anal. Chem. **69**, 461A–467A (1997)
100. Svec, F., Peters, E.C., Sykora, D., Yu, C., Frechet, J.M.J.: Monolithic stationary phases for capillary electrochromatography based on synthetic polymers: designs and applications. J. High Resolut. Chromatogr. **23**, 3–18 (2000)
101. Fujimoto, C.: Charged polyacrylamide gels for capillary electrochromatographic separations of uncharged, low molecular weight compounds. Anal. Chem. **67**, 2050–2053 (1995)
102. Fujimoto, C., Fujise, Y., Matsuzawa, E.: Fritless packed columns for capillary electrochromatography: separation of uncharged compounds on hydrophobic hydrogels. Anal. Chem. **68**, 2753–2757 (1996)
103. Palm, A., Novotny, M.V.: Macroporous polyacrylamide/poly(ethylene glycol) matrixes as stationary phases in capillary electrochromatography. Anal. Chem. **69**, 4499–4507 (1997)
104. Gusev, I., Huang, X., Horvath, Cs: Capillary columns with in situ formed porous monolithic packing for micro high-performance liquid chromatography and capillary electrochromatography. J. Chromatogr. A **855**, 273–290 (1999)
105. Peters, E.C., Petro, M., Svec, F., Frechet, J.M.J.: Molded rigid polymer monoliths as separation media for capillary electrochromatography. 1. Fine control of porous properties and surface chemistry. Anal. Chem. **70**, 2288–2295 (1998)
106. Peters, E.C., Petro, M., Svec, F., Frechet, J.M.J.: Molded rigid polymer monoliths as separation media for capillary electrochromatography. 2. Effect of chromatographic conditions on the separation. Anal. Chem. **70**, 2296–2302 (1998)
107. Wulff, G.: Molecular imprinting in cross-linked materials with the aid of molecular templates: a way towards artificial antibodies. Angew. Chem. Int. Ed. Engl. **34**, 1812–1832 (1995)
108. Liao, J.-L., Chen, N., Ericson, C., Hjerten, S.: Preparation of continuous beds derivatized with one-step alkyl and sulfonate groups for capillary electrochromatography. Anal. Chem. **68**, 3468–3472 (1996)

109. Tanaka, N., Nagayama, H., Kobayashi, H., Ikegami, T., Hosoya, K., Ishizuka, N., Minakuchi, H., Nakanishi, K., Cabrera, K., Lubda, D.: Monolithic silica columns for HPLC, Micro-HPLC, and CEC. J. High Resolut. Chromatogr. **23**, 111–116 (2000)
110. Li, S., Lloyd, D.K.: Direct chiral separations by capillary electrophoresis using capillaries packed with an alpha.1-acid glycoprotein chiral stationary phase. Anal. Chem. **65**, 3684–3690 (1993)
111. Wistuba, D., Czesla, H., Roeder, M., Schurig, V.: Enantiomer separation by pressure-supported electrochromatography using capillaries packed with a permethyl-β-cyclodextrin stationary phase. J. Chromatogr. A **815**, 183–188 (1998)
112. Carter-Finch, A.S., Smith, N.W.: Enantiomeric separations by capillary electrochromatography using a macrocyclic antibiotic chiral stationary phase. J. Chromatogr. A **848**, 375–385 (1999)
113. Wikstrom, H., Svensson, L.A., Torstensson, A., Owens, P.K.: Immobilisation and evaluation of a vancomycin chiral stationary phase for capillary electrochromatography. J. Chromatogr. A **869**, 395–409 (2000)
114. Mayer, S., Schurig, V.: Enantiomer separation using mobile and immobile cyclodextrin derivatives with electromigration. Electrophoresis **15**, 835 (1994)
115. Mayer, S., Schleimer, M., Schurig, V.: Dual chiral recognition system involving cyclodextrin derivatives in capillary electrophoresis. J. Microcol. Sep. **6**, 43–48 (1994)
116. Armstrong, D.W., Tang, Y., Ward, T., Nichols, M.: Derivatized cyclodextrins immobilized on fused-silica capillaries for enantiomeric separations via capillary electrophoresis, gas chromatography, or supercritical fluid chromatography. Anal. Chem. **65**, 1114–1117 (1993)
117. Schweitz, L., Andersson, L.I., Nilsson, S.: Capillary electrochromatography with predetermined selectivity obtained through molecular imprinting. Anal. Chem. **69**, 1179–1183 (1997)
118. Peters, E.C., Lewandowski, K., Petro, M., Svec, F., Frechet, J.M.J.: Chiral electrochromatography with a "molded" rigid monolithic capillary column. Anal. Commun. **35**, 83–86 (1998)
119. Lämmerhofer, M., Peters, E.C., Yu, C., Svec, F., Fréchet, J.M.J., Lindner, W.: Chiral monolithic columns for enantioselective capillary electrochromatography prepared by in situ copolymerization of a monomer with quinidine functionality: 1. Optimization of polymerization conditions, porous properties, and chemistry of the stationary phase. Anal. Chem. **72**, 4614–4622 (2000)
120. Lämmerhofer, M., Svec, F., Fréchet, J.M.J., Lindner, W.: Chiral monolithic columns for enantioselective capillary electrochromatography prepared by copolymerization of a monomer with quinidine functionality: 2. Effect of chromatographic conditions on the chiral separations. Anal. Chem. **72**, 4623–4628 (2000)
121. Koide, T., Ueno, K.: Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with charged polyacrylamide gels incorporating chiral selectors. Anal. Sci. **14**, 1021–1023 (1998)
122. Koide, T., Ueno, K.: Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with β-cyclodextrin-bonded charged polyacrylamide gels. Anal. Sci. **15**, 791–794 (1999)
123. Koide, T., Ueno, K.: Enantiomeric separations of cationic and neutral compounds by capillary electrochromatography with monolithic chiral stationary phases of beta-cyclodextrin-bonded negatively charged polyacrylamide gels. J. Chromatogr. A **893**, 177–187 (2000)
124. Koide, T., Ueno, K.: Enantiomeric separations by capillary electrochromatography with charged polyacrylamide gels incorporating chiral selectors. Anal. Sci. **16**, 1065–1070 (2000)
125. Guttman, A., Paulus, A., Cohen, A.S., Grinberg, N., Karger, B.L.: Use of complexing agents for selective separation in high-performance capillary electrophoresis: chiral resolution via cyclodextrins incorporated within polyacrylamide gel columns. J. Chromatogr. A **448**, 41–53 (1988)
126. Verleysen, K., Vandijck, J., Schelfaut, M., Sandra, P.: Enantiomeric separations in capillary electrophoresis using 18-crown-6-tetracarboxylic acid ($18C6H_4$) as buffer additive. J. High Resolut. Chromatogr. **21**, 323–331 (1998)
127. Behr, J.-P., Girodeau, J.-M., Hayward, R.C., Lehn, J.-M., Sauvage, J.-P.: Molecular receptors. Functionalized and chiral macrocyclic polyethers derived from tartaric acid. Helv. Chim. Acta **63**, 2096–2111 (1980)
128. Kuhn, R., Wagner, J., Walbroeh, Y., Bereuter, T.: Potential and limitations of an optically active crown ether for chiral separation in capillary zone electrophoresis. Electrophoresis **15**, 828–834 (1994)
129. Nishi, H., Nakamura, K., Nakai, H., Sato, T.: Separation of enantiomers and isomers of amino compounds by capillary electrophoresis and high-performance liquid chromatography utilizing crown ethers. J. Chromatogr. **A757**, 225–235 (1997)
130. Mori, Y., Ueno, K., Umeda, T.: Enantiomeric separations of primary amino compounds by nonaqueous capillary zone electrophoresis with a chiral crown ether. J. Chromatogr. A **757**, 328–332 (1997)
131. Koide, T., Ueno, K.: Enantiomeric separations of primary amino compounds by capillary electrochromatography with monolithic chiral stationary phases of chiral crown ether-bonded negatively charged polyacrylamide gels. J. Chromatogr. A **909**, 305–315 (2001)