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Series of homologous displacers for preparative chiral displacement chromatographic separations on Cyclobond-II columns

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Abstract

A series of homologous displacers have been synthesized to aid the development of chiral displacement chromatographic separations on Cyclobond-II cyclodextrin silica stationary phases. The displacers contain an anchor group, derived from mandelic acid, a H-bonding mid-section (carboxyl and C = O groups) and a solubility adjusting tail section (*n*-alkanoate groups). The capacity factors and the individual excess adsorption isotherms of these displacers were measured in acetonitrile-citrate buffer eluents. Both the *k'* values and the isotherm parameters vary regularly with both the acetonitrile concentration of the eluent and the length of the alkanoate chain. Because the capacity factors and the adsorption strengths of these compounds cover a sufficiently broad range, they can serve as displacers in a variety of displacement chromatographic separations on Cyclobond-II columns.

1. Introduction

In earlier papers we demonstrated that preparative displacement chromatographic separations of geometrical isomers, positional isomers and enantiomers can be achieved using columns packed with commercially available cyclodextrin silica stationary phases operated in the reversed-phase mode [1-5], and on Pirkle-type naphthylalanine silica stationary phases operated in the normal-phase mode [6,7]. One of the most serious impediments to the development of a

displacement chromatographic separation is the lack of readily available displacers. Traditionally, displacers are selected by trial-and-error, using previous experience with similar analytes and potential displacer as guides. On cyclodextrin silicas, operated in the reversed-phase mode, ionic detergents, alkylphenols, nitrophenols and cyclic alcohols have been used successfully as displacers [1-5].

The displacers must satisfy divergent demands: they must be highly soluble in the carrier solvent to form concentrated displacer solutions, they must adsorb competitively on the stationary phase and must do so more strongly than the solutes, yet they must allow easy and fast regeneration of the column once the separation is complete. Unfortunately, no single compound

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class possesses all these characteristics. However, in the case of Pirkle-type stationary phases, a generic displacer structure could be identified and three series of homologous compounds were synthesized and studied in detail: the 3,5-dinitrobenzoyl esters of alcohols, the 3,5-dinitrophenylcarbamates of alcohols, and the N-3,5-dinitrophenylamidoethyl-1-alkanoates [6]. These displacer families are based on a common core structure and can be synthesized using inexpensive reagents and simple preparative methods.

Based on the success of the above approach to displacer selection, we attempted to identify generic displacer structures for cyclodextrin silica stationary phases operated in the reversed-phase mode, and to synthesize several homologous displacers using a simple reaction scheme. The objective of this paper is to describe such a family of closely related displacers, intended for use on the Cyclobond-II α -cyclodextrin silica stationary phases [8,9].

The proposed generic displacers for α -cyclodextrin silica stationary phases contain an anchor group which fits the cavity of α -cyclodextrin (phenyl group), a section that can form multiple hydrogen bonds with the secondary hydroxyl groups of α -cyclodextrin (carboxyl and carbonyl groups), and a solubility-adjusting tail section which regulates displacer solubility (alkanoate group of variable chain length).

2. Experimental

Three 250 mm \times 4.6 mm I.D. Cyclobond-II columns, packed with a 5- μ m α -cyclodextrin (CD) silica stationary phase, were obtained from Astec (Whippany, NJ, USA). One of the columns was used for the retention studies, the

adsorption isotherm determinations and the fraction analysis work to obtain the reconstructed displacement chromatograms. The other two columns, connected in series, were used for the actual displacement chromatographic separations.

HPLC-grade acetonitrile (ACN) and tetrahydrofuran (THF) were purchased from J.T. Baker (Phillipsburg, NJ, USA). Water was obtained from a Milli-Q unit (Millipore, Bedford, MA, USA). All chemicals used for the synthesis of the displacers were obtained from Aldrich (Milwaukee, WI, USA), and used as received, without further purification.

The ester type displacers were synthesized [10] according to the general Schotten–Baumann reaction scheme (Fig. 1). Briefly, 8 mmol acid halide, dissolved in 10 ml tetrahydrofuran (THF), was added dropwise over a period of 30 min to a 50-ml round-bottom flask containing 4 mmol of mandelic acid, 10 ml of THF and 2 ml of pyridine. While stirred, the mixture was refluxed for 2 h. Subsequently, THF was removed using a Buchi RotaVapor-R (Fisher Scientific, Pittsburgh, PA, USA), 30 ml of 0.1 M HCl solution was added to the residue and extracted three times with 10-ml portions of CH_2Cl_2 . The combined CH_2Cl_2 extracts were washed three times with 10-ml portions of 0.01 M HCl solution, dried over magnesium sulfate, and allowed to evaporate slowly to obtain a crystalline product. Finally, the product was purified by recrystallizing it twice from CH_2Cl_2 . The structure of the final product was verified by ^1H NMR spectroscopy (XL-200E; Varian, Palo Alto, CA, USA) and its purity was checked by HPLC using the Cyclobond-II column.

The retention measurements and adsorption isotherm determination methods were described in detail in Ref. [6]. The custom-built, multi-

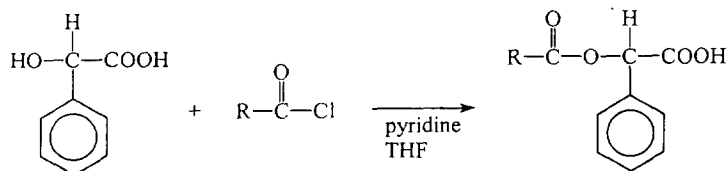


Fig. 1. Reaction scheme for the synthesis of the mandelic acid-based displacers.

purpose liquid chromatographic system [1–5] was used for the displacement chromatographic separations. All measurements were made at 30°C. The columns were regenerated after the isotherm determinations and the displacement chromatographic separations by running a 5%/min solvent gradient starting with the carrier solvent composition and ending with pure ACN. Finally, ACN was pumped through the column until the UV absorbance of the effluent became equal to that of the wash ACN.

3. Results

The capacity factors (k') of the potential displacers were determined in several ACN–citrate buffer eluents as shown in Fig. 2. The log k' values increase regularly with the length of the alkanooate chain in the displacers, as usual in reversed-phase HPLC.

The adsorption isotherms of the synthesized displacers were also measured in eluents of increasing ACN concentration. As an example, the individual excess adsorption isotherms measured in a 3% (v/v) acetonitrile in 40 mM citrate buffer (pH 6) are shown in Fig. 3. In the concentration ranges tested, the isotherms can

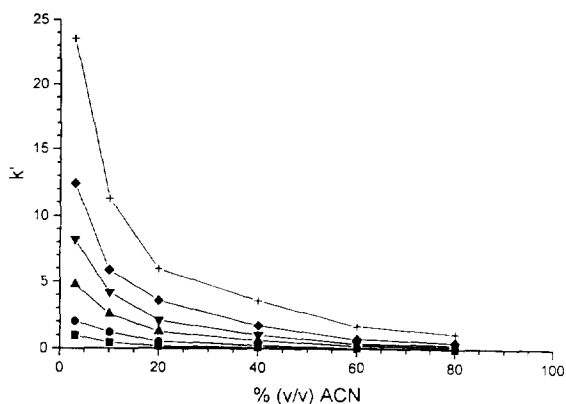


Fig. 2. Capacity factors of the mandelic acid-based displacers as a function of the ACN concentration in the pH 6, acetonitrile: 1 mM citrate eluents on the Cyclobond-II column at 30°C. ■ = Mandelic acid; ● = acetate derivative; ▲ = butyrate derivative; ▼ = pentanoate derivative; ◆ = hexanoate derivative; + = octanoate derivative.

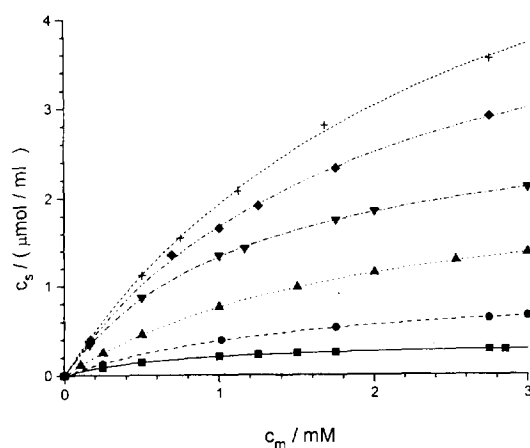


Fig. 3. Adsorption isotherms of the mandelic acid-based displacers in the pH 6, acetonitrile–1 mM citrate (3:97, v/v) eluents on the Cyclobond-II column at 30°C. ■ = Mandelic acid; ● = acetate derivative; ▲ = butyrate derivative; ▼ = pentanoate derivative; ◆ = hexanoate derivative; + = octanoate derivative. The lines show the isotherms calculated with the Langmuir isotherm equation and the parameters shown in Table 1. c_s = Stationary phase concentration; c_m = mobile phase concentration.

be described adequately with the simple Langmuir isotherm equation [6]. The isotherm parameters, determined by the Origin Version 3 software package, are listed in Table 1. The b parameters do not change regularly with the carbon number as was the case with the 3,5-dinitrobenzamido-type displacers developed for the Pirkle-type chiral stationary phases [6]. The a parameters increase as the chain length is increased, indicating that the adsorption behavior of these homologous displacers on Cyclobond-II is similar, and that their adsorption strengths cover a broad enough range to be of use for

Table 1
Langmuir isotherm parameters for the mandelic acid-based displacers of increasing side-chain length

C_n	a	b
0	0.482 ± 0.001	1.317 ± 0.004
2	0.62 ± 0.02	0.60 ± 0.03
4	1.137 ± 0.005	0.490 ± 0.005
5	2.40 ± 0.04	0.80 ± 0.02
6	2.55 ± 0.07	0.52 ± 0.03
8	2.70 ± 0.06	0.39 ± 0.02

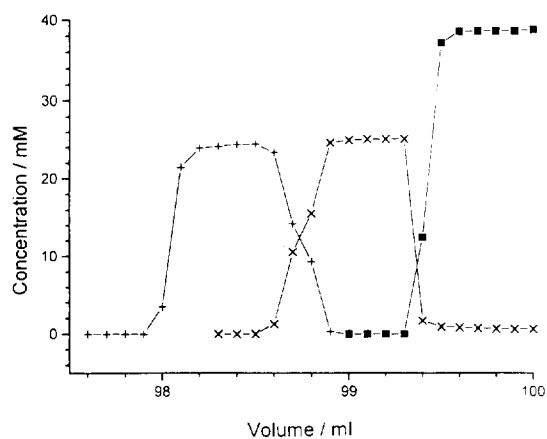


Fig. 4. Reconstructed displacement chromatogram of a 6.85- μ mol racemic tryptophane sample at 30°C, on two Cyclobond-II columns connected in series. Carrier solution: pH 3, acetonitrile-40 mM citrate buffer (3:97, v/v), displacer: 38 mM mandelic acid, flow-rate: 0.2 ml/min, fraction size: 100 μ l. + = D-Tryptophan; x = L-tryptophan; ■ = mandelic acid displacer.

actual displacement chromatographic applications.

To demonstrate the utility of these new displacers, two reconstructed displacement chromatograms are shown in Figs. 4 and 5. The enantiomers of underivatized tryptophan can be

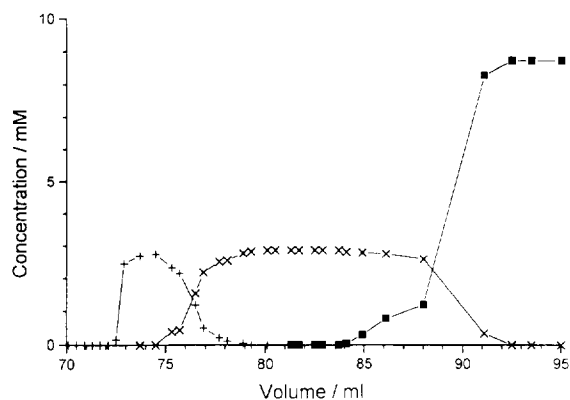


Fig. 5. Reconstructed displacement chromatogram of a sample containing 3.61 μ mol 2-nitrotoluene and 5.61 μ mol 3-nitrotoluene at 30°C, on two Cyclobond-II columns connected in series. Carrier solution: pH 3, acetonitrile-40 mM citrate buffer (3:97, v/v), displacer: 8.5 mM 2-phenyl-2-butyroylacetic acid, flow-rate: 0.2 ml/min, fraction size: 450 μ l. + = 2-Nitrotoluene; x = 3-nitrotoluene; ■ = 2-phenyl-2-butyroylacetic acid displacer.

separated using 3% ACN in 40 mM citrate buffers (pH 3), resulting in moderate retention of the later-eluting L-enantiomer ($k'_2 = 3.2$). Even the first member of the displacer series, mandelic acid (which has a higher capacity factor, 5.8, in this carrier solution) is adsorbed sufficiently strongly and can be considered a potential displacer. The reconstructed displacement chromatogram of a 6.85- μ mol tryptophan sample obtained with a 38 mM mandelic acid solution as displacer is shown in Fig. 4. The yields of D- and L-tryptophan at the 95% enantiomeric purity level were 85 and 73%, respectively.

The nitrotoluene isomers are retained more strongly in the same eluent ($k'_2 = 8.1$) and require the use of a higher member of the displacer series. The reconstructed displacement chromatogram of a sample containing 3.61 μ mol of 2-nitrotoluene and 5.61 μ mol of 3-nitrotoluene, obtained with an 8.5 mM solution of 2-phenyl-2-butyroylacetic acid as displacer, is shown in Fig. 5. At the 95% purity level, the yield for 2-nitrotoluene is 61% and for 3-nitrotoluene it is 63%.

4. Conclusions

A displacer family, to be used with the Cyclobond-II chiral stationary phase, was designed and synthesized according to the concept of generic displacers. Members of the displacer family contain a phenyl group as anchoring group, a carboxyl group as hydrogen bonding group and an alkanolic group as solubility adjusting group. The retention and adsorption properties of these displacers were determined and found to follow regular patterns as a function of the length of their alkanolic side chain: longer chains result in larger capacity factors and increased adsorption. This regular adsorption behavior facilitates displacer selection for the Cyclobond-II columns and eliminates much of the trial-and-error effort traditionally involved in the development of a displacement chromatographic separation on cyclodextrin silica stationary phases.

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