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Note

Liquid chromatography on triacetylcellulose*

Preparative separation of enantiomers on an axially compressed column

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Separation of enantiomers on a scale of a few hundred milligrams has been described for only a few sorbents, probably because at least 100 g of stationary phase are required. Synthetic sorbents based on silica (40 and 58 μm) containing optically active amino acids[3] have been used. One such material is available commercially, but its high price shows that it costs a lot to prepare. This is also true for optically active polyacrylamides[4]. Lactose hydrate[5], starch[6], cellulose bearing 2.5 acetyl groups per D-glucose unit[7], and triacetylcellulose[8] are based on cheap natural materials and have also served for preparative liquid chromatography in some cases. As the times required for separations on triacetylcellulose are considerable (*ca.* 15–50 h per run)[8], we wished to optimize the linear velocity, *i.e.* time of separation, particle size, and column loading for this versatile[1] sorbent.

EXPERIMENTAL

For the preparative separations, 130 g of triacetylcellulose (20–30 μm), corresponding to columns B and C in ref. 9 except for particle size, were packed by axial compression[10] at 8 bar into the column (40 mm I.D.) of a Chromatospac Prep 10 instrument (Jobin Yvon, Longjumeau, France). A length of 243 mm resulted. The column was used at 23°C at an eluent pressure of *ca.* 3 bar, ethanol–water (96:4) being the eluent and volumes of 9 ml being injected. A dead volume of 191 ml was determined by means of 1,3,5-tri-*tert.*-butylbenzene as a non-retained substrate[9]. Absorbance at 254 nm was monitored by a Gilson Spectrochem M detector (Abimed Analysentechnik, Düsseldorf, F.R.G.).

Details of the analytical measurements are given in Figs. 1 and 2.

The substrates were: (±)-N,N,2,3,4,6-hexamethylthiobenzamide[9], (±)-1-(9-anthryl)-2,2,2-trifluoroethanol (1) from Aldrich (Milwaukee, WI, U.S.A.), and (±)-2-methyl-3-(2′-methylphenyl)-4(3H)-quinazolinone[9] (2).

* Part 12. For Part 11, see ref. 1. For Part 10, see ref. 2.

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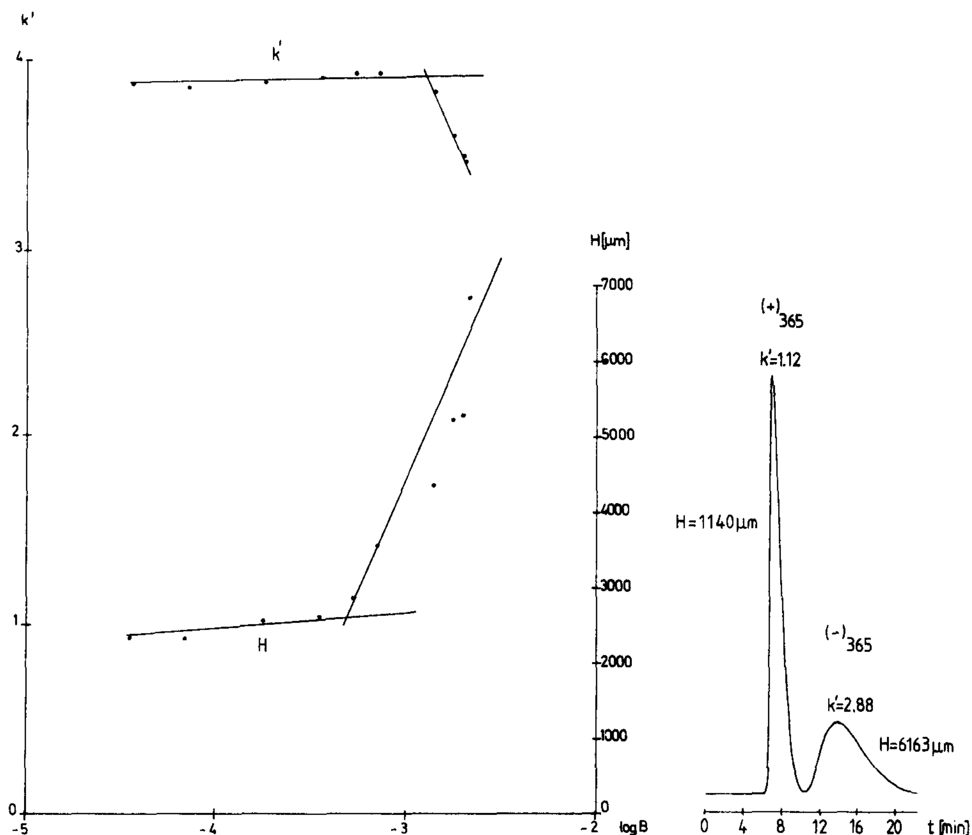


Fig. 1. Loadability of triacetylcellulose by $(+)_{436}$-N,N,2,3,4,6-hexamethylthiobenzamide, which is the more retained of the enantiomers (*cf.* ref. 9). Capacity factor, k', and plate height, H, are given as functions of log B, where B represents the load, *i.e.* weight in grams of substrate per 5.5 g of triacetylcellulose (8–15 μm). Column, 250 × 8 mm I.D.; eluent, ethanol–water (96:4); flow-rate, 4 ml/min; dead volume, 7.53 ml; temperature, 23°C.

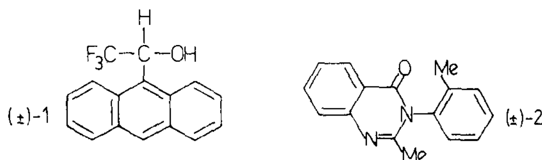
Fig. 2. Chromatogram of 15 μg of (±)-2. Sorbent, triacetylcellulose (8–15 μm); column, 250 × 8 mm I.D.; eluent, ethanol–water (96:4); flow-rate, 2 ml/min; dead volume, 7.12 ml; temperature, 23°C, absorbance at 254 nm; α = 2.57; R_s = 1.16.

RESULTS AND DISCUSSION

To begin with, we investigated the loadability of triacetylcellulose. The capacity factor and the plate height both remained nearly constant up to a load B (weight of substrate per weight of sorbent) of *ca.* $5 \cdot 10^{-4}$, *i.e.* log $B = -3.3$ in Fig. 1, if the plate height is considered. This corresponds to 0.5 mg of substrate per gram of sorbent (*cf.* ref. 9). Higher loads generated the expected decrease of k' and increase of H.

The quality of the separation is also sensitive to the linear velocity, u, and its influence on the plate height, *i.e.* the Van Deemter relationship. For triacetylcellulose[1,9], minimum plate heights were found to occur at *ca.* u = 0.1 mm $\sec^{-1}$. The

particle size is also significant[1], and we chose the range 20–30 μm, a compromise between the accessibility of large amounts and the performance of the sorbent.



Our preparative separations of the enantiomers of (±)-1 and (±)-2 (Table I) were performed taking into account the above conditions. Analytical chromatograms (see ref. 1 and Fig. 2, respectively) helped us to decide the elution time at which the two fractions should be fractionated. For instance, an overload of (±)-1 (log B = −2.72) was cut between the two peaks, at the minimum of the detection curve, thus yielding pure enantiomers (Table I, first line). On strong overloading (log B = −2.11), fractionation was performed after the minimum of the detection curve, which still resulted in a pure second enantiomer (Table I, second line). The enantiomers (+)- and (−)-1 have also been separated preparatively on sorbents[3] derived from optically active amino acids. The compound (±)-2 is a non-planar heterobiaryl, the racemate (methaqualone) and the enantiomers[11] of which exhibit unequal anticonvulsive activity. Its enrichment (Table I) was performed by overloading and cutting at the curve minimum.

TABLE I

PREPARATIVE SEPARATIONS

B is the load, *i.e.* the weight in grams of substrate per 130 g of triacetylcellulose; u is the linear velocity; t is the time required for separation; P is the enantiomeric purity.

Substrate	*Weight (mg)*	*log B*	*u (mm sec⁻¹)*	*t (min)*	*First fraction*		*Second fraction*	
					Weight (mg)	*P (%)*	*Weight (mg)*	*P (%)*
(±)-1	250	−2.72	0.11	190	115	100*	133	99*
(±)-1	1000	−2.11	0.10	220	815	7*	182	99*
(±)-2	250	−2.72	0.11	190	97	87**	152	56**

* Determined by analytical chromatography (cf. ref. 1).

** Determined by polarimetry, using $[\alpha]^{21}_{365}$ = 675° ml g^{-1} dm^{-1}, based on ref. 13.

We used a commercial preparative chromatograph with axial compression[10,12] in preference to a normal glass column because such instruments are available in many organic chemistry laboratories and result, in our experience, in easy and reproducible packing, a condition that is not always met by conventional preparative columns.

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