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Short communication

Enantiomer separations by supercritical fluid chromatography on a chiral stationary phase physically anchored to porous graphitic carbon

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Abstract

A chiral anthrylamine derivative was prepared and adsorbed on the surface of pre-packed porous graphitic carbon, and was evaluated as a chiral stationary phase in packed-column supercritical (and sub-critical) fluid chromatography. It successfully separated the enantiomers of two commercial anti-inflammatory agents and also a series of racemic tropic acid derivatives.

1. Introduction

Porous graphitic carbon (PGC) is a new packing for chromatographic columns that has been developed in the last decade [1,2]. Its main advantage over silica is its pH stability across the whole range 0–14. The order of retention on PGC is not governed by the order of solute polarity and, as supercritical fluids with polar modifiers are able to solvate many polar species, it is believed that the use of PGC in supercritical fluid chromatography (SFC) may expand the scope of applications in SFC [3]. Good efficiency, peak shapes and selectivity have been demonstrated on PGC in SFC but there is also one major drawback, namely that of the high affinity of the packing for large, planar molecules.

Chiral separations have been effected on such a packing in HPLC but only when a chiral additive is used in the mobile phase [4,5]. It is the high affinity of PGC for large, planar molecules that provides a possible means of producing a carbon-based chiral stationary phase (CSP). In this study, anthracene was used to anchor a chiral selector (**I**), derived from (*R*, *R*)-(+)-tartaric acid, to the carbon surface.

2. Experimental

2.1. Instrumentation

The SFC instrumentation consisted of a Gilson SFC2 cooler, a Gilson Model 305 CO_2 pump, a Gilson model 306 modifier pump, a Gilson Model 805S manometric module, a Gilson Model 811B dynamic mixer, a Rheodyne Model 7125 manual injector valve, a Pye Unicam Model 740592 oven, an Applied Biosystems Model 757 absorbance detector and a Tescom Series 26-1700 back-pressure regulator. The UV instru-

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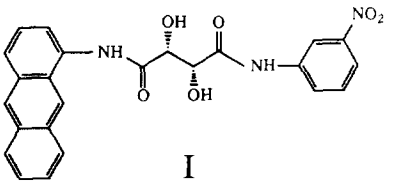

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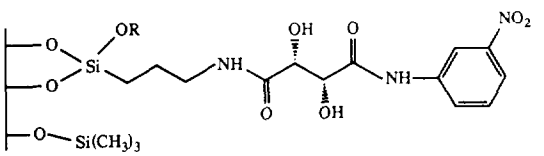

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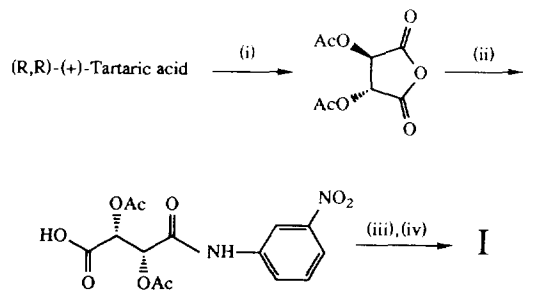


Fig. 1. Synthesis of chiral selector I. Reagents: (i) Ac_2O, H_2SO_4; (ii) $3\text{-}NO_2C_6H_4NH_2$; (iii) 1-aminoanthracene, peptide coupling; (iv) $NaHCO_3$, CH_3COCH_3.

mentation consisted of a Shimadzu (UV-2101PC) UV–Vis scanning spectrophotometer.

2.2. Materials

The solvents used included methanol, 2-propanol (Rathburn, HPLC grade) and carbon dioxide with liquid offtake dip-tube (Air Products). The porous graphitic carbon was purchased in the form of a Hypercarb HPLC column (10 cm × 3 mm I.D.; Shandon No. 59863752). The silica-based CSP (25 cm × 4.5 mm I.D.) and tropic acid amides were synthesized at UMIST. Ibuprofen and flurbiprofen were purchased from Sigma and benzoin from Fisons.

2.3. Preparation of chiral selector

The chiral selector **I** chosen for the carbon-based CSP was designed to be analogous to the selector in the silica-based CSP **II**, previously prepared at UMIST [6]. A comparison of the performance of the same selector on these two packings would then be possible. In order to anchor the chiral selector to the Hypercarb, anthracene was directly attached to the tartaric acid moiety via an amide linage. The procedure for the synthesis of **I** is shown in Fig. 1.

Table 1
Analysis of achiral compounds on uncoated and coated PGC

Compound	Uncoated PGC			Coated PGC		
	t_R (min)	N (plates/m)	A_S	t_R (min)	N (plates/m)	A_S
Phenol	1.11	4740	3.3	0.87	4193	1.9
p-Nitrophenol		not eluted		40.50	1850	3.0
p-Cresol	1.95	4350	5.0	1.15	6055	2.4
Naphthalene	5.99	10750	4.0	0.63	4098	4.3
Acetophenone	1.06	12710	1.7	0.58	3982	2.5
Benzoic acid	5.60	2720	0.2	3.08	1073	6.1
Aniline	0.68	5230	2.5	0.59	1594	9.4

Conditions: 1% MeOH–CO_2, 3 ml/min, 200 atm (1 atm = 101 325 Pa) 20°C, 254 nm.

2.4. Coating of chiral selector onto PGC

A solution (5 mg/ml) of **I** in tetrahydrofuran (THF) was pumped and recycled through the column and the decrease in concentration in the reservoir was monitored off-line-by UV spectrophotometry over a 7-day period, after which little further uptake appeared to occur.

3. Results and discussion

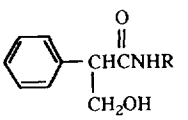
The surface coverage of the selector on Hypercarb, determined from the measured uptake of the selector, was 80 mg (400 μmol/g). If the molecular area of anthracene is taken to be 0.202 nm^2, this result suggests a 50% complete monolayer coverage.

Table 1 shows the SFC analysis of some achiral compounds on the coated PGC and a separate uncoated PGC column. Coating PGC with the chiral selector evidently resulted in a decrease in both retention and column efficiency. There is no general trend observed for asymmetry values (A_S) [7] from uncoated to coated PGC and the decrease in efficiency obtained for similar or lower A_S values has been attributed to a relative increase in peak width (values not given). Benzoic acid and aniline were eluted fairly quickly but the peak shapes were poor, presumably owing to strong hydrogen bonding interactions with the selector.

A series of tropic acid amides (Fig. 2a) were resolved by SFC on both the carbon and silica-based CSP. Although the two columns are not of identical dimensions, CSP **II** being used in a longer column, 25 cm × 4.5 mm I.D., similar trends for retention (k'_2) and selectivity values (α) were obtained (see graphs in Fig. 2). The greater values of α obtained on the shorter PGC CSP are promising and should encourage further study of this approach to chiral separations on porous graphitic carbon.

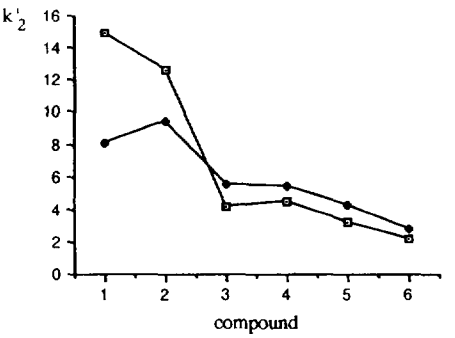
Even more promising are the separations of benzoin, ibuprofen and flurbiprofen (Fig. 3). The last two demonstrate the successful separation of unprotected acid substances under

a



Compound	R
1	$-C_6H_5$
2	$-CH_2C_6H_5$
3	$-CH_2CH_2CH_3$
4	$-CH_2CH{=}CH_2$
5	$-CH(CH_3)_2$
6	$-C(CH_3)_3$

b



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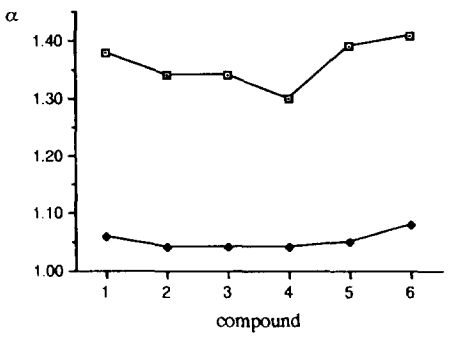

Fig. 2. Comparison of (□) PGC with (▲) silica. (a) Structures of tropic acid amides; (b) retention (k'_2) and (c) selectivity values (α) of compounds **1–6**. Conditions: 6% 2-propanol–CO_2, 3 ml/min, 200 atm, 20°C, 217 nm.

normal-phase conditions. Peak tailing is evident, however, resulting in decreased resolution of the enantiomers. Again, this is thought to be due to hydrogen bonding interactions with the selector.

After continuous use of the CSP, a decrease in retention and resolution was noted. At first this

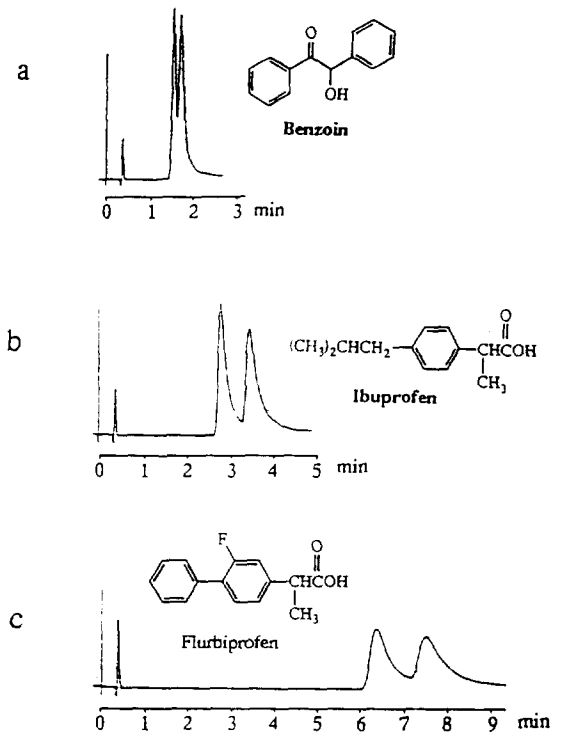

Fig. 3. Separations of (a) benzoin, (b) ibuprofen and (c) flurbiprofen. Conditions: 2% 2-propanol–CO_2, 3 ml/min, 200 atm, 20°C, 217 nm.

was believed to be due to the leaching of the chiral selector from the column. Flushing the column with a mobile phase of 20% THF–CO_2 at room temperature, however, reproduced the original separation. This suggests that the decrease in retention and resolution could be due to the masking of attractive interaction sites by substances highly retained on the column, which can subsequently be washed from the column, leaving the chiral selector undisturbed. Although we have not yet completed a thorough study of the durability and ruggedness of this anthracene-anchored Hypercarb-supported CSP, we have found that the selector is rapidly removed by washing the column with THF.

4. Conclusions

Anthracene has proved to be successful in anchoring the chiral selector **I** to PGC, yielding a satisfactory carbon-based CSP. Superior chiral separations have been demonstrated on this PGC-based CSP than on the corresponding silica-based CSP **II** in SFC and the resolution of unprotected, acidic drugs, e.g., ibuprofen, has been achieved.

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