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Imprintable brush-type chiral stationary phase

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Abstract

A chiral stationary phase (CSP) based upon a previously studied naphthamide atropisomer is described. Although CSPs based upon imprinted polymers have been known for some time, this naphthamide-derived CSP is the first reported example of an imprintable brush-type CSP. Deracemization of the racemic CSP using a soluble enantiopure imprint molecule affords a CSP which resolves the enantiomers of the imprint molecule and related compounds. A time-dependent HPLC study of the reversion of the imprinted stationary phase to the racemic form was performed, with a decrease in enantioselectivity being noted over a period of two weeks at room temperature, after which time the stationary phase was no longer capable of resolving enantiomers. Speculations about the potential utility of such CSPs are offered.

1. Introduction

The idea that a material could be somehow "imprinted" by a probe molecule so as to display at some later time an enhanced retention for that compound was originally conceived by Pauling [1], who suggested in 1940 that some such process might account for the ability of the immune system to mount an antibody response to an exceedingly wide variety of antigen structures. Realizing that a single polypeptide chain could exist in a multitude of low energy conformations, Pauling suggested that in the presence of different imprint molecules a single polypeptide chain could fold to form a variety of different antibody conformers, each with specific affinity for their imprint molecules. Later research showed that the diversity in immune response is in fact due to a more or less random splicing of bits of DNA coding for antibody structure, with a positive selection for those cells which produce antibodies which bind to the invading antigen [2,3]. Nevertheless, Pauling's hypothesis has given rise to a number of investigations of imprinted stationary phases for chromatographic separations [4–10]. Typically these stationary phases are produced by polymerizing a monomer in the presence of a imprint compound, milling and sizing the resulting polymer, and removal of the imprint compound by leaching (Fig. 1).

From the outset, much of the work in this field has focused on the production of chiral stationary phases (CSPs) for the chromatographic separation of enantiomers. Many of these CSPs have shown high levels of enantioselectivity, although the irregular nature of the stationary phase particles and the wide distribution of binding site affinities has historically resulted in poor chromatographic efficiency, particularly for the more retained enantiomer. Nevertheless, there has been continuing progress in producing more

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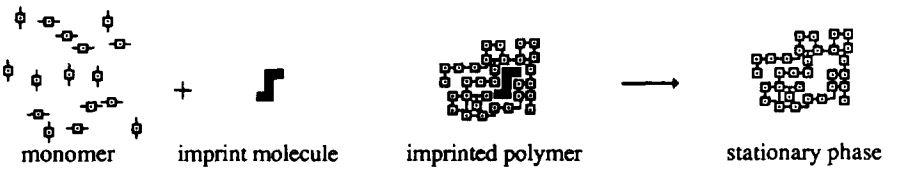

Fig. 1. Typical production of an imprinted polymer stationary phase involves polymerization of a monomer in the presence of an imprint compound followed by removal of the imprint compound by leaching.

efficient stationary phases. In the present study the first example of a silica-based imprintable brush-type CSP is presented.

A previous study of the chromatographic separation of the slowly interconverting enantiomers of a series of naphthamide atropisomers showed that separation factors in the range of 2–3 were obtained for many of these analytes using the Whelk-O 1 CSP (Fig. 2) [11]. This relatively high degree of enantioselectivity, combined with an enantiomer interconversion half life on the order of a day, permits a demonstration of enantioenrichment by on-column deracemization [11]. In this procedure, a racemic sample is applied to the CSP, the flow is stopped and several half lives allowed to elapse. The analyte is then eluted from the column, affording a sample which is enriched in the more retained enantiomer. The degree of enantioenrichment approaches the level of enantioselectivity observed in the chromatographic separation. A schematic depiction of the deracemization process is presented in Fig. 3.

The concept of reciprocity has been of great importance in the development of Pirkle-type CSPs [12]. Consequently, the design of a reciprocal CSP based upon the naphthamide atropisomer structure was a natural extension of the previously described study. Such a CSP cannot conveniently be prepared in enantiomerically pure form, owing to the relatively rapid racemization of the naphthamide enantiomers. However, the racemic CSP 1 (Fig. 4) (actually a mixture of two racemic diastereomers owing to the slow rate of rotation about both the $C_{(carbonyl)}-N_{(amide)}$ and the $C_{(carbonyl)}-C_{(aryl)}$ bonds [11]) is readily prepared by a simple extension of the procedure used in the previous study [11] (reaction of 2-methyl-1-naphthoyl

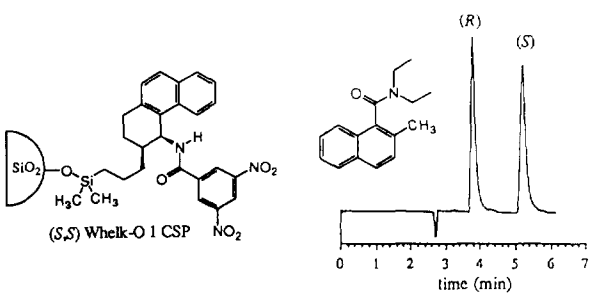

Fig. 2. Separation of the slowly interconverting enantiomers of *N,N*-diethyl-2-methyl-1-naphthylenecarboxamide on the Whelk-O 1 CSP. Mobile phase = ethyl acetate; flow rate = 1 ml/min; detection = UV 254. $k'_1 = 0.46$; $\alpha = 2.17$.

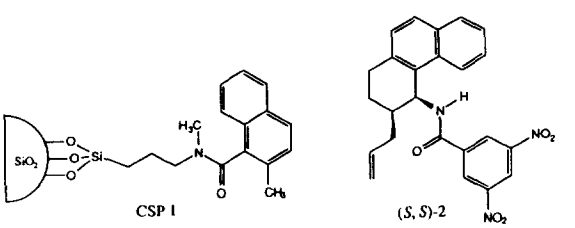

Fig. 4. The imprintable brush-type CSP 1 and the imprint molecule (*S,S*)-**2** used in its deracemization.

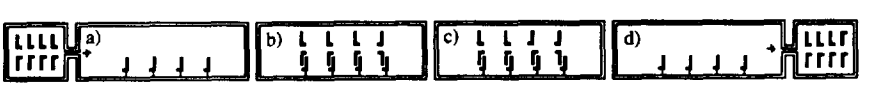

Fig. 3. On-column deracemization experiment. (a) racemate pumped onto column. (b) selective adsorption of one enantiomer leaves an excess of the opposite enantiomer in the mobile phase. (c) racemization of the analyte in the mobile phase. (d) column flushed to afford sample enriched in more retained enantiomer.

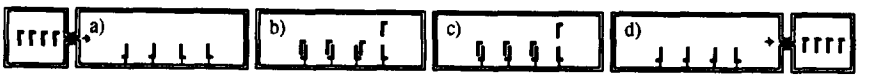

Fig. 5. Imprinting of CSP 1. (a) Enantiopure (*S,S*)-2 pumped onto column. (b) selective adsorbate formation with (*S*) form of CSP selector. (c) deracemization of CSP selector to favor (*S*) form. (d) column flushed to afford enantioenriched CSP.

chloride with the commercially available N-methyltrimethoxyaminopropyl silane followed by bonding of the resulting naphthamide silane to silica gel). This racemic CSP can be readily deracemized using (*S,S*)-**2**, a soluble analogue of the Whelk-O 1 CSP, as an imprint compound (Fig. 5).

2. Experimental

2.1. Apparatus

Chromatographic analysis was performed using a Kratos Spectroflow 400 pump, a Rheodyne Model 7125 injector fitted with either a 5-ml or a 20-μl sample loop, a Kratos Spectroflow 757 variable-wavelength absorbance monitor set at 254 or 280 nm, and a Hewlett-Packard HP 3394 integrating recorder.

2.2. Materials

The (*S,S*)-Whelk-O 1 CSP was obtained from Regis Technologies (Morton Grove, IL, USA). CSP 1 was prepared by reaction of 2-methyl-1-naphthoyl chloride (prepared by the method of Adams and Binder [13]) with N-methyltrimethoxyaminopropyl silane (Hüls America, Bristol, PA, USA), followed by bonding of the resulting silane on 5 μm/100 Å Rexchrom silica (Regis). Combustion analysis (C) revealed a selector surface coverage of $4.6 \cdot 10^{-4}$ mol/g (2.3 μmol/m^2). HPLC-grade ethyl acetate was obtained from EM Science (Gibbstown, NJ, USA). Racemic and (*S,S*)-**2** were prepared as described previously [14].

2.3. Deracemization of CSP 1 using (*S,S*)-2

Racemic CSP 1 was deracemized by first equilibrating the column with ethyl acetate at a flow-rate of 1 ml/min, then filling a 5-ml sample loop with a 20 mg/ml solution of (*S,S*)-**2** in ethyl acetate, and injecting this solution onto the column for a period of 4 min, at which time the flow was stopped, column end caps were inserted, and the column stored at room temperature for a period of 17 days. The column was then flushed with ethyl acetate at a flow-rate of 2 ml/min until a stable baseline was observed (3 h).

2.4. Evaluation of CSP 1

Separation of the enantiomers of a racemic sample of **2** spiked with 1,3,5-tri-*tert.*-butylbenzene as a void time indicator was studied on CSP 1 as a function of time at room temperature using a mobile phase of ethyl acetate at a flow-rate of 2 ml/min with 280 nm detection. During the course of the protracted study the column was intermittently stored in a freezer at −15°C, so as to effectively "freeze" the racemization process. This was done solely for operator convenience.

3. Results and discussion

Fig. 6 shows representative chromatograms which were obtained for the separation of the enantiomers of a racemic sample of **2** on the imprinted CSP 1. Independent injections allow identification of the less retained peak as (*R,R*)-**2** and the more retained peak as (*S,S*)-**2**. A relatively large separation factor is noted after 4 h ($\alpha = 2.54$) with a rapid decrease in enantioselectivity being observed until only one peak is observed at 119 h. A decrease in column flow-rate to 0.5 ml/min at 119 h revealed a marginal separation ($\alpha = 1.09$), which decreased over time until no trace of enantioseparation was seen at 194 h.

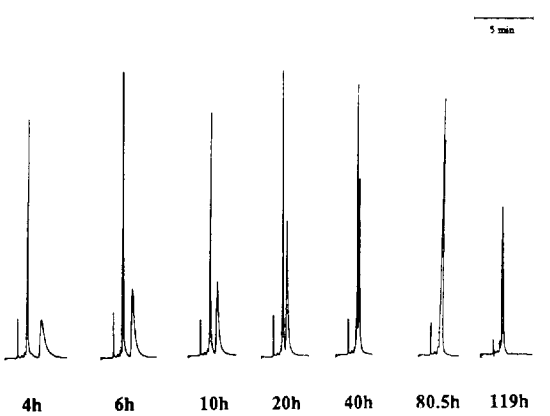

Fig. 6. Time dependent separation of the enantiomers of **2** on imprinted CSP 1. Conditions: Sample = racemic **2** spiked with 1,3,5 tri-*t*-butylbenzene; Column–CSP 1 following 17 day imprinting with 80 mg (*S,S*)-**2** in ethyl acetate; mobile phase = ethyl acetate; flow rate = 2.00 ml/min, detection = UV 254 nm; room temperature.

A plot of the time-dependent change in retention factors (k'_1, k'_2) for the two enantiomers of compound **2** on CSP 1 is shown in Fig. 7. As stated previously, the data after 119 h were collected at a flow-rate of 0.5 ml/min. In order to permit convenient data collection the column was periodically stored in a freezer at −15°C for the time periods marked with an asterisk. The results indicate that racemization of the CSP was almost completely suppressed at this temperature. The decrease in enantioselectivity can be seen to be almost entirely due to a decrease in the retention factor for the more retained enantiomer, a somewhat surprising result. A plot of the decrease in separation factor (α) for the two enantiomers of compound **2** on CSP 1 is shown in Fig. 8. Analysis of such data can reveal the kinetic parameters for enantiomer interconversion. In the present case, this analysis is somewhat complicated by the fact that CSP 1 contains four stereoisomeric components whose interconversion has been shown to be somewhat complex [11].

Several factors which influence the "charging" of an imprintable brush-type CSP can be identified or postulated. Under optimum circumstances, the limiting enantioenrichment obtained in the deracemization of the CSP can be expected to be roughly equivalent to the enantioselectivity observed for the enantiomers of the chiral selector by the imprinting agent. In the deracemization of CSP 1 by (*S,S*)-**2**, higher concentrations of the imprinter lead to a greater degree of CSP deracemization. This situation will be general, for the extent of deracemization is influenced by the position of the complexation equilibrium. Thus, a highly soluble imprint molecule which has both a high affinity for and a high enantioselectivity toward the CSP selector is preferred.

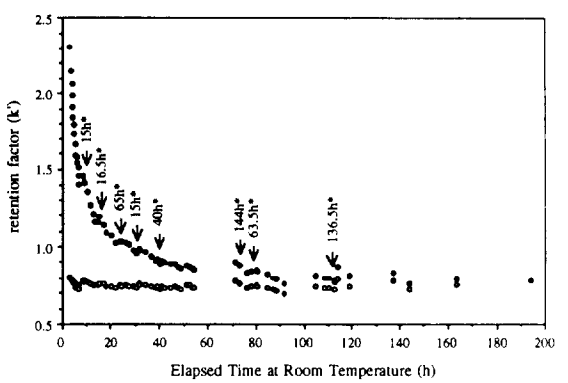

Fig. 7. Time dependent change in retention factors for separation of enantiomers of **2** on CSP 1. Conditions: as for Figure 6, except that data points greater than 120 h were collected at a flow rate of 0.5 ml/min. * denotes column storage time at −15°C.

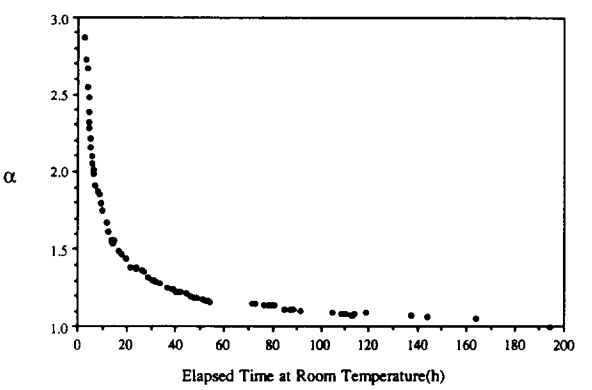

Fig. 8. Time dependent change in separation factor for separation of enantiomers of **2** on CSP 1. Conditions: as for Figure 7.

4. Conclusions

CSP 1 exhibits a number of undesirable properties which preclude its practical utility. Foremost among these is the rapidly changing characteristics of the column at room temperature and its relatively short lifespan once imprinted. However, longer lived analogues could be prepared by choice of a chiral selector with a slower rate of enantiomer interconversion, an approach which would also require increased time for the imprinting process. The time required to imprint such a CSP can be decreased by conducting the deracemization at an elevated temperature, where enantiomer interconversion is more rapid. However, owing to the fact that enantioselectivity typically decreases at higher temperatures, less enantioenrichment would be expected in the resulting CSP.

An interesting extension of this work, one that harkens back to Pauling's 1940 hypothesis, is the possibility of a general-purpose CSP which could accommodate a variety of imprint molecules. For example, a CSP structure containing only five slowly interconverting structural elements can exist in as many as 2^5 or 32 stereoisomeric forms, each of which could be expected to display differing levels of enantioselectivity for different racemates. By judicious choice of structure and appropriate disposition of interacting groups, an imprintable CSP with broad generality could perhaps be produced.

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