Journal of Chromatography, 167 (1978) 171–186

CHROM. 11,450

PREPARATION OF CHROMATOGRAPHIC SORBENTS WITH CHIRAL CAVITIES FOR RACEMIC RESOLUTION*

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SUMMARY

A procedure is described for preparing chromatographic sorbents with high specificity for particular substances. For this purpose, with the aid of a chiral template molecule functional groups were placed in a highly crosslinked polymer in such a way that they were present in a chiral cavity in a given stereochemistry.

For example, 4-nitrophenyl-α-D-mannoside-2,3:4,6-di-O-(4-vinylphenylboronate) (1) was copolymerized to a macroporous polymer, from which the template 4-nitrophenyl-α-D-mannopyranoside (2) could be split off. These polymers were used for the chromatographic resolution of the racemate of the template molecule 2. A high specificity for this separation, with separation factors up to 2.32, was obtained. The optical yield of the separation reached 87%.

The number of theoretical plates for this type of column is low. Slow kinetics of the exchange equilibria and some unusual properties of the chromatographic system were observed. Apparently a peculiar separation mechanism is operative.

INTRODUCTION

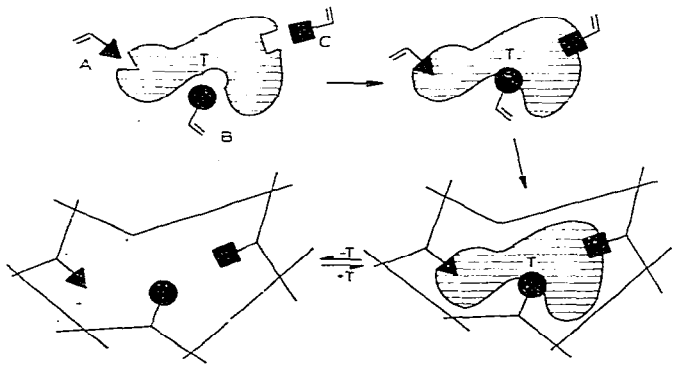
In chromatography, it is of considerable interest to prepare sorbents with a high specificity for particular substances. This type of chromatography has been called affinity chromatography[1,2]. The most specific sorbents can be obtained by binding receptors of biological systems, *e.g.*, antibodies or enzymes, to polymers. These sorbents are highly specific for the antigens of the antibodies or the substrates of the enzymes. The disadvantage of such systems is that they are liable to be modified by chemicals, solvents, temperature, etc. Moreover, only for a limited number of substances are specific receptors known that can be isolated in a pure state.

With a view to preparing more stable synthetic polymers having specific receptor structures, we tried to construct cavities of specific shape containing functional groups with binding ability. The functional groups should possess a stereochemically exact defined arrangement (discontinuate word arrangement)[3]. The

* Enzyme-analogue Built Polymers, Part VIII. For Part VII, see G. Wulff and J. Schulze, *Angew. Chem.*, 90 (1978) 568; *Angew. Chem. Int. Ed. Engl.*, 17 (1978) 537.

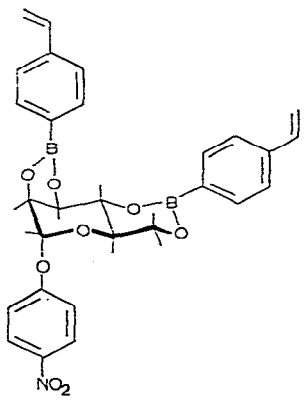
remainder of the polymer is less specific in its function and serves to support the structure of the cavities, in a similar manner to the function of the biopolymer in enzymes and antibodies.

We have previously described a new approach for the preparation of such cavities containing functional groups by polymerization in the presence of template molecules[3–7]. For that purpose the functional groups to be introduced (A, B and C in Scheme 1) were bound in the form of polymerizable vinyl derivatives to a suitable template molecule. This monomer was then copolymerized with a crosslinking comonomer in the presence of an inert solvent to obtain a macroporous polymer with good accessibility to the template. The polymer was highly crosslinked with chains in a fixed arrangement. After removal of the template a polymer was formed with cavities stamped by the template and with functional groups in a fixed stereochemistry that corresponded to the chemical structure of the template (see Scheme 1).



Scheme 1.

The exact arrangement of the functional groups within the cavities was confirmed by the ability of the binding sites to distinguish between enantiomers. Thus one antipode of a chiral template was used for the preparation of the polymer and the difference in the binding ability for the two enantiomers of the template was



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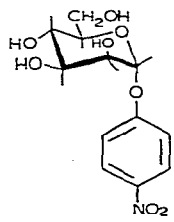
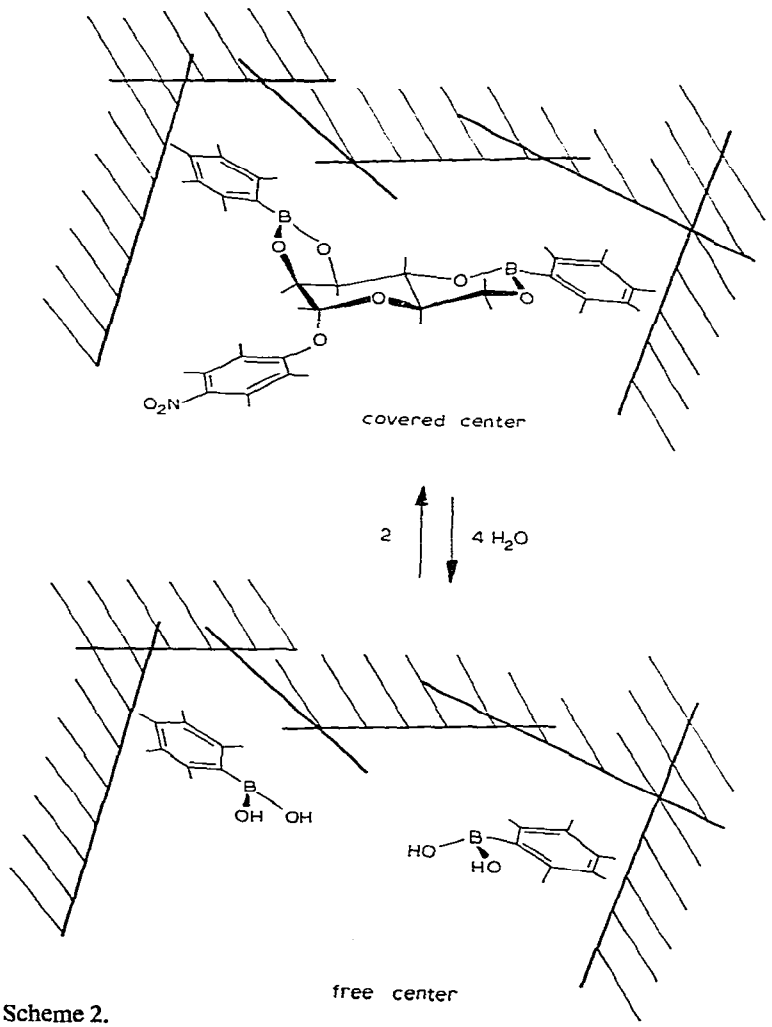


2

measured. During the polymerization the interaction between the template and the functional groups should be as strong as possible and covalent bonds should be chosen. The interactions have to be much weaker if the same functional groups are to be used in a chromatographic process. In this instance the functional groups should undergo an easily reversible interaction with the substrate. Further, it should be possible to influence the position of the equilibrium over a wide range. An example that fulfils these critera to a certain extent is the esterification equilibrium between boronic acids and diols. Therefore, as an example of the new principle, *p*-nitrophenyl-α-D-mannopyranoside (2) was chosen as a template. It was esterified with 2 mole of *p*-vinylphenylboronic acid to yield (1)[6]. This monomer was then copolymerized to a macroporous polymer.

After removal of the templates, the free boronic acids should be retained in a stereospecific arrangement within the chiral cavities formed (see Scheme 2). This can



Scheme 2.

be tested by determining the ratio of the amounts of the two enantiomers of the template embedded in the cavities of the polymer. By equilibrating in the batch procedure in suitable solvents, it was shown that these polymers were able to resolve the racemates of the template[6]. In this paper, the use of these polymers for the chromatographic separation of the racemates of 2 is described. In particular, we discuss how the particular separation mechanism influences the chromatographic behaviour.

EXPERIMENTAL

Preparation of polymers[6]

For the preparation of the polymers commercial divinylbenzene was used. After being distilled in a nitrogen atmosphere it contained, according to gas chromatographic analysis, 18.1% of *p*-divinylbenzene, 35.8% of *m*-divinylbenzene, 11.8% of 4-ethylstyrene, 29.2% of 3-ethylstyrene and 4.6% of diethylbenzenes, naphthalene, etc. This mixture of divinylbenzenes was copolymerized with 1 and styrene in the presence of an inert solvent with azobis(isobutyronitrile) (0.7% of the monomeric mixture) as initiator for 4 days at 80°.

For the preparation of polymer Z, a mixture of divinylbenzene enriched with the *p*-isomer[8] was used. The composition was 73.2% of *p*-divinylbenzene, 14.8% of *m*-divinylbenzene, 4.1% of 4-ethylstyrene and 5.4% of 3-ethylstyrene. After polymerization, the polymer was milled (Janke-Kunkel A10 or Alpine Contraplex 63C mill) and wet sieved under acetonitrile. Polymer with a grain size of 32–45 μm (in the swollen state) was used for chromatography.

Chromatography

Columns were filled by the slurry packing method under pressure in methanol as solvent. Thick-walled glass columns of length 200 mm and I.D. 4 mm equipped with a water-jacket (Quickfit) were filled with approximately 0.6 g of polymer at pressures up to 100 bar.

The chromatographic system consisted of a Waters Model 6000 high-performance liquid chromatography (HPLC) pump, and a UV detector with variable wavelength (Zeiss PM 2 DLC). The detector was used at the absorption maximum of 2 (λ_{max} = 300 nm, log ε = 4.07). The template of the polymer packing was split off at 50° with methanol containing 5% of water during 20 h at a pumping rate of 0.4 ml/min. The eluents used for chromatography and the conditions applied are given in Table III. For very low pumping rates, the control electronics of the Waters Model 6000 pump were modified so that the original pumping rate could be switched down to one fifth, *i.e.*, to 0.02 ml/min. The retention volumes were obtained by injecting the D- and L-forms of 2 separately. The dead volume (V_0) was estimated by injecting acetone, as it gave nearly the same retention volume as 2 in methanol–water (9:1), where no boronic esters can be formed.

For the gel permeation chromatographic (GPC) experiments the polymer was swollen in tetrahydrofuran (THF) and slurry packed as described above. Polystyrene standards were supplied by Waters Assoc. (Milford, Mass., U.S.A.) or Knauer (Oberursel, G.F.R.). The effective diameter of the polymer coils used in Fig. 3 is twice the diameter of an equivalent hydrodynamic sphere, which can be calculated[9] from Flory's equation and the Mark–Houwink parameters[10].

The volume of the whole chromatographic system was 2.7 ml, the interstitial

volume was 0.9–1.6 ml and the volume of solvent imbibed in the pores of the column packing was 0.7–1.4 ml.

Electron micrography was carried out by Dr. A. Maas (Study Group for Solid State Surfaces. University of Bonn). The samples for the scanning electron microscope (S4 Stereoscan, Cambridge Scientific Instruments) were coated with carbon and 30 nm of gold in an E5000 SEM coating unit (Polarson Equipment).

The polymers for the EM 300 transmission electron microscope (Philips) were treated with lead acetate to give a better contrast. For this purpose the polymers were impregnated under vacuum with a solution of lead acetate in glacial acetic acid and dried. The microscopic objects were prepared by scraping off small pieces from the polymer grain. The polymer A3 in Fig. 2 originates from a different series of tests, which was based on methyl methacrylates. The monomer mixture had the composition 51.0% of ethylene dimethacrylate, 23.3% of methyl methacrylate and 25.7% D-mannitol-1,2;3,4;5,6-tri(4-vinylphenylboronate).

The inner surfaces of the polymers were measured according to the B.E.T. method; the swellability factor was determined, as described earlier[6], from the specific bulk volume and the specific gel bed volume.

RESULTS AND DISCUSSION

Characterization of the sorbents

Sorbents with cavities of specific shape and with functional groups in a "discontinuate word" arrangement should possess the following properties:

(i) The shape of the cavity and the arrangement of the functional groups should be preserved after splitting off the template. For this, extensive crosslinking and a low swellability are desirable.

(ii) The cavities and the functional groups therein should have some degree of flexibility to ensure a fast uptake and release of the template in the cavity in an equilibrium reaction. This requirement is contradictory to (i).

(iii) It should be possible to remove a high proportion of the template after the polymerization. Therefore, good accessibility to the cavities is necessary. In particular, this is necessary during the chromatographic process that should not be hindered by slow diffusion processes.

(iv) The sorbent should be mechanically stable so that it can be used in chromatographic columns under pressure.

A rigid and mechanically stable polymer can be obtained by introducing a high degree of crosslinking. If the polymerization is carried out in the presence of an inert solvent, macroporous polymers with large inner surface areas and good accessibility are obtained. According to the results of Millar[11] and Häupke and Hoffmann[12], 40–50% of crosslinking agent in the monomeric mixture and a ratio of monomer to solvent of 1:1 yield polymers with considerable macroporosity. As we had to use other solvents for solubility reasons and the monomer 1 acted as a crosslinking agent as well, the results in the literature could not be adopted directly. Moreover, it could not be foreseen how the polymers would behave in a chromatographic process, where for kinetic reasons some flexibility is necessary. Therefore, polymers E–Q (see Table I) with different compositions were prepared; in particular, the amount of crosslinking agent was varied (L–Q).

TABLE I

COMPOSITION OF THE POLYMERIZATION MIXTURE FOR THE PREPARATION OF POLYMERS E–Q

Polymer	*Composition of polymer mixture*				*Volume of solvent per gram of monomer mixture (ml)*	
	2 (%)	*mmole of 2 per gram of mixture*	*DVB* (%)*	*Styrene (%)*	*Benzene*	*Acetonitrile*
E	16.7	0.317	45	–	0.50	0.50
I	16.7	0.317	45	–	1.00	–
K	16.7	0.317	45	–	–	1.00
F	11.1	0.212	48	–	0.50	0.50
G	5.6	0.106	51	–	0.50	0.50
H	5.0	0.095	51.3	–	0.50	0.50
L	5.6	0.106	10	75.9	0.50	0.50
M	5.6	0.106	20	57.4	0.50	0.50
N	5.6	0.106	30	38.9	0.50	0.50
O	5.6	0.106	40	20.4	0.50	0.50
P	5.6	0.106	50	1.8	0.50	0.50
Q	5.6	0.106	80	5.9	0.50	0.50

* DVB = commercial divinylbenzene mixture.

The polymers thus obtained, with the exception of L and M, show the typical structure of macroporous polymers (I shows only swelling porosity). The scanning electron micrograph for polymer G (Fig. 1) shows that it consists of primary particles grown together with diameters of 100–200 nm. Between these particles, a system of pores (macropores) is formed, which can be seen in the transmission electron micro-

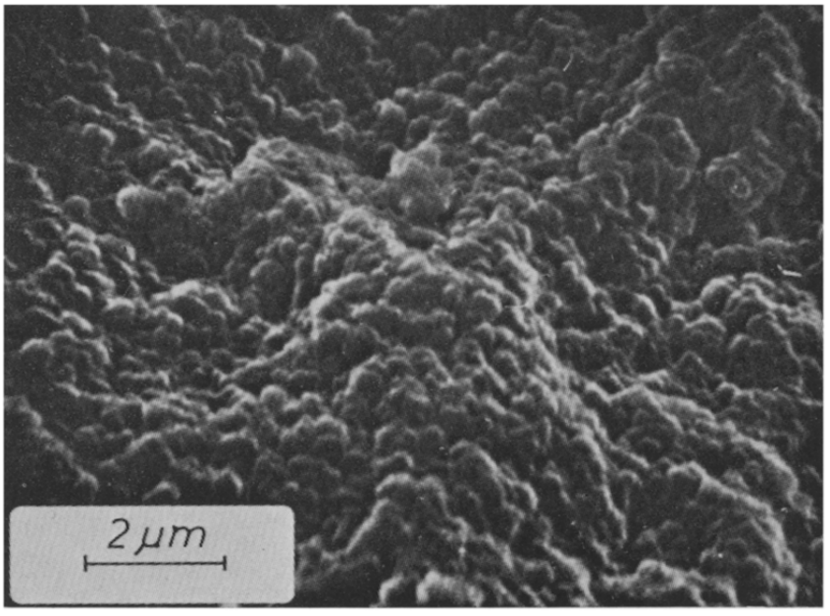


Fig. 1. Scanning electron micrograph of polymer G. The sample was coated with carbon and 30 nm of gold. Magnification 10,600 ×.

graph (Fig. 2). A better insight into the porous structure of the polymers, especially in the swollen state, can be obtained from GPC. Fig. 3 shows the usual calibration graphs obtained with polystyrene standards for a series of polymers. With an increasing degree of crosslinking the molecular weight of the exclusion limit also increases. The size of the largest macropores is indicated by the effective diameter of a polymer coil with the molecular weight of the exclusion limit. As can be seen, with small amounts of crosslinking agent only small pores (< 5 nm) are present, whereas with larger amounts more and more macropores (up to 50 nm) are formed. Polymers L and M are not macroporous, and only micropores exist.

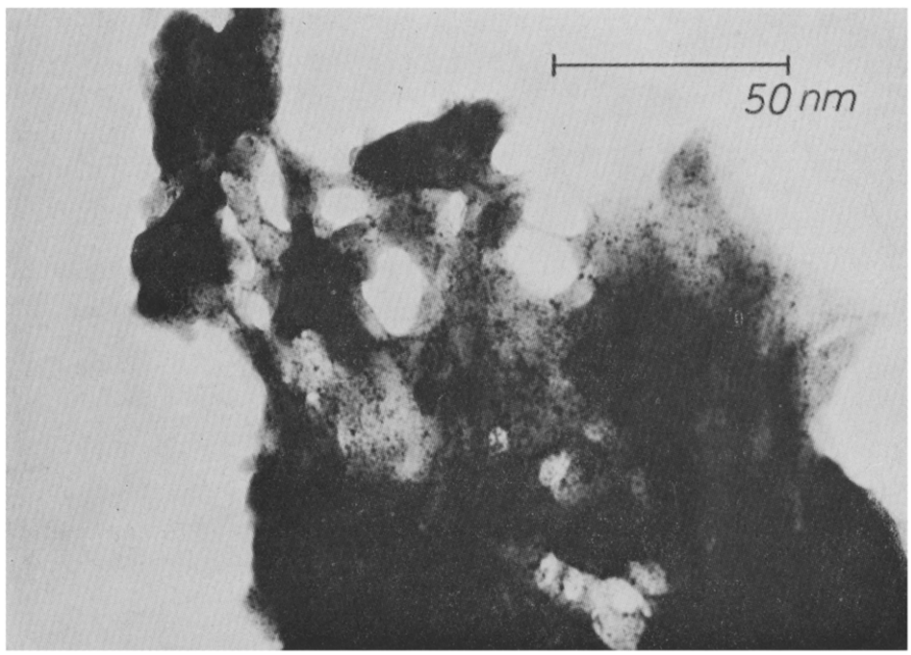


Fig. 2. Transmission electron micrograph of polymer A3. The sample was treated with lead acetate for a better contrast. Magnification 670,000 : 1.

For the accessibility of a macroporous polymer both the pore structure and the inner surface area are determining factors. As in all instances of higher crosslinking high inner surface areas (112–625 m^2/g) (see Table II) are present and nearly all pores are penetrable for the template 2, sufficient accessibility seems to be reached.

The pore diameters depend on the degree of swelling of the polymers. GPC was performed in tetrahydrofuran as solvent and the separation of the racemates by column chromatography with methanol as eluent. Therefore, it is important to know the degree of swelling in both solvents. Further, the degree of swelling indicates the rigidity of the arrangement in the cavities.

Information on the swellability could be obtained from the proportion of the specific gel bed volume in the corresponding solvent to the bulk volume according to Heitz and Platt[13] (see Table II). Fig. 4 shows these ratios for methanol and tetra-

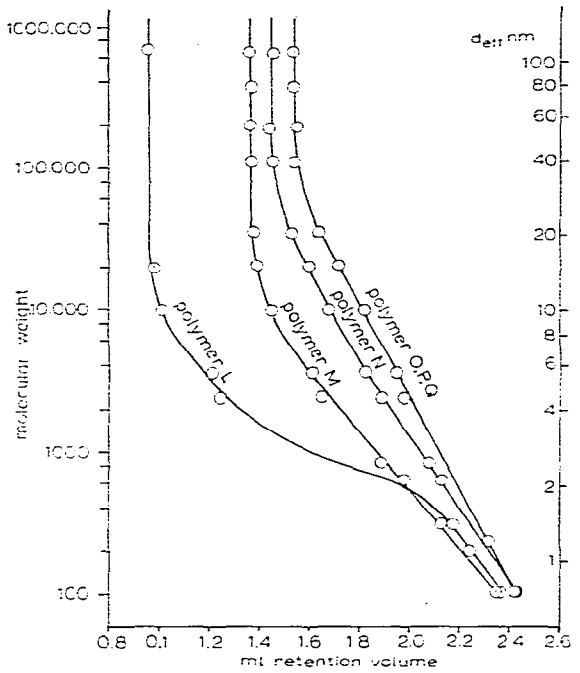


Fig. 3. GPC calibration graphs for polymers L–Q in THF with polystyrenes of different molecular weight. On the right-hand ordinate the effective diameters of the polymer coils are given.

TABLE II

PROPERTIES OF THE POLYMERS

Polymer	*Specific surface area (m^2/g)*	*Specific bulk volume (ml/g)*	*Specific gel bed volume in methanol (ml/g)*	*Swellability factor in methanol*
E	112	1.82	3.26	1.79
I	<5	1.80	2.93	1.63
K	212	3.22	4.35	1.35
F	19	2.03	4.07	2.00
G	227*	2.31	4.22	1.83
H	199	1.90	4.01	2.11
L	<5	1.59	1.60	1.02
M	<5	1.48	1.62	1.10
N	197	2.05	3.54	1.72
O	375	2.17	4.39	2.02
P	455	2.22	4.48	2.02
Q	620	2.75	4.11	1.50

* New measurements yielded this value, which is different from that reported earlier[6].

hydrofuran as a function of the degree of crosslinking. Polystyrene is soluble in tetrahydrofuran and with increasing degree of crosslinking of the polymers the swellability decreases. On the other hand, polystyrene is insoluble in methanol and in this solvent the swellability increases with increasing degree of crosslinking in the polymer in the region of 10–40% of crosslinking agent and at higher percentages it decreases

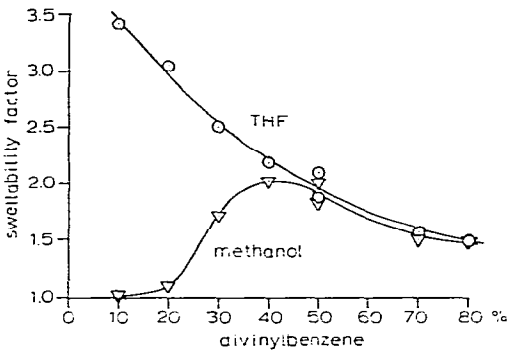


Fig. 4. Dependence of the swellability factors in methanol and THF on the amount of crosslinking agent in polymers L–Q.

again. This swellability is connected with the inner surface area of the polymers and can be explained by solvation of the functional groups within the cavities. With a higher degree of DVB the effect of the increasing inner surface area is overcompensated by the strong rigidity of the polymer.

Chromatographic results

The polymers were sieved to a grain size of 32–45 μm and filled by the slurry packing method into the column of an HPLC set-up. With methanol–water (95:5) the boronic diester bonds were hydrolysed and the templates were split off: approximately 80% of the templates could be removed. Complete removal of the templates cannot be expected with macroporous polymers, as the dense, highly crosslinked parts (the so-called nuclei[14]) formed during the heterogeneous polymerization are not fully penetrable. If the water content of the methanol is reduced, it is possible to adjust the equilibrium between the template and the boronic acids in the cavities in such a way that on chromatography with the template relative retentions (k') between 3 and 10 are reached. Under these conditions, mainly a trans-esterification between boronic dimethyl esters and the templates occurs[6].

For a precise determination of the racemic resolution both enantiomers of the template were injected separately. The ratio of the relative retentions for the D- and L-forms is expressed by the separation factor, α:

$$\alpha = \frac{k'_D}{k'_L} = \frac{t_D - t_0}{t_L - t_0} = \frac{V_D - V_0}{V_L - V_0}$$

where t_D = retention time of the D-form, V_D = retention volume of the D-form, k'_D = relative retention of the D-form, t_L, V_L and k'_L are corresponding properties for the L-form and V_0 = dead volume of the column. The separation factor may serve as a measurement of the specificity of the arrangement within the cavities.

Table III shows some results of the chromatographic investigation of the polymers. It can be seen that the α values, *i.e.*, the specificity of the sorbents, are high, ranging from 1.05 to 2.32. The specificity is strongly dependent on the degree of crosslinking, as can be seen from Fig. 5. Polymers with a low degree of crosslinking show no specificity. The specificity increases with increasing degree of crosslinking.

TABLE III

CHROMATOGRAPHIC RESULTS

Polymer	Eluent composition (%)*				Flow-rate (ml/min)	Column temperature (°C)	V_0 (ml)	Retention volume (ml)		Separation factor, α	Number of theoretical plates		Optical yield (%)**
	AN	MeOH	H_2O	Pip				L-form	D-form		n_L	n_D	
E	80	19.8	0.2	—	0.4	60	1.82	25.6	57.0	2.32	6	3	67
I	80	19.5	0.5	—	0.2	60	1.92	22.8	34.8	1.57	14	6	52
K	80	19.8	0.2	—	0.2	60	2.0	48.4	82.4	1.73	3	2	51
F	80	19.8	0.2	—	0.2	60	2.05	25.1	41.5	1.72	4	3	38
G	—	97.98	0.02	2	0.2	60	2.36	8.75	13.3	1.71	33	10	62
H	—	97.98	0.02	2	0.02	65	2.18	4.63	7.71	2.26	95	14	87
L	—	97.97	0.03	2	0.4	60	1.63	2.36	2.36	1.00	114	114	0
M	—	97.97	0.03	1	0.2	50	1.65	5.56	5.61	1.01	41	39	< 5
N	—	97.97	0.03	1	0.2	50	2.27	11.2	12.2	1.10	68	35	<30
O	—	97.97	0.03	2	0.2	60	2.27	5.50	6.55	1.33	43	21	<30
P	—	97.97	0.03	2	0.2	50	1.70	12.4	16.9	1.42	28	12	<30
Q	—	97.97	0.03	2	0.2	50	1.70	9.6	13.8	1.53	26	10	<30

* AN = acetonitrile; Pip = piperidine.

** Optical yields were calculated by graphical integration of the chromatograms according to Lüttringhaus[15].

If the amount of 1 in the monomeric mixture is decreased (polymers E, F, G and H) the specificity is increased.

It should be noted that the racemates of substances other than the templates (*e.g.*, mandelic acid) show no racemic resolution, whereas more closely related substances (*e.g.*, glyceric acid benzyl ester) exhibit a small but significant effect ($\alpha = 1.08$).

For a good chromatographic resolution not only a high separation factor but also narrow peaks, *i.e.*, a large number of theoretical plates, are needed. Unfortunately, the peaks in our chromatograms are broad and strong tailing is shown, especially by

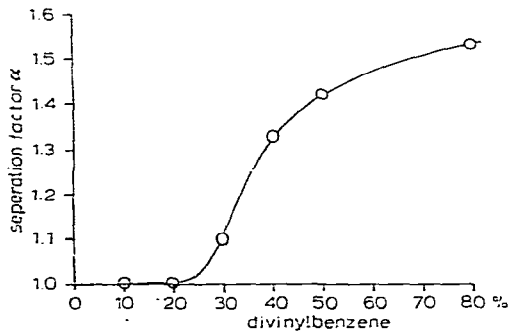


Fig. 5. Dependence of the separation factors, α, in chromatography on the amount of crosslinking agent in the polymer (L–Q). Chromatographic conditions: temperature, 50°; eluent, methanol–2% piperidine; flow-rate, 0.2 ml/min; sample, 20 μg of D- or L-form of 2 injected separately.

the more strongly bound D-form. Therefore, it is difficult to calculate the number of plates[16]. From the peak width at half-height ($b_{1/2}$), $n_{theor.}$ was roughly estimated:

$$n_{theor.} = 8 \ln 2 \left(\frac{V_R}{b_{1/2}}\right)^2$$

As this method was used throughout in spite of errors in the estimation of n, it can be taken for comparison purposes (see Table III).

Fig. 6 shows a chromatogram of the resolution experiments on a polymer, D- and L-*p*-nitrophenyl mannoside being injected separately. Although the separation factor is high ($\alpha = 2.32$) the peaks are very broad. In Fig. 7 the separation factor is a little bit smaller ($\alpha = 2.26$) but the number of plates is considerably higher. In this instance the separation of the racemic mixture was carried out and an optical yield of 87% was obtained. Most of the first eluted L-form is obtained in optically pure form. It is striking that the more specifically bound D-form shows broad peaks with long tailing.

We investigated how the separation factor and the number of plates are influenced by the chromatographic conditions. From the results it was hoped to be able to optimize the separation and to obtain some information on the separation mechanism.

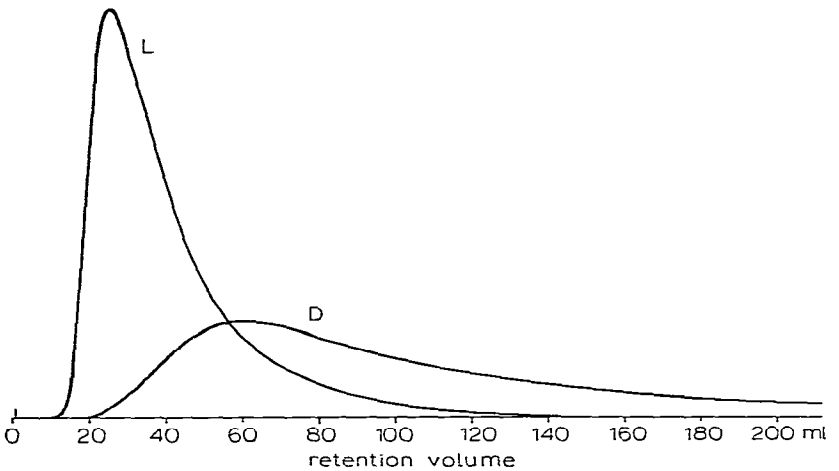


Fig. 6. Chromatography of D- and L-4-nitrophenyl-α-mannopyranoside on polymer E (0.6 g). Flow-rate, 0.4 ml/min; solvent, acetonitrile–methanol–water (80:20:0.02); sample, 100 μg of L- or D-form of 2 injected separately; temperature 60°; α value, 2.32; optical yield, 68%.

(a) Influence of the type of solvent. If the solvent is changed, two effects have to be taken into account. Firstly, the equilibrium of the substance between the sorbent and solution may be changed, giving a different k' value, and secondly, the degree of swelling may be influenced. This phenomenon would also alter the k' values, as the number of accessible binding sites is changed.

If the water content in methanol as eluent is reduced, the k' values increase,

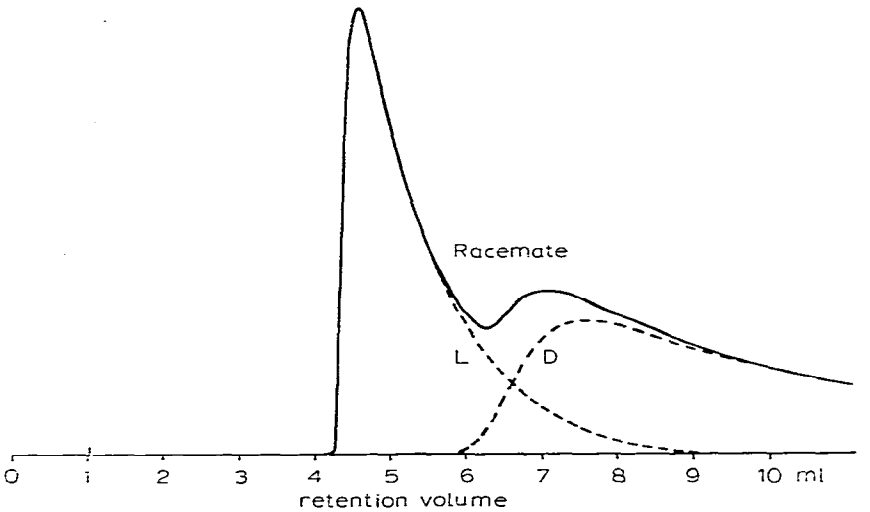
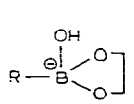


Fig. 7. Chromatography of the racemate of 2 on polymer H (0.6 g). Flow-rate, 0.02 ml/min; solvent, methanol–piperidine (98:2); sample, 100 μg of racemate of 2; temperature, 65°; α value, 2.26; optical yield, 87%.

the increase being more pronounced for the D- than for the L-form, thus leading to an increase in the α values. At the same time the number of plates also decreases, thus worsening the resolution.

With water as eluent little binding and no racemic resolution are observed. It is known that in alkaline media basic boronic diesters with tetrahedral boron (3) instead of esters with trigonal boron are formed. Therefore, the chromatography of diols on gels containing boronic acids is possible in water at pH > 9[17,18]. In our work no separation between D- and L-forms could be observed under these conditions.

Whereas the addition of acids to methanol as eluent has no significant effect, the addition of 0.5% of ammonia solution increases k' three-fold. The effect of piperidine is even more pronounced. It can be assumed that in these instances basic esters of the type 4 are formed.



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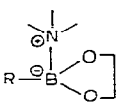
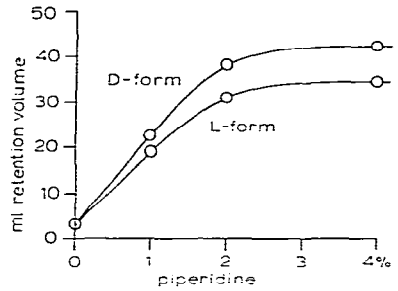


(4)

Fig. 8 shows the dependence of the retention volumes of the D- and L-forms on the amount of piperidine present in the eluent. As can be seen from Fig. 9, the separation factor, α, also improves as the amount of piperidine is increased.

In tetrahydrofuran virtually no retention of 2 is observed; it appears at a position on the chromatogram that would be expected from its molecular weight. Possibly owing to swelling of the matrix but not of the cavities, the latter are not accessible to 2.



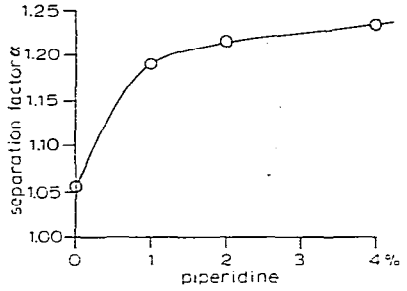
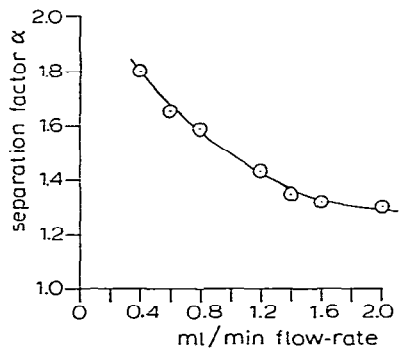


Fig. 8. Influence of amount of piperidine on retention volume, V_R. Chromatographic conditions: polymer E (0.6 g); flow-rate, 1.0 ml/min; solvent, methanol with increasing amount of piperidine; sample, 100 μg of D- or L-form of 2 injected separately; temperature, 60°.

Fig. 9. Influence of amount of piperidine on separation factor, α. Conditions as in Fig. 8.

(b) Sample capacity. At low ratios of substance to sorbent (3.5–28 μg on 0.6 g of sorbent), V_R and α remain constant. At 220 μg of substance V_R begins to decrease, although α still remains constant.

(c) Influence of flow-rate. At lower flow-rates the separation factor improves markedly, as can be seen from Fig. 10. This can be explained by the fact that the equilibria are now better established. In accordance with this, Fig. 11 shows that the retention volumes also increase. Surprisingly, the number of plates of the column does not change significantly. This is in contrast to the chromatography of substances that give no interaction with boronic acids, such as acetone and nitrobenzene, and which show high n values (800–1500 per 20 cm), increasing with decreasing pumping rate. This effect will be discussed later. Such non-ideal behaviour is also shown on elongation of the columns. Columns of length 1 m show nearly the same n value for 2 as does a column of length 20 cm, whereas for acetone n increases proportionally with column length, as one would expect.



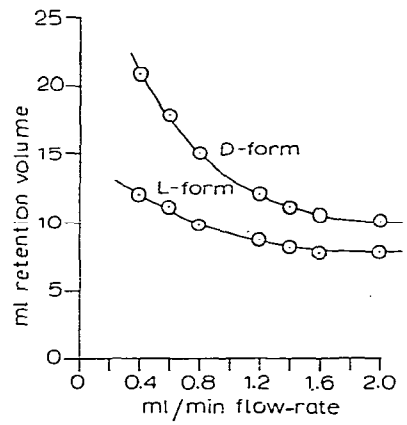


Fig. 10. Dependence of separation factor, α, on flow-rate. Chromatographic conditions: polymer E (0.6 g); solvent, methanol–acetonitrile–water (19.5:80:0.5); sample, 100 μg of D- or L-form of 2 injected separately; temperature, 60°.

Fig. 11. Dependence of retention volume, V_R, on flow-rate. Conditions as in Fig. 10.

(d) Influence of temperature. With methanol or methanol–acetonitrile as eluent, V_R increases with increasing temperature, the increase being greater for the the D- than for the L-form. This results in a considerable increase in the separation factor, α, as is indicated in Figs. 12 and 13. At higher temperatures (above 60°), V_R drifts to lower values during the measuring time. It could be shown that after use of a column for 6 weeks at 60° the boron content was reduced from 1.03 to 0.60%. This means that the B–C bond is cleaved during chromatography, possibly by oxygen dissolved in the eluent. This effect is markedly reduced by carrying out the chromatography in a nitrogen atmosphere.

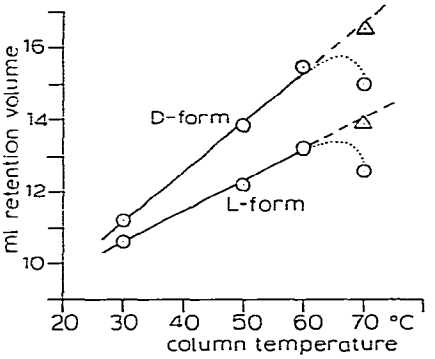


Fig. 12. Influence of temperature on retention volume, V_R. Chromatographic conditions: polymer C (0.6 g); flow-rate, 0.4 ml/min; solvent, methanol–0.2% water; sample, 27 μg of D- or L-form of 2 injected separately. At 70° V_R begins rapidly to decrease during the time of measurement. The values indicated by △ have been extrapolated to the time of the change of temperature from 60 to 70° from the known rate of decrease at 70°.

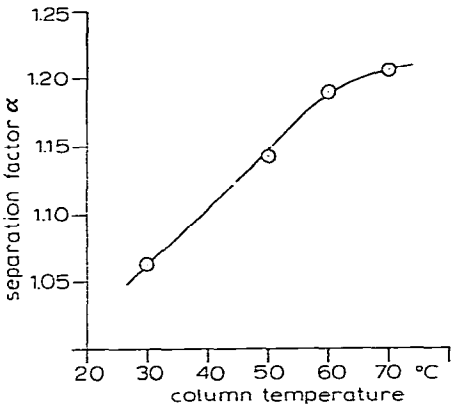


Fig. 13. Influence of temperature on separation factor, α. Conditions as in Fig. 12.

CONCLUSION

The results indicate that it is possible to prepare chromatographic sorbents with cavities of a specific shape and with functional groups therein in a stereospecific arrangement. These polymers bear structural analogies to biological receptors. In a chromatographic process these polymers possess a pronounced difference in the binding ability for the two enantiomers of the template. The separation factors achieved are high, ranging from 1.05 to 2.32, and are higher than those obtained in the batch procedure[6]. This can be explained by the fact that the microcavities possess a broad distribution of specificity[7], and in chromatography with very low loadings only the most specific cavities are responsible for the separation. In contrast, in the batch procedure higher ratios of substance to sorbent have generally been used[6,7].

For chromatographic purposes these sorbents have to be improved, as broad peaks with strong tailing occur. The small number of theoretical plates cannot be ascribed to the performance of the column, as the results are reproducible on different columns with the same sorbent and as substances such as acetone and nitrobenzene show high n values.

Apparently this type of chromatography has a particular binding mechanism that is different from that found in common chromatography, and this mainly affects the kinetics of the exchange equilibria. Hence it is surprising that n is not substantially affected by the flow-rate, the temperature or the length of the columns. Further, at higher temperature the retention volume increases. The kinetics will be discussed below, considering our investigations on the structure of the polymers and the literature data on macroporous polymers[11,14].

Fig. 14 shows the structure of the polymers schematically. There are highly crosslinked parts (the so-called nuclei) (diameter d = 100–200 nm) connected by parts with a lower degree of crosslinking. Between the nuclei a system of macropores (d = 20–50 nm) is formed. It can be assumed that the diffusion within the macropores is not hindered in any way. The microcavities formed by splitting off the templates are of the order of 1–2 nm, and are situated near to the inner surface (100–400 m^2/g) or inside the denser nuclei. Templates inside a highly crosslinked part of the nuclei will be entrapped and cannot be split off. Accessibility to the microcavities in the nuclei will be strongly dependent on the degree of swelling of the polymer. Diffusion within the crosslinked part is possible to some extent, but may be hindered. The pores formed within the nuclei are called micropores and their sizes are of the same as the microcavities or somewhat larger.

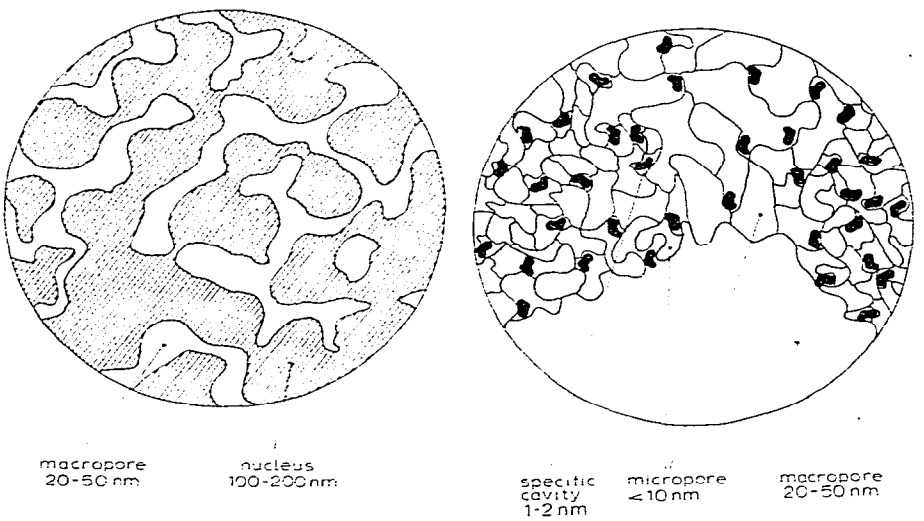


Fig. 14. Schematic diagrams of a macroporous polymer with chiral microcavities at two magnifications.

The kinetics of the exchange equilibria may be influenced by the kinetics of the reaction of the boronic acids with the template to form the boronic diester. From low-molecular-weight analogues it was shown that the half-life of the forward reaction of benzeneboronic acid with propanediol and also that of the reverse hydrolysis reaction was of the order of 100–200 sec[19]. For a chromatographic process this is not very fast, but as on slowing down the flow-rate the plate number, n, is only slightly improved, the main reason for peak broadening must be sought elsewhere.

It is to be expected that it is difficult to bind the template in highly specific microcavities if the chains in the surroundings possess high rigidity. Some flexibility of the arrangement should be present. The larger retention volumes and the higher separation factors at higher temperatures may be attributed to the fact that owing to greater swelling and greater flexibility, highly specific microcavities not accessible at lower temperatures can now bind the template.

The embedding of the substrate in the microcavities will be further complicated, as the microcavities are stamped by the end-product of the reaction, *i.e.*, boronic diesters with trigonal boron. However, as the transition state of the reaction of the boronic acids with diols to form the cyclic diesters is assumed to contain a tetrahedral boron atom, the slow reaction kinetics may also be attributed to the different stereochemistry of the transition state.

We are at present studying possibilities of improving the chromatographic behaviour of our sorbents, along the following lines:

(i) the use of highly crosslinked polymers with more flexible crosslinking agents;

(ii) the use of functional groups with a faster exchange equilibrium; this can be done by suitable neighbouring group effects;

(iii) the use of monomers in which the stereochemistry of the transition state of the reaction is similar to that of the end-product.

ACKNOWLEDGEMENTS

Thanks are due to the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie for financial support.

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