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Article

A Novel Method for the Preparation of a Chiral Stationary Phase Containing an Enantiopure Acridino-18-Crown-6 Ether Selector

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This paper reports a novel method for the preparation of chiral stationary phases (CSPs) using an acridino-18-crown-6 ether selector as a model compound. Chiral stationary phase (*R,R*)-CSP-2A was obtained by *in situ* continuously recirculating the solution of carboxyl-substituted acridino-18-crown-6 ether (*R,R*)-4, dicyclohexylcarbodiimide and 3-(triethoxysilyl)propylamine through a high-performance liquid chromatography (HPLC) column containing blank silica gel in elevated pressure and temperature. The enantiomer separating ability of chiral stationary phase (*R,R*)-CSP-2A was investigated by HPLC using mixtures of enantiomers of 1-(1-naphthyl)ethylamine hydrogen perchlorate, 1-(2-naphthyl)ethylamine, 1-(4-bromophenyl)ethylamine and 1-(4-nitrophenyl)ethylamine hydrogen chloride. The best results were found for the separation of the mixtures of enantiomers of Br-PEA.

Introduction

Enantiomeric recognition, which can be regarded as a special type of molecular recognition, is an essential phenomenon in living organisms. Examples of its action can be found in many processes such as the metabolism of single enantiomeric forms of amino acids and sugars, enzyme–substrate interactions, immunological responses and effects of drugs. Different enantiomers of biologically active compounds may possess different pharmacological and toxicological properties, therefore the determination of the enantiomeric composition of organic compounds is of great importance especially in pharmaceutical, pesticide, food and cosmetic industries. Apart from capillary electrophoresis (CE) (1–4), one of the most frequently used methods for separation of enantiomers is liquid chromatography on chiral stationary phases (CSPs) (4–15).

Cram and his coworkers prepared the first CSPs containing chiral crown ethers by successfully immobilizing optically active bis(1,1′-binaphthyl)-22-crown-6 ethers on silica gel (16) and polystyrene resin (17), and tested them for the resolution of primary amines, amino acids and amino acid esters. Since then CSPs containing chiral crown ethers as selectors have been widely used in the resolution of various chiral compounds containing a primary amino group. Stationary phases based on enantiopure crown ethers containing a pyridine (18–24) or an acridine (25, 26) unit (see Figure 1) were capable of separating the enantiomers of primary aralkylamines. Primary amines, amino acids and their derivatives are significant compounds of biological relevance. Primary amines serve as neurotransmitters, and amino acids are the building blocks of proteins (27).

In order to prepare CSPs the chiral selector is usually bound to silica gel using mechanical stirring, then the modified adsorbent is compressed in an empty stainless-steel high-performance liquid chromatography (HPLC) column (traditional batch process) (15, 22–26). It is conventionally accepted that the most reliable packing method for HPLC columns is the slurry-pack technique (28).

In this paper, we demonstrate a novel method for the preparation of a previously reported (26) acridino-18-crown-6 ether based chiral stationary phase ((*R,R*)-CSP-**2B**, see Figure 1) by continuously recirculating the solution of (*R,R*)-**4**, dicyclohexylcarbodiimide (DCC) and 3-(triethoxysilyl)propylamine (TSPA) through a blank HPLC column in elevated pressure and temperature. Here we also present studies and a comparison with previously reported (25, 26) acridino-crown ether based CSPs on its enantiomeric separation ability of the mixtures of enantiomers of primary amino compounds containing an aromatic moiety.

Experimental

Instrumentation and reagents

Reagents were purchased from Sigma-Aldrich Corporation unless otherwise noted. Solvents were dried and purified according to well-established methods (29). The blank HPLC columns (Superspher Si-60, 4 μm mean particle size, 6 nm mean pore size, 125 × 4 mm geometry) were obtained from Merck Ltd., Budapest, Hungary. The preparation of the CSP was performed by using a Syrris Asia 130 Flow Chemistry System. Chromatography was performed on a Hitachi HPLC system, involving an L-2450 UV-detector, an L-2130 pump, an L-2200 autosampler and an L-2300 column oven. Elemental analyses were performed on a Vario EL III instrument (Elementanalyze Corporation).

Procedure for the preparation of (R,R)-CSP-2A

A mixture of the acridino-crown ether derivative containing a carboxyl end group (*R,R*)-**4** (26) (80 mg, 0.18 mmol), DCC (1.1 equiv.) and TSPA (1 equiv.) was dissolved in pure CH_2Cl_2.The concentration of the solution of the crown ether was 4 mg/mL. The solution was circulated through a blank HPLC column (SP-**3**) (flow rate: 0.1 mL/min) for 5 h at 60°C, 20 bar, then for 2 h at 100°C, 20 bar by using a Syrris Asia Flow Chemistry System. The bounding process was monitored by a diode array detector of the HPLC system. The detector showed that the reaction finished after 7 h. Before the separation studies, the new CSP was flushed with isopropyl alcohol at 40°C (flow rate: 0.3 mL/min).

Characterization of (R,R)-CSP-2A

After the HPLC studies, the columns containing (*R,R*)-CSP-**2A** and blank silica gel were disassembled. The silica gel containing the bound crown ether was dried in a vacuum oven at 80°C for 16 h. A sample of blank silica gel was dried in the same way and it gave a combustion analysis of C, 0.39; H, 2.01; N, 0.00. The combustion analysis of (*R,R*)-CSP-**2A** gave C, 2.92; H, 2.14 and N, 0.22. This result shows that each gram of (*R,R*)-CSP-**2A** contained 0.078 mmol (by C%) and 0.079 mmol (by N%) of chiral crown ether.

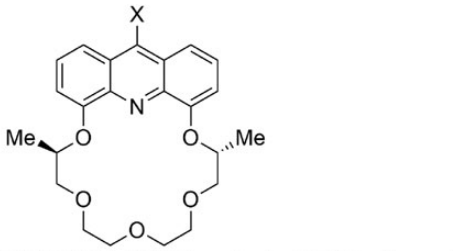
Results

Synthesis

Chiral stationary phase (*R,R*)-CSP-**2A** was prepared starting from chiral acridino-crown ether (*R,R*)-**4** (26) as outlined in Scheme 1. To carboxylic acid (*R,R*)-**4**, TSPA and DCC were added, the solution was continuously recirculated through a HPLC column containing blank silica gel (SP-**3**) until the immobilization to the HPLC quality silica gel by covalent bonds finished.

High-performance liquid chromatography

The HPLC column containing (*R,R*)-CSP-**2A** was tested for the separation of the enantiomers of compounds listed in Figure 2. A column containing blank silica gel (SP-**3**) was used as a reference. During the separation studies, isocratic elution was applied with solvent systems containing HCOOH and triethylamine (TEA) or diethylamine (DEA) in a 1:4 mixture of MeOH/CH_3CN (Table I). Optimal analysis times and effective resolutions were achieved with the above-mentioned eluents applying a flow rate of 1.0 mL/min at 40°C. Acidic modifier (HCOOH) in the mobile phase is necessary to form the protonated primary amino group of the analytes (30). TEA and DEA in the mobile phase were used for the deactivation of the free silanol groups (31). For



(*R,R*)-CSP-**1**: X = $CONH(CH_2)_3S(CH_2)_3$-spherical HPLC silica gel
Prepared by traditional batch process
(*R,R*)-CSP-**2A**: X = $CONH(CH_2)_3$-spherical HPLC silica gel
Prepared by continuous recirculation method
(*R,R*)-CSP-**2B**: X = $CONH(CH_2)_3$-spherical HPLC silica gel
Prepared by traditional batch process

Figure 1. Schematic representation of chiral stationary phases based on enantiopure acridino-18-crown-6 ethers as selectors.

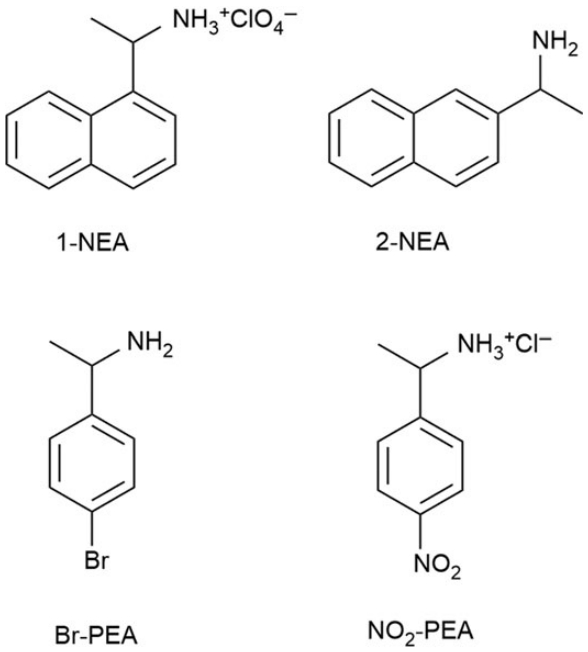
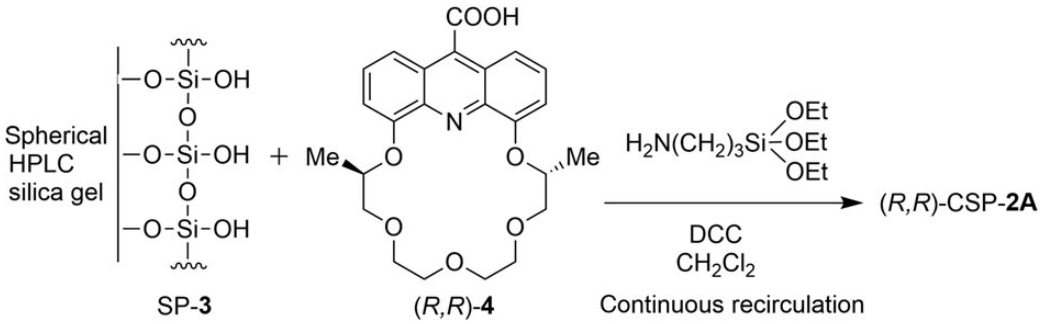


Figure 2. Structures of the aralkylamines used in the enantiomer separation studies.

Table I
Modifiers of the Mobile Phases

Method	Solvent system modifiers		
	HCOOH (%)	TEA (%)	DEA (%)
A01	0.08	0.16	–
A02	0.08	0.12	–
A03	0.08	0.08	–
A04	0.08	–	0.12
A05	0.08	–	0.08
A06	0.12	–	0.08



Scheme 1. Preparation of chiral stationary phase (*R,R*)-CSP-**2A**.

Table II
Chromatographic Data for the Separation of the Mixtures of Enantiomers of Protonated Primary Aralkylamines on (*R*, *R*)-CSP-**2A**

Method	Analytes							
	1-NEA				2-NEA			
	t (R), min	*t* (S), min	R_s	α	*t* (*R*), min	*t* (*S*), min	R_s	α
A01	12.00	21.50	2.85	1.79	14.04	24.76	2.89	1.76
A02	10.72	18.67	1.54	1.74	13.69	25.46	2.53	1.86
A03	11.13	22.96	1.82	2.06	14.04	26.77	3.34	1.91
A04	8.06	10.67	1.63	1.32	9.03	12.19	2.21	1.35
A05	6.75	12.56	2.06	1.86	7.99	14.08	3.05	1.76
A06	6.91	12.87	2.53	1.86	8.09	14.31	3.31	1.77

Table III
Chromatographic Data for the Separation of the Mixtures of Enantiomers of Protonated Primary Aralkylamines on (*R*, *R*)-CSP-**2A**

Method	Analytes							
	Br-PEA				NO_2-PEA			
	t (*R*), min	*t* (*S*), min	R_s	α	*t* (*R*), min	*t* (*S*), min	R_s	α
A01	10.15	18.42	2.95	1.82	5.68	9.03	1.56	1.59
A02	10.66	20.13	2.11	1.89	6.24	10.26	1.51	1.64
A03	11.55	24.27	2.70	2.10	7.27	12.73	1.53	1.75
A04	7.37	10.24	2.30	1.39	4.49	6.00	1.39	1.34
A05	6.59	12.12	2.64	1.84	4.07	6.07	1.24	1.49
A06	6.98	13.23	3.41	1.89	4.83	7.54	1.36	1.56

the determination of the elution order of the enantiomers, we used a mixture of the (*R*)- and (*S*)-enantiomers in the ratio of 1:2.

Discussion

The reference column containing blank silica gel (SP-**3**) did not discriminate between the enantiomers of the test compounds, while (*R,R*)-CSP-**2A** did (Tables II and III). Upon studying the enantiomeric recognition ability of (*R,R*)-CSP-**2A**, it was found that for all tested compounds the (*R*)-enantiomer eluted with a shorter retention time than that of its antipode. This behavior is in full agreement with the generally observed higher stability of heterochiral complexes [(*R,R*)-crown ether-(*S*)-ammonium salt and (*S,S*)-crown ether-(*R*)-ammonium salt] compared with that of the homochiral ones [(*R,R*)-crown ether-(*R*)-ammonium salt and (*S,S*)-crown ether-(*S*)-ammonium salt] in the cases of optically active acridino-18-crown-6 (25, 26) and pyridino-18-crown-6 ethers (18–24, 32).

Chiral stationary phase (*R,R*)-CSP-**2A** could effectively separate the enantiomers of all the studied test compounds with baseline separation (resolution factor R_s is above 1.5, Tables II and III). Composition of the mobile phase has significant effect on the enantiomeric recognition ability of CSPs. Acidic and basic additives can enhance the eluting ability of mobile phases, resulting in appreciable enantioseparation of the analytes (33).

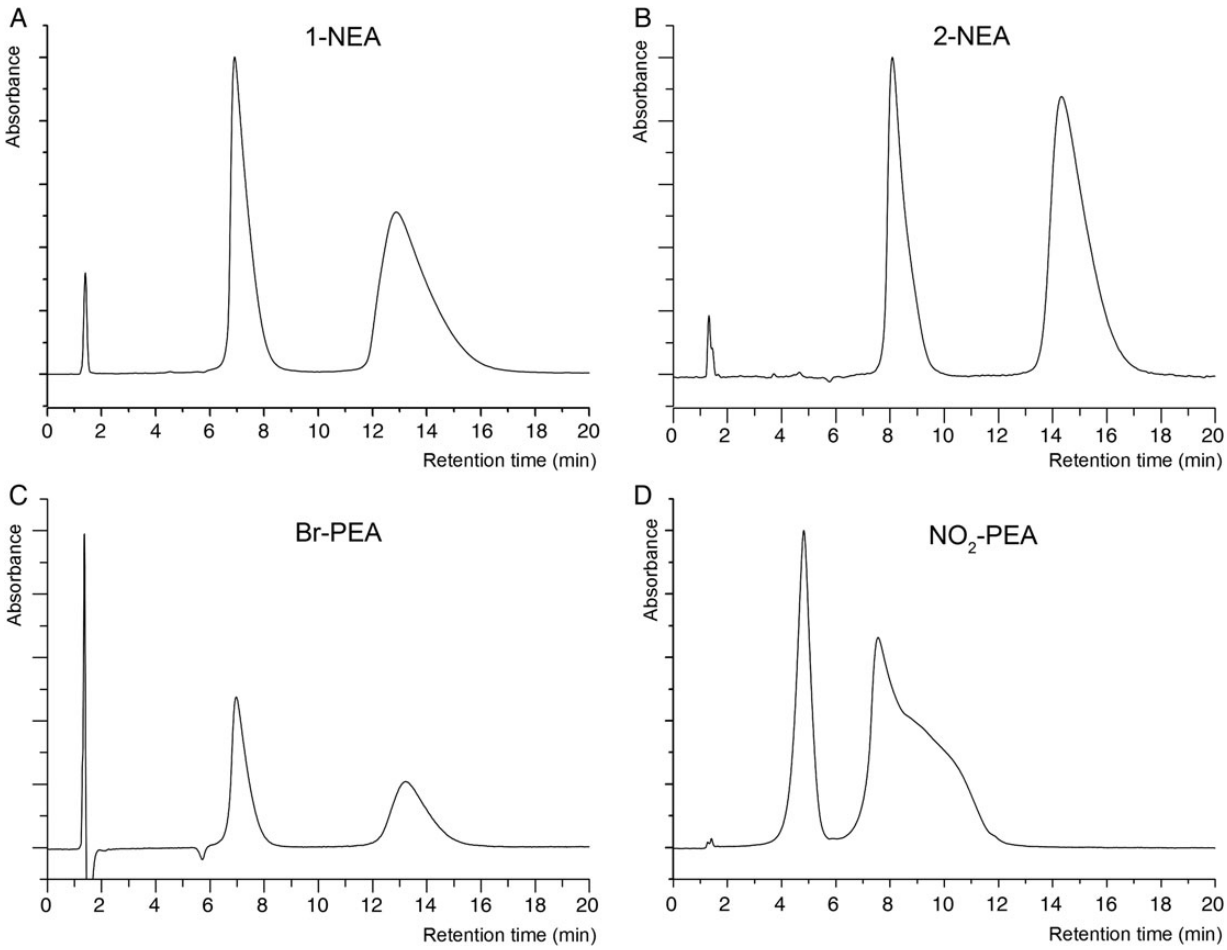


Figure 3. Chromatograms of enantioseparations of mixtures of 1-NEA (A), 2-NEA (B), Br-PEA (C) and NO_2-PEA (D) enantiomers using (*R,R*)-CSP-**2A**.

Upon changing the basic modifier from TEA to DEA, the decrease of retention time was found. Upon decreasing the content of TEA or DEA, the separation factor (α) is increased. Increasing the content of HCOOH separation and resolution factors are also increased. Highest retention times were observed for the separation of 1-(2-naphthyl)ethylamine (2-NEA) enantiomers which may be attributed to the favorable overlap (better $\pi-\pi$ interaction) of the naphthalene ring meaning that the stability constant of the complex is higher. The highest enantioselectivity was achieved in the case of 1-(4-bromophenyl)ethylamine (Br-PEA). For the separation of 1-(4-nitrophenyl)ethylamine hydrogen chloride (NO_2-PEA) enantiomers we observed increased tailing (Figure 3D), this can be explained by the strong $\pi-\pi$ interaction, namely that the electron-rich acridine ring system forms strong secondary attractive interaction with the electron-poor nitrophenyl moiety, resulting in greater retention and tailing. Chiral stationary phase (*R,R*)-CSP-**2A**, prepared by a continuous recirculation method, gave longer retention times, better separation and resolution factors for 1-(1-naphthyl)ethylamine hydrogen perchlorate (1-NEA), 2-NEA and Br-PEA than the reported (25, 26) acridino-crown ether-based CSPs, prepared by traditional batch process, (*R,R*)-CSP-**1** ($R_s < 1.5$, $\alpha < 2$) and (*R,R*)-CSP-**2B** ($R_s < 2.5$, $\alpha < 1.8$).

Conclusion

A new and effective continuous recirculation (flow) method has been elaborated for the preparation of acridino-18-crown-6 ether-based chiral stationary phase (*R,R*)-CSP-**2B** (26). We demonstrated that chiral stationary phase (*R,R*)-CSP-**2A** could separate the mixtures of protonated primary aralkylamine enantiomers efficiently. The chiral stationary phase showed the best enantioseparation factors for the separation of the mixtures of enantiomers of Br-PEA. Experiments are currently in progress to use (*R,R*)-CSP-**2A** for the enantioseparation of other mixtures of enantiomers of protonated primary amines, amino acids and their derivatives and to prepare new CSPs with the novel method described here.

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