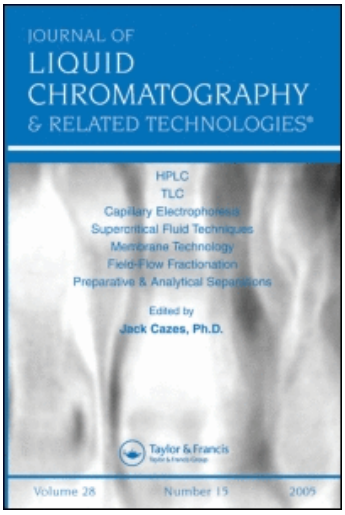

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ENZYME-ANALOGUE BUILT POLYMERS. XIX. RACEMIC RESOLUTION ON POLYMERS CONTAINING CHIRAL CAVITIES

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ABSTRACT

With the aid of a chiral template molecule binding groups could be placed in a highly crosslinked polymer in such a way that they are present in a chiral cavity of specific shape in a given stereochemistry. Polymers of this type possessed a high selectivity for racemic resolution of the racemate of the template. In the batch procedure separation factors α as high as 3.65 were obtained. In chromatography similar to other chromatographic racemic resolutions a slow mass transfer was observed. This is not due to resistance to intra-particle diffusion since similar polymers coated as thin layers to wide pore silica showed comparable behaviour.

New chromatographic investigations on glycoldimethacrylate based polymers with high selectivity in the batch procedure are described. Systematic investigations showed that resolutions of R_S = 1.2 can be obtained. At elevated temperature the separations were even better and higher retention was observed. The amount of cavities on the surface was varied and an optimum for chromatographic separations was found.

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* For Part 18, see Ref. 25)

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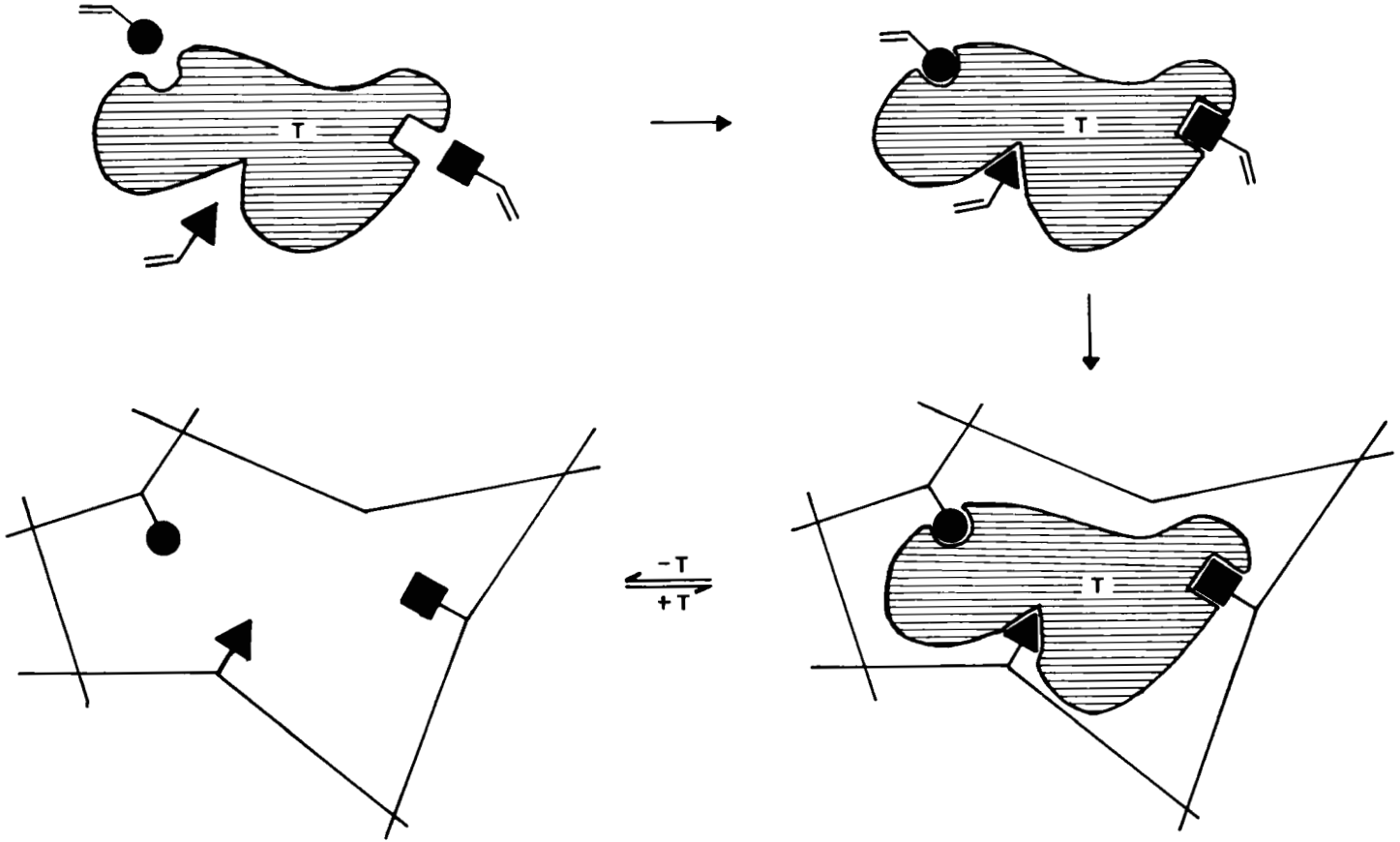
INTRODUCTION

The chromatographic separation of racemates has reached considerable progress during the last 15 years (for recent reviews see e.g. [1-4]). Polymers or silicas with pendent chiral groupings which interact more or less selectively with the solutes have been used (see e.g. [1-7]). Ligand exchange chromatography was especially successful in the separation of the racemates of amino acids (see e.g. [8-10]). Highly useful materials in racemic resolution proved to be triacetyl-cellulose (see e.g. [11,12]) and optically active poly(tritylmethacrylate)(see e.g. [13]). Low molecular weight compounds forming chiral cavities, like cyclodextrins (see e.g. [14-16]) or certain crown ethers (see e.g. [17]), were bound to polymers or silica and used for effective chromatographic separation.

In this paper it will be reported on the use of polymers with binding groups located in a definite spatial proximity and cooperativity in cavities of specific shape. This arrangement is similar to those of natural receptor sites. It could be verified by an imprinting procedure using suitable template molecules (recent reviews see [18-20]).

REVIEW

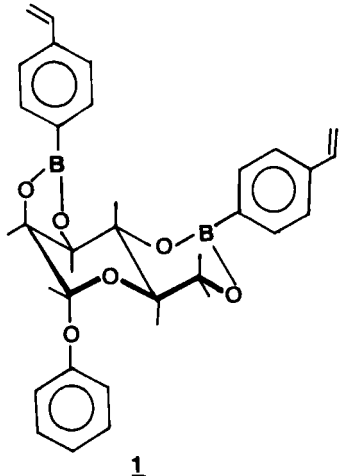
The Preparation of Polymers with Chiral Cavities. Several years ago we introduced a new approach to the preparation of highly selective binding sites [21-23]. The binding groups which were to be introduced were bound in the form of polymerizable vinyl derivatives to suitable template molecules (see Scheme 1). This monomer was then copolymerized under conditions such that highly crosslinked polymers with chains in a fixed arrangement were formed. After removal of the template, free cavities resulted with a shape and an arrangement of functional groups which corresponded to those of the template. The functional groups on this polymer are located at quite different points of the polymer chain, they are held in a spatial relationship by the crosslinking of the polymer. In most cases we used optically active template



Scheme 1. Schematic picture of the preparation of microcavities with functional groups in a defined arrangement.

molecules, the resulting cavities, therefore, were chiral as well. This could be demonstrated by the ability of these polymers to resolve the racemate of the template used. Numerous examples for this approach using different templates have been published by our group, and later also by a number of other groups (for some recent papers see e.g. [24-30]). A comprehensive review is given in l.c.[19]. This method resembles to some extent that of Dickey [31] who precipitated silicic acid in the presence of certain dyes and found a small but detectable preference for adsorption of these substances to the resulting silica.

For the optimization of this procedure in our laboratory, the monomer 1 was mainly used [24]. The template is phenyl-α-D-mannopyranoside to which two 4-vinylbenzeneboronic acids were bound by two diester linkages. The boronic acid groups act as the binding group. The monomer 1 was copolymerized with radical initiation in the presence of an inert solvent with a high amount of a bifunctional crosslinking agent. Under these conditions, macroporous polymers were obtained which possess a permanent pore structure and a high inner surface area. Consequently a good accessibility and a low swelling ability, and therefore a limited mobility of the polymer chains, can be expected.

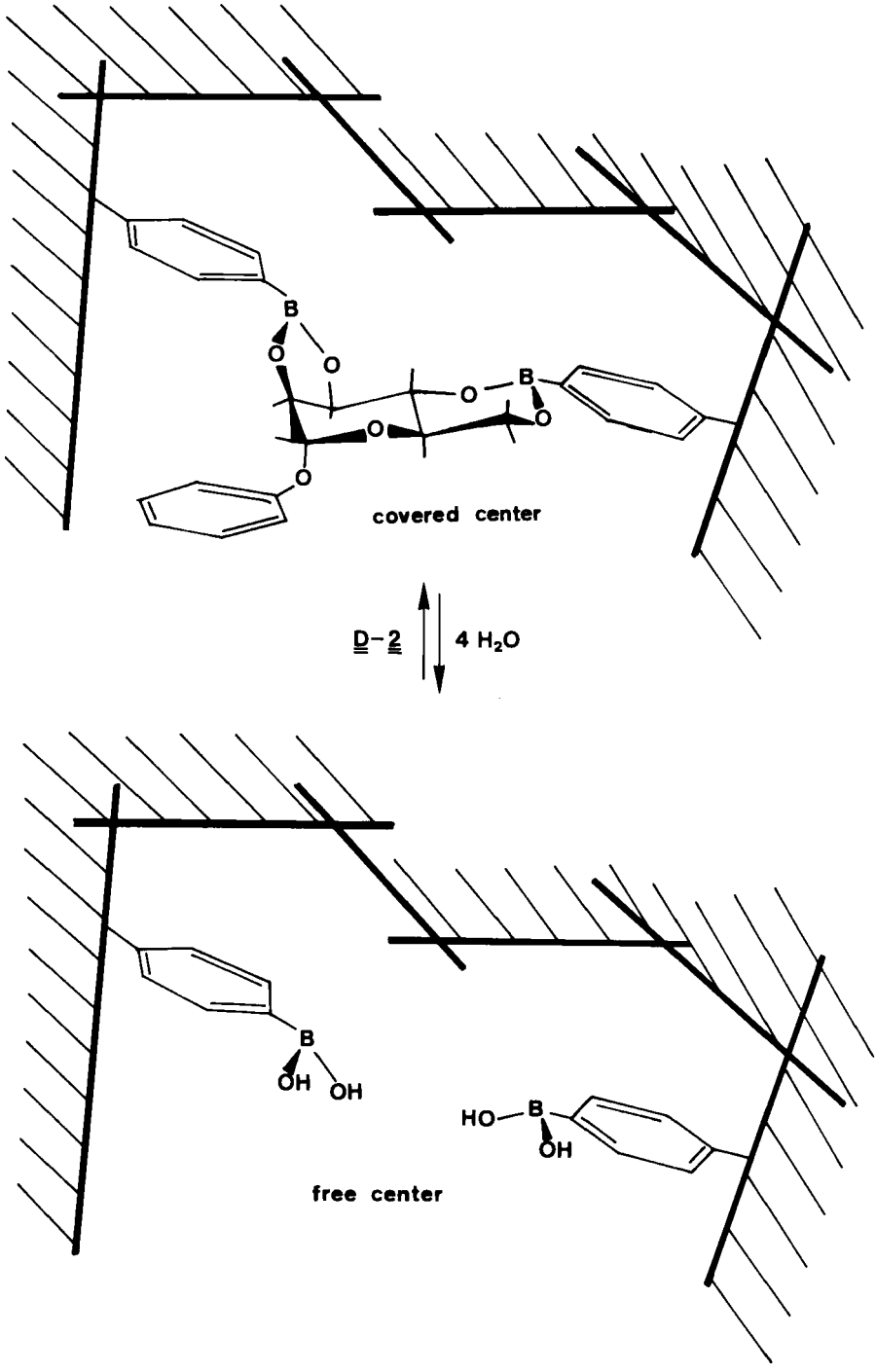


1

From this type of polymer the templates could be split off at a degree of 40-90% with water or alcohol (see Scheme 2). During optimization the specificity was tested in a batch procedure. The polymer was treated with the racemate of the template under equilibrium conditions. The enantiomer which was used for the preparation of the polymer, was preferably absorbed. The specificity was expressed by the separation factor α, which is the ratio of the distribution coefficients between solution and polymer of L- and D-form. The highest α-value obtained after optimization of the polymer structure was $\alpha = 3.65$ [24]. In this case an enrichment of the L-form in the filtrate of 12.8% and of the D-form at the polymer of 40.4% was observed in the batch procedure.

During the polymer optimization it was found that both the kind and the amount of the crosslinking agent have the strongest influence on the specificity of the polymers [24,32]. Figure 1 shows the selectivity for racemic resolution of the polymers in dependence on the amount of the crosslinking agents p-divinyl-benzene, ethylene glycol dimethacrylate and butylenediol dimethacrylate. Below 10% crosslinking the specificity disappears, at higher crosslinking the specificity increases. Ethylene glycol dimethacrylate behaved in a remarkable way. Up to 50% crosslinking, an increase to an α-value of 1.50 was observed, from 50% to 66.7% a dramatic increase from 1.50 to 3.04 occured, which means a fourfold increase in selectivity in this range. Butylenediol dimethacrylate and especially p-divinyl-benzene as crosslinking agents showed a much lower increase.

The Function of the Binding Groups. In the method described of preparing binding sites by an imprinting procedure, the binding groups have a twofold function. First, during polymerization a strong interaction between template and binding groups should be present, so that the template molecule can fix the binding groups to the growing polymer chains in a defined stereochemistry. Second, after splitting off the template, the binding groups should be able to undergo an easily reversible binding interaction with the template. For the first purpose binding should be strong, for the second the activation energy should be low.



Scheme 2. Removal and uptake of the template.

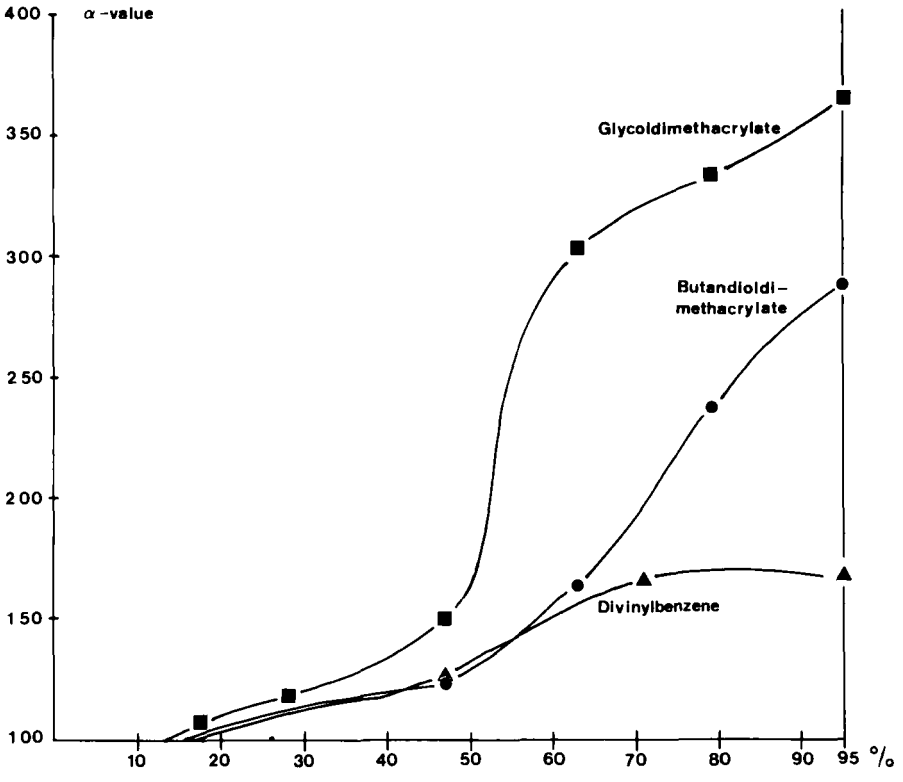


Figure 1. Dependence of the specificity of the polymers on the kind and the amount of crosslinking agent.

We have chosen in most cases covalent binding since it is strictly oriented in space during polymerization [33]. For a fast and reversible binding reaction, as necessary in chromatography, however, the activation energies of covalent bonds are too high in most cases. They could be lowered by the addition of suitable catalysts (e.g. piperidine or ammonia in the case of boronic acids)[34,35], or by the introduction of appropriate neighboring groups[36,37].

Earlier Chromatographic Work [23,35,38]. Polymers of the type discussed above were prepared with techn. divinylbenzene as

the crosslinking agent [38]. Fractions of 32-45 µm particle size were used for chromatography in a usual HPLC set up. Under optimal conditions a pronounced difference in the binding ability for the two enantiomers of the template was observed. The separation factors observed, $\alpha = 2.32$, were higher than in the batch procedure with the same polymer ($\alpha = 1.20 - 1.63$ depending on the coverage). This difference was explained by the fact that the microcavities possessed a broad distribution of specificity, and in chromatography with very low loadings only the most specific cavities were responsible for the separation. Furthermore, the chromatographic separations were performed at 60°C whereas the batch procedure was performed at room temperature. At higher temperature the selectivity increases [38].

In spite of the high α-value the separations were rather poor, as could be seen by the occurence of strong peak broadening. Apparently the mass transfer was relatively slow. Some peculiar properties of the chromatographic system were observed. It was found that the number of theoretical plates was not substantially affected by the flow rate, the temperature, or the length of the columns. Furthermore, at a higher temperature, the retention volume increased.

Several reasons for the slow kinetics can be discussed:

a) The formation and hydrolysis of the covalent bonds during chromatography could be too slow.
b) The cavities are too rigid so that the embedding process might be kinetically hindered.
c) The cavities are stamped by the end-product of the binding reaction (i.e. boronic diesters with trigonal boron), whereas the transition state of the binding has a different stereochemistry (a tetrahedral boron). This might slow down the exchange equilibrium.
d) The diffusion of the substances to be separated through the polymer matrix to reach a chiral cavity may be rate-limiting.

The kinetics of the covalent binding have been studied with low molecular weight analogues. By the introduction of suitable neighbouring groups in phenylboronic acids, the establishment of the equilibrium can be enhanced by several orders of magnitude [36,37]. Polymers with such binding groups

showed a similar behaviour to that described above. It can therefore be concluded that covalent binding itself is not the slow step. It cannot, however, be excluded that due to steric hindrance, or because the transition state has another geometry, the binding in a cavity is much slower.

In order to study the influence of the diffusion on the kinetics within the polymer matrix, thin layers of polymers were prepared on a solid support [35,39-41]. So the commercially available 3-(trimethoxysilyl)-propyl methacrylate was bound via siloxane bonds to the surface of wide pore silica (see Scheme 3). On this surface thin layers of monomers (10-150 Å) of a similar composition as in the case of bulk polymers containing 1 were polymerized. After removal of the template (see Scheme 3), these polymers on silica showed a high selectivity for racemic resolution in the batch procedure comparable to the best macroporous polymers described earlier. In this way it was easily possible to prepare non-swellable particles of a desired particle size. These particles can be packed in columns with high efficiency for non-retarded or non-specifically retarded substances.

For selective racemic resolutions in chromatography, a slow mass transfer was again observed. This cannot, therefore, be attributed to diffusion resistance in the polymer matrix, since these very thin layers should not show substantial diffusion hindrance.

NEW RESULTS

In the course of the optimization of the polymer structure, polymers which much higher selectivity for racemic resolution in the batch procedure were prepared [24,32]. These polymers were now investigated for their chromatographic behaviour.

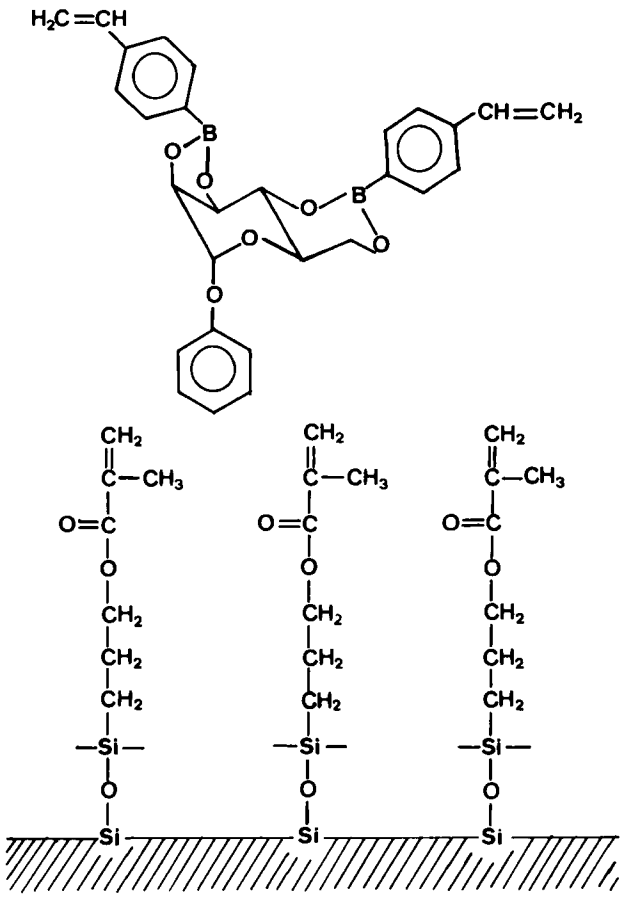
EXPERIMENTAL

Polymers: The preparation of the polymers was performed as described earlier [23]. The composition of the polymerization mixtures is given in Table 1. Macroporous polymers are thus obtained. Splitting percentage, inner surface area, swella-

Table I. Composition of the Polymerization Mixtures and Properties of the Polymers E-1 - E-5

Polymer	Monomer Composition EGDM [in g]	1 [in g]	[% 1]	Splitting Percentage	Surface Area [m^2/g]	Swellability in Aceto-nitrile	Separation Factor α
E-1	14.9	0.375	[2.5]	80.5	322	1.19	2.49
E-2	15.0	0.74	[4.7]	81.7	362	1.63	3.54
E-3	15.0	2.28	[13.2]	85.0	212	2.07	3.04
E-4	14.9	3.01	[16.8]	83.9	210	2.16	2.27
E-5	15.0	4.50	[23.1]	86.0	111	2.50	1.86

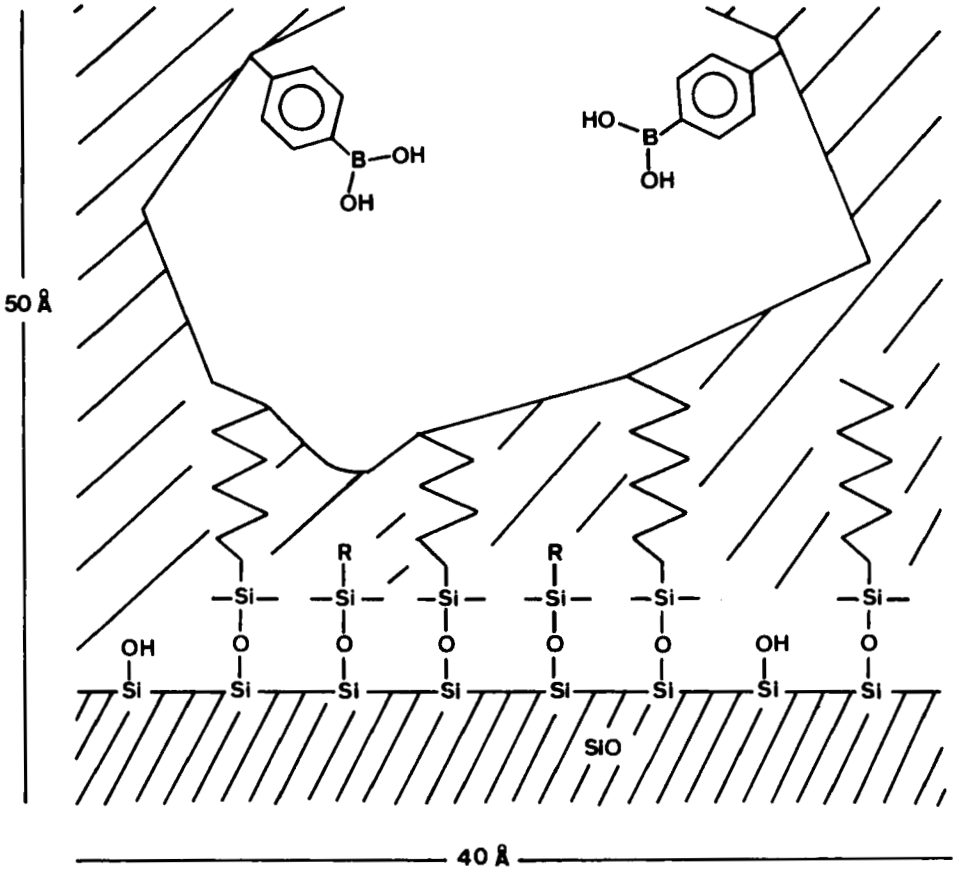
Polymerization was performed in presence of 15 g tetrahydrofurane with the addition of 50 mg azobis(isobutyronitrile). EGDM = ethylene glycol dimethacrylate.



Scheme 3. Schematic picture of a polymer coated silica imprinted by 1. a before polymerization, b after polymerization and splitting off the template.

bility, and separation factor α in the batch procedure were determined in the usual manner [23].

Preparation of polymer packings: The obtained polymer blocks were crushed, ground in a coffee mill and then milled in an Alpine mill (Contraplex 63 C). Depending on the amount prepared, the polymer sample was then classified either by sieving (sieve set of 32-90 μm), or by a wind siever (Alpine



Scheme 3b.

Multiplex 100 MRZ). Fractions used for chromatography were either 32-45 μm or 8-16 μm. After final elimination of the rest of the fines by repeated sedimentation in methanol, the columns were packed in stirring suspension (methanol/THF 2:1) at final pressure of 350-400 bar with a flow of up to 40 ml/min.

Chromatography. The apparatus consisted of a Gilson pump model 302 with microflow accessory (0.5-5000 μl/min), a Rheodyne variable volume sample injector, and as detectors

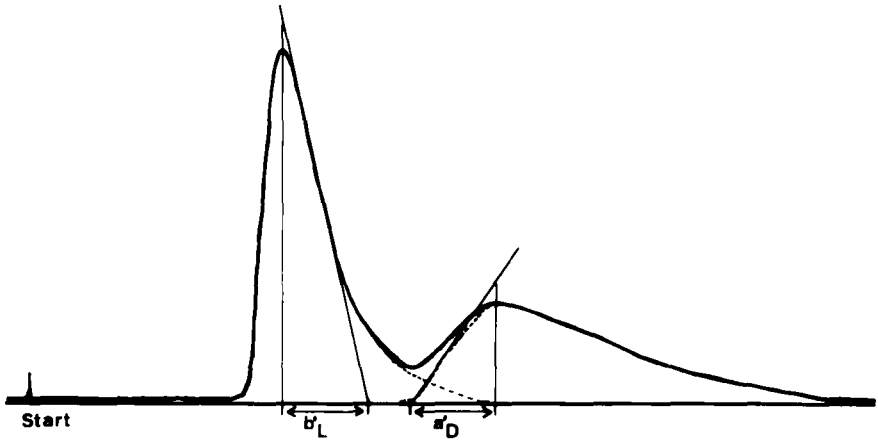


Figure 2. Chromatography of the racemate of 2 on E-3. Flow-rate: 0.1 ml/min; solvent: acetonitrile + 4% aqueous NH_3 (25%) + 5% H_2O. Sample: 10 µl of a solution of each 10 mg D-2 and L-2 per ml solvent. Room temperature; resolution R_s = 1.2

$$R_s = \frac{V_{RD} - V_{RL}}{b_L' + a_D'}$$

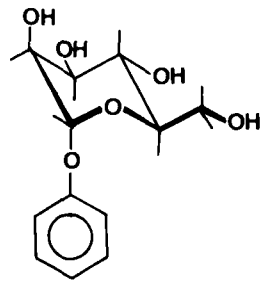
an Altex UV-254 and a Waters-RI-detector R 401. Solutions of solutes (1-5 mg/ml) were injected in volumes of 2-10 µl. The columns had dimensions of 250 x 4 mm or 125 x 4 mm, respectively. Retention volumes were approximated by peak maxima volumes and for the description of the dynamics of the chromatographic process resolution R_s was estimated. This was done by measuring the baseline from the rear to the vertex of the first peak and from the front to the vertex of the second peak of a triangle constructed of the baseline and lines tangent to the chromatogram at the two points of inflection (see Figure 2). Since the peaks are strongly asymmetric, this procedure for a semiquantitative description seems to be most reliable.

RESULTS AND DISCUSSION

Properties of the Polymers. In the present investigation a number of polymers with increasing content of the template

monomer 1 (2.5-23.1%) were prepared. The templates could be removed from all polymers to a high extend (80-86%) by hydrolysis with methanol-water (see Table 1). The crosslinking of the polymers freed from the template decreases with an increasing content of template monomer. Therefore the flexibility of the polymer chains should increase at higher content of 1. This could be one reason for the strong increase in swellability (from 1.19-2.50). The other reason for higher swellability with an increasing content of 1 is the high amount of free boronic acids at the polymers that could be solvated by the swelling solvent. The macroporous structure of the polymers changes to some extent as well. The inner surface areas obtained by BET measurements are reduced from 322 to 111 m^2/g with increasing amount of 1 in the polymerization mixture. The selectivity for the racemic resolution of the racemate of the template was first determined in the batch procedure in methanol. There appears to be an optimum of specificity at a lower content of 1.

The Role of the Mobile Phase Composition in Chromatography. To choose the right eluent for chromatography, the binding constants of the template D 2 and its enantiomer L 2 were determined in various solvents (see Table 2). These binding constants reflect mainly the influence of the solvent on the ester bond formation within chiral cavities.



D-2

The ratio of the binding constants of L- and D-form is again expressed as the separation factor α. The α-values indicate the exactness of the cavity fitting. It can be assumed that the D-form (the template) is mainly bound by two boronic ester bonds (two point binding) whereas the L-form only by one.

Table II. Binding Constants of D-2 and L-2 at E-2 in Various Solvents

Solvent	Binding Constant of L-2	Binding Constant of D-2	Separation Factor α
MeOH	0.37	1.31	3.54
MeOH	0.14	0.52	3.67
MeOH 0.1% H_2O	0.17	0.39	2.31
MeOH 2% NH_3 gas	0.46	0.78	1.68
MeOH 1% H_2O, 1% NH_3*	0.32	0.40	1.22
MeOH 3% piperidine	0.91	1.06	1.16
AcN	5.48	20.28	3.72
AcN 5% H_2O	0.1	0.26	2.60
AcN 10% H_2O	0.056	0.090	1.65
AcN 4% H_2O, 1% NH_3*	0.31	0.47	1.54
THF	0.15	0.21	1.37

NH_3* = 25% NH_3 in H_2O; MeOH = methanol; AcN = acetonitrile; THF = tetrahydrofurane.

As already known from earlier work [23,32], methanol and acetonitrile are good solvents for the equilibration in the batch procedure, whereas tetrahydrofurane is a rather poor one. Addition of water decreases binding because the equilibrium of esterification is shifted to the educts. The selectivity in presence of water is reduced.

Retention can be increased by the addition of certain nitrogen bases which can form a B-N-interaction as in the case of NH_3 or piperidine. These bases form a tetrahedral B-N-addition product. Esters of this type are more stable towards water than the trigonal ones [34]. But since these esters exhibit another geometry than those used during the imprinting procedure, the selectivity decreases. In chromatography the addition of these bases could have an important advantage. It is known [34] that the kinetics of the achievment of the esterification equilibria are greatly enhanced.

Chromatographic separations were first investigated in methanol as the eluent. In methanol the retention was rather

low but could be increased by the addition of aqueous NH_3. Under these conditions the selectivity decreased strongly so that other solvents were tried.

Chromatography in acetonitrile and tetrahydrofurane showed a striking difference. Chromatography of D 2 on polymer E 2 showed in THF virtually no retention. The same was true for a THF/AcN (1:1) mixture. Further increase of the AcN content in the mobile phase resulted in incomplete recovery of the substance, and at a AcN/THF ratio of 8:2 no substances could be removed. This should be due to the fact that no equilibria in AcN can be established during a chromatographic run. The water formed on esterification is quickly removed. At the moment the peculiar behaviour of THF cannot be explained.

Further chromatographic experiments were performed with AcN as the main eluent. In order to obtain equilibria, some water has to be added. The addition of NH_3 increases the retention. Whereas both H_2O and NH_3 reduce the selectivity, the mass transfer is enhanced by the addition of ammonia and sharper peaks are observed. NH_3 accelerates the esterification reaction of the boronic acids with diols. Thus most of the separations were performed with acetonitrile with the addition of 6% NH_3 (25% in water) and 4% H_2O.

The Role of Sorbent Composition. Polymers E-1 - E-5 having an increasing concentration of cavities containing boronic acid, were investigated (see Table 3). The selectivity (as expressed by the separation factor α) is highest in the case of polymer E-1 with the lowest concentration of the cavities. With increasing concentration of cavities the selectivity is reduced. They are no longer isolated from each other and an intercavital binding of both enantiomers can occur.

On the other hand, higher concentration of active centers improves the chromatographic efficiency of the column, so that an optimum of the resolution is observed with polymers E-2 and E-3. Resolutions of R_s = 1.2 on E-3 and R_s = 1.1 on E-2 were obtained [42]. Figure 2 shows the separation of the racemate of 2 on polymer E-3.

These polymers can also be used for semipreparative separations. In one example 30 mg of the racemate was

Table III. Chromatographic Investigations on Polymers of Different Composition.

	Polymer	Flow Rate [ml/min]	Eluent Acetonitrile with the Addition of [in %]*	k_L'	k_D'	α	R_S
1	E-1	0.03	6 NH_3 4 H_2O	1.5	4.00	2.67	0.8
2	E-1	0.04	8 NH_3 4 H_2O	1.7	4.70	2.76	0.5
3	E-1	0.05	8 NH_3 6 H_2O	1.9	4.8	2.53	0.6
4	E-2	0.05	6 NH_3 4 H_2O	4.25	7.5	1.76	1.1
5	E-2	0.2	6 NH_3 4 H_2O	4.30	6.3	1.47	0.7
6	E-2	0.4	6 NH_3 4 H_2O	3.80	5.3	1.39	-**
7	E-3	0.1	4 NH_3 5 H_2O	22.3	44.0	1.97	1.2
8	E-3	0.2	2 NH_3 5 H_2O	24.0	46.5	1.94	1.0
9	E-4	0.3	6 NH_3 4 H_2O	7.7	11.2	1.45	0.4
10	E-5	0.3	6 NH_3	15.0	21.7	1.44	0.5

* NH_3 is a 25% solution in H_2O

** Due to poor resolution not determined.

Chromatography of the racemate D,L-2 on polymers E-1 - E-5. Sample 10 µl of a solution of 20 mg per ml acetonitrile; room temperature; R_s was estimated according to formula

$$R_S = \frac{V_{RD} - V_{RL}}{a_D' + b_L'}$$ (see Figure 4)

Table IV. Temperature Dependence of the Rentention Volume of D-2 and L-2 on Polymer E-2

Flow Rate	Substance	25°C V_R	25°C α	35°C V_R	35°C α	45°C V_R	45°C α	55°C V_R	55°C α
1 ml/min	L-2	6	1.4	6	1.8	6	2.9	6.9	3.3
	D-2	8		10		15		20	
0.3 ml/min	L-2	6.5	3.0	6.9	3.7	7.6	4.5	7.6	4.5
	D-2	17		22		30		30	

Chromatography with methanol as the eluent. Retention volumes (V_R in ml) for L-2 and D-2 were determined by injecting each substance separately.

chromatographed at 20 g of polymer E-3. Substances at the peak maxima of the separation were optically pure.

The Kinetics of the Chromatographic Separation. Entries 4-6 in Table 3 clearly show a strong flow-rate dependence of the separation of the phenyl-mannosides on this type of sorbent. The peak broadening was much larger than that of non-binding solutes of similar chemical structure, and was not significantly affected by the change of particle diameter of column packing. This phenomenon indicates that the dominant contribution of plate height in this system is the kinetic term [43,44].

In accordance with this, the column efficiency is greatly increased at elevated temperatures. Table 4 shows the retention of L-2 and D-2 on polymer E-2 in methanol as a mobile phase at different temperatures. At higher temperature retention is higher and the selectivity increases considerably. This effect is especially remarkable at high flow rate. The α-values increased from 1.33 to 2.90. Since in spite of stronger retention, the peak width did not increase considerably either, the separations were much better.

These results on the kinetics are not surprising, since relatively slow kinetics were observed in most affinity chromatography systems [45], as well as in chromatography with enantio-

selective sorbents. Broad peaks are obtained in chromatography on triacetylcellulose [11] and in ligand exchange chromatography on polymers or on silica bonded phases [8,46]. In ligand exchange chromatography, ligand complex formation itself is not the limiting factor, since sharp peaks were obtained when the complexation occurs in solution and the diastereoisomers were separated on a reversed phase [8,47,48].

CONCLUSION

Highly crosslinked polymers with chiral cavities obtained by polymerization in the presence of suitable templates can be used for the chromatographic separation of the racemate of the template. The mass transfer of the separation is relatively slow, but due to the very high selectivity, separations with R_s = 1.2 could be obtained. Further optimization is possible by increasing the temperature. The amount of cavities on the surface was varied and an optimum for chromatographic separations was found.

Earlier investigations had shown that matrix diffusion within the crosslinked polymer is not the rate limiting step in the separation [35,39]. Sorption-desorption kinetics of the cavity-bound substrates should be decisive for the relative slow mass transfer. Although the covalent bond formation in boronic esters in solution is fast, in an exactly fitting cavity this appears to be a slower process. This is especially pronounced for a two-point-binding in the cavity. Thus, the wrong enantiomer, usually only bound by an one-point-binding, showed a faster mass transfer and therefore less peak broadening.

Further improvements of the chromatographic separation are separations at higher temperature, the use of more flexible polymers and the use of faster and less sterically demanding binding reactions.

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