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HIGH-PRESSURE LIQUID CHROMATOGRAPHY ON TRIACETYLCELLULOSE

CHARACTERIZATION OF A SORBENT FOR THE SEPARATION OF ENANTIOMERS*

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SUMMARY

Microcrystalline triacetylcellulose (d_p = 10–20 μm) was used for analytical liquid chromatography at pressures around 50 bar. 1,3,5-Tri-*tert.*-butylbenzene is proposed as a compound which is not retained on this sorbent. Thus, reliable information about porosity, linear flow-rates, u, and relative retentions become available for the first time. An attempt was made to obtain the plate height, H, dependence upon u in ethanol. For three racemates, comparable data with four eluents were obtained and discussed with a view to future separtions of enantiomers. A general increase of H values of the enantiomers with increasing k' is found for triacetylcellulose as a sorbent.

INTRODUCTION

Microcrystalline swollen triacetylcellulose[2] is capable of separating enantiomers belonging to many different classes of organic compounds, *e.g.*, refs. 3–10, including materials devoid of aryl substituents and molecules lacking reactive groups, *e.g.*, hydrocarbons[1,11–13]. All of these studies were carried out with relatively large columns, large particles and low pressures, because the predominant motivation was the semipreparative separation of enantiomers. These conditions result in considerable consumption of time per chromatographic analysis, which, in turn, did not represent an encouragement to characterize the sorbent and to optimize the separations at low pressures.

In 1980 we showed[14] that triacetylcellulose can be obtained in particle sizes d_p = 5–10 μm and, contrary to published[3,15,16] opinions, can be used successfully at pressures of at least 100 bar. To our knowledge, no further publication on small-particle microcrystalline triacetylcellulose has appeared. As will be described, a non-retained compound was not known. This lack prevented, *inter alia*, the determination of linear flow-rates and the calculation and comparison of relative retentions. As far as eluents are concerned, some semiquantitative results obtained by Hesse and Hagel[2,11] led to the predominant use of ethanol–water. Therefore, we

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* Liquid Chromatography on Triacetylcellulose, Part VII. For Part VI, see ref. 1.

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decided to utilize our finding[14] of pressure-stability for a less time-consuming and more quantitative characterization of triacetylcellulose, including the use of other eluents.

EXPERIMENTAL

Microcrystalline triacetylcellulose, prepared and milled as described[3], was air-separated (Zickzacksichter A 100 MZR; Alpine, Augsburg, F.R.G.) and 2.5 g of the fraction with d_p = 10–20 μm (measured under a microscope) were swollen and slurry-packed with ethanol–4% water, as described[3], into a steel column (250 × 4.6 mm) at 40 bar (column A). Triacetylcellulose, received from I. Vanden Eynde (Union Chimique Belge, Leuven, Belgium) was milled and air-separated in the same way and 8 g of this material, d_p = 10–20 μm, were packed into a steel column (250 × 8 mm) at 110 bar (column B). Column C was prepared in exactly the same way.

The eluents were distilled before use, except methyl *tert.*-butyl ether (for HPLC; Fluka, Buchs, Switzerland). The exchange of eluents was performed after packing with ethanol–4% water.

The Model 6000 A pump, Model U 6K injector and Model 440 absorbance detector (254 nm), were all from Waters (Königstein, F.R.G.). Separations were carried out at 22°C. Column A showed Δp $\approx$30 bar at 0.5 ml/min; columns B and C showed $\approx$70 bar at 2 ml/min.

1,3,5-Tri-*tert.*-butylbenzene (1), m.p. 72–74°C, served as a non-retained compound. Its elution volumes in ethanol–4% water were 2.05 ml (column A), 7.45 ml (column B) and 7.56 ml (column C).

The solutes were: (±)-2,4-di-*tert.*-butyl-5,7-dimethylphenanthrene (2)[1], m.p. 90–91.5°C; (±)-N,N,2,3,4,6-hexamethylbenzenecarbothioamide (3)[17], m.p. 68–70°C, a small impurity was recognized by liquid chromatography (LC) only (Fig. 1); (±)-1,3,6-trimethyl[5]helicene (4)[1], m.p. 171–172.5°C; (±)-2-methyl-3-(2′-methylphenyl)-4(3H)-quinazolinone (5)[18], m.p. 116–117°C.

RESULTS AND DISCUSSION

The dependence of the volume flow-rate, *F*, of ethanol–4% water upon pressure, Δp, was determined for our triacetylcellulose column A and found to be linear up to at least 0.6 ml/min. At higher flow-rates pressures could not be measured accurately with our manometer.

Hesse and Hagel[3] recommended an extract of green leaves for the determination of V_0, the elution volume of a non-retained compound, in ethanol–4% water as eluent. However, they did not give further details. According to Schlögl and Widhalm[7] the elution volume determined by this method[3] depends upon the temperature and thus does not represent V_0. We have found that the 1,3,5-tri-*tert.*-butylbenzene (1) peak on triacetylcellulose appears earlier than the first peak of a green leaf extract. Our assumption that compound 1 is not reatained is supported by our finding that ethyltriphenylphosphonium bromide and benzylethylmethylphenylammonium methylsulphate exhibit elution volumes identical with that of this compound. The V_0 values on our columns are given in the Experimental. From these values we calculated the porosity, $\varepsilon_T = V_0/V$, of the sorbent, where V is the volume of the empty column.

The results are $\varepsilon_T = 0.5$ for column A and $\varepsilon_T = 0.6$ for columns B and C. The last two columns contained identical material. This material and that in column A originated from different sources, but their particle sizes were the same (d_p = 10–20 μm). The above results for ε_T indicate porosities for our sorbent which are roughly intermediate between the extreme values[19] of ε_T = 0.42 and 0.84.

Column B was used to obtain some information about the load capacity of the sorbent. The capacity factors, k'_1 and k'_2, as well as the plate heights* H_1 and H_2, of (+)- and (−)-3 (see Table III for structure) in ethanol–4% water remained roughly constant up to a load of $4 \cdot 10^{-4}$ g of (±)-3 per g of sorbent. Higher loads generated the expected decrease of k' and increase of H. Thus the capacities of triacetylcellulose, of silica[19,20] and of reversed-phase sorbents[20] are of similar magnitude.

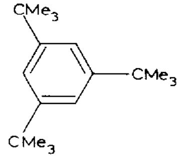
The influence of the linear velocity, u, upon plate height, H, was examined by using the non-retained compound 1 (Table I). The usual decrease of H upon decreasing u was observed. Some indication of the expected minimum plate height was detected around 0.1 mm/sec; unfortunately, the type of pump used for this study was unable to generate flow-rates lower than 0.04 mm/sec. H = 37 μm (Table I) corresponds to a reduced plate height of roughly 2.5, indicating a suitable sorbent and a well-packed column. Under identical conditions, the enantiomers of the helical hydrocarbon 2 are separated and show an analogous decrease in plate heights, H_1 and H_2, upon decreasing u (Table II). Therefore, the resolution, R_s, is highest at u = 0.10 mm/sec. The plate height H_2 of the enantiomer which is eluted last was found to be higher than H_1 for the early enantiomer (Table II). We shall see below that this is a general observation for LC on triacetylcellulose.

The experiments with different eluents (Table III) were carried out by using the enantiomers of the following substrates: helicene (4)[1], a further representative of

TABLE I

INFLUENCE OF FLOW-RATE UPON PLATE HEIGHT

Column B; 20 μg of compound 1 injected; eluent ethanol–4% water.



1

u(mm/sec)	*H(μm)*
2.2	227
0.57	82
0.28	56
0.22	49
0.16	44
0.10	37
0.04	38

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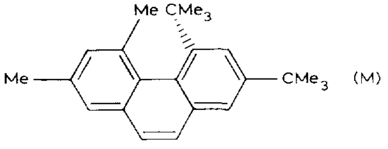
* All plate heights were determined via widths at half-height, all resolutions via base-widths.

TABLE II

INFLUENCE OF FLOW-RATE UPON CHROMATOGRAPHY OF (–)- AND (+)-2

Column B; 10 μg of (±)-2 injected; eluent ethanol–4% water. Capacity factors, k'_1 and k'_2, and plate heights, H_1 and H_2, refer to $(-)_{546}$- and $(+)_{546}$-2, respectively. See Table I for corresponding plate heights of non-retained 1.



(ref. 26)

2

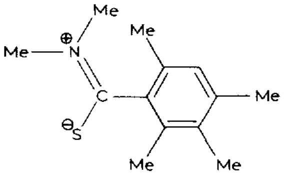
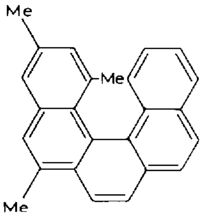
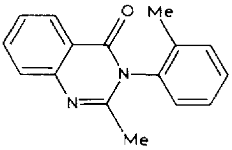
u(mm/sec)	k'_1	k'_2	α	H_1(μm)	H_2(μm)	R_s
0.57	0.15	0.36	2.4	210	≈1350	≈0.7*
0.22	0.21	0.45	2.1	123	980	1.1
0.10	0.17	0.42	2.5	109	576	1.2

* Partial overlap of the two peaks.

TABLE III

INFLUENCE OF ELUENTS UPON CHROMATOGRAPHY OF ENANTIOMERS

10 μg of substrate injected; column C; $F = 2$ ml/min. Capacity factors, k'_1 and k'_2, and plate heights, H_1 and H_2, refer to enantiomers eluted early or late, respectively.

Substrate	Eluents	k'_1	k'_2	α	H_1(μm)	H_2(μm)	R_s
 (±)–3	Methanol	0.35	0.71	2.0	102	255	1.9
	Ethanol–4% water*	0.77	4.26	5.5	568	1605	3.4
	Isopropanol	1.44	6.29	4.4	1312	≈3600	2.1
	Methyl *tert.*-butyl ether	0.92	1.65	1.8	1059	1947	1.0
 (±)–4	Methanol	0.66	1.03	1.6	328	445	1.1
	Ethanol–4% water*	0.77	1.22	1.6	912	1445	0.8
	Isopropanol	1.54	2.53	1.6	2079	≈3000	≈0.7**
	Methyl *tert.*-butyl ether	0.39	0.59	1.5	385	≈ 700	≈0.7**
 (±)–5	Methanol	0.90	2.50	2.8	340	4110	1.6
	Ethanol–4% water*	1.08	2.63	2.4	804	8000	1.0
	Isopropanol	2.94	4.26	1.5			<0.6**
	Methyl *tert.*-butyl ether	3.71	4.76	1.3	800 –1000	1900 –2400	<0.6**

* Orders of elution: $(-)_{436}$-3 before $(+)_{436}$-3; $(-)_{436}$-4 before $(+)_{436}$-4; $(+)_{365}$-5 before $(-)_{365}$-5.

** Partial overlap of the two peaks.

helical hydrocarbons; thiobenzamide (3)[17], containing two non-coplanar π-systems[9]; quinazolinone (5)[9,18], a heterocyclic non-planar biaryl, the racemate (methaqualone)[21] and the enantiomers[22] of which exhibit hypnotic and anticonvulsive activity. If we assume that the order of elution of the two enantiomers is not reversed by a change of eluent, the k' values of all six molecules increase on changing from methanol to ethanol to isopropanol (*cf.*, Fig. 1). This sequence is supported by qualitative statements concerning Tröger's base[2] and mandelic acid[11], and may prove useful for future analytical and preparative applications. It is identical to the sequence found in adsorption liquid chromatography[20]. However, this does not necessarily mean that the polarity of the eluents is decisive, because their elution power may be determined by their steric size if sorption operates by inclusion[3] of substrate molecules into the cavities or channels of triacetylcellulose. The capacity factors determined by using methyl *tert.*-butyl ether as a solvent (Table III) do not fit into the above sequence.

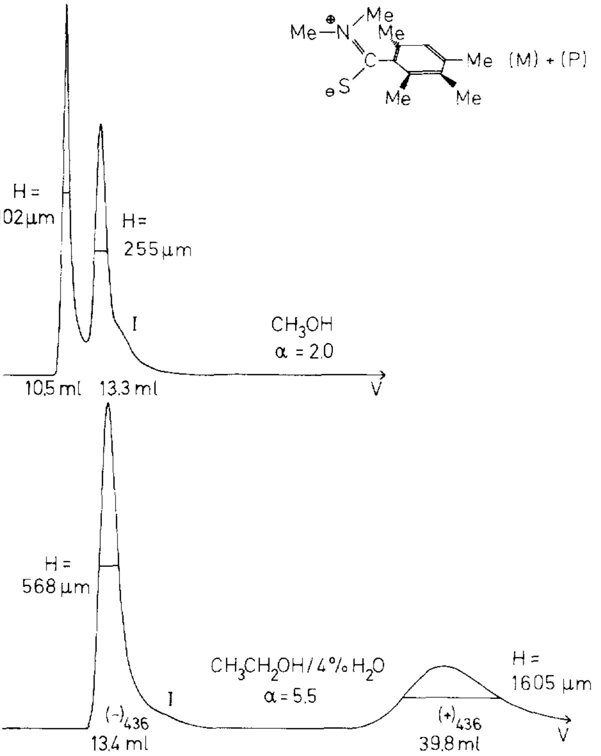


Fig. 1. Chromatograms of 10 μg of compound (MP)-3 (ref. 26) on column C. Eluents: methanol (top) and ethanol–4% water (bottom); F = 2 ml/min. Absorbance at 254 nm plotted *vs.* V, the volume after injection. I = Unknown impurity in 3.

As observed for the enantiomers of compound 2 (Table II), all plate heights, H_2, in Table III are higher than their corresponding H_1 values. In addition, the size of this difference varies for different compounds. The behaviour of other racemates[13] shows this phenomenon to be a general one for triacetylcellulose. For several sorbents there are cases of a decrease, of constancy and of an increase of the plate height with increasing capacity factor. Among the last cases, we found two different publications[23,24] in which the higher k' value corresponded to the higher H value and where retention was most probably brought about by inclusion. Sorption on triacetylcellulose may be accomplished by inclusion; slow sorption or desorption, *i.e.*, mass transfer, may generate the observed $H(k')$ dependence[25]. Further data are needed to confirm these suggestions.

As far as the resolutions (Table III) are concerned, R_s for compound 3 is indeed highest in ethanol–4% water, as may be concluded from the preference[2,11] for this eluent. However, this is not a general rule, as may be seen from compounds 4 and 5, which are best resolved in methanol.

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