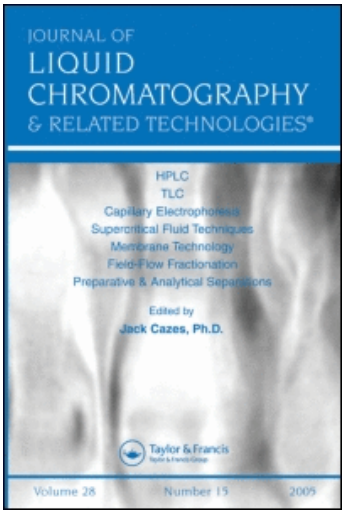


Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Template Imprinted Polymers for HPLC Separation of Racemates

GÜNter Wulff[a]; Milan Minarik[b]
[a] Institute of Organic Chemistry and Macromolecular Chemistry Heinricn-Heine-University Düsseldorf, Federal Republic of Germany [b] Institute of Chemical Process Fundamentals Czechoslovak Academy of Sciences, Prague, CSSR

To cite this Article Wulff, GÜNter and Minarik, Milan(1990) 'Template Imprinted Polymers for HPLC Separation of Racemates', Journal of Liquid Chromatography & Related Technologies, 13: 15, 2987 — 3000
To link to this Article: DOI: 10.1080/01483919008049082
URL: http://dx.doi.org/10.1080/01483919008049082

JOURNAL OF LIQUID CHROMATOGRAPHY, 13(15), 2987-3000 (1990)

TEMPLATE IMPRINTED POLYMERS FOR HPLC SEPARATION OF RACEMATES

GÜNTER WULFF[1] AND MILAN MINARIK[2]
[1]*Institute of Organic Chemistry*
and Macromolecular Chemistry
Heinrich-Heine-University
Düsseldorf, Federal Republic of Germany
[2]*Institute of Chemical Process Fundamentals*
Czechoslovak Academy of Sciences
165 02 Prague, CSSR

ABSTRACT

A novel approach to the separation of enantiomers using polymeric sorbents imprinted with optically active template molecules has been examined for use in HPLC. The racemate of the template molecule can be separated whereby covalent interactions are responsible for retardation in the chromatographic process. This covalent interaction governs the overall kinetics of the separation. Increasing the reaction rate of this binding reaction by optimizing the experimental conditions improves the separation considerably. The template form of the enantiomeric mixture is thus strongly retarded and separated from the non-template form. A further substantial improvement of the chromatographic pattern is obtained by

gradient elution when resolution, analysis time and peak shape approximate to the usual high performance liquid chromatographic separation.

INTRODUCTION

In previous papers (1-4) we have demonstrated the possibility of separating enantiomers using tailor-made polymeric sorbents possessing chiral cavities of specific shape. These polymeric sorbents were prepared by a novel approach (5-7) in which functional groups carrying polymerizable vinyl groups were bound to an optically active template molecule. This template monomer was subsequently copolymerized with a large amount of crosslinking agent in the presence of an inert solvent to yield a macroporous polymer. After cleavage of the template from the polymer, microcavities are formed whose shape and arrangement of functional groups correspond to the template molecule. These polymers can be used for the racemic resolution of the racemate of the template.

It has been proven that relatively strong binding of the templates to the growing polymer chains during polymerization results in the production of materials showing high selectivity for racemic resolution. This is also true for the chromatographic process itself. Covalent bonds of a special type can therefore be used advantageously (1-7). However, it is also possible to use non-covalent interactions, as e.g. electrostatic and/or hydrogen-bonding (8,9).

When using covalent bonds the applicability of this procedure is somewhat restricted since the substances to be

chromatographed have to carry suitable functional groups. These groups should be able to undergo fast and reversible covalent interactions with binding site groupings. These interactions have to be fast enough for HPLC conditions. As an example for such an interaction, polymer bound phenyl boronic acid interacting with diol groups has been studied in detail and the following conclusions were drawn (1-4):

(i) The chromatographic conditions could be optimized by accelerating the kinetics of the interaction. This can be achieved by adding a suitable nitrogene containing base as NH_3, and by increasing the column temperature.

(ii) Under optimized conditions a different sorption mechanism of the two enantiomeric forms is noticeable. The peak width of the non-template enantiomer is sufficiently narrow, i.e. the kinetic term becomes negligible. In contrast to that the template enantiomer shows a relatively broad peak width, which has been ascribed to the slow kinetics of a two-point interaction within the chiral cavity (10).

(iii) Further improvement of the chromatographic separation can be brought about by increasing the accessibility of the adsorbents, e.g. by increasing the flexibility of the cavities and of the functional groups and thus accelerating the sorption and desorption process of the template analogue molecules in the column.

The main purpose of this paper is to examine the above mentioned factors in detail and to further improve this chromatographic model.

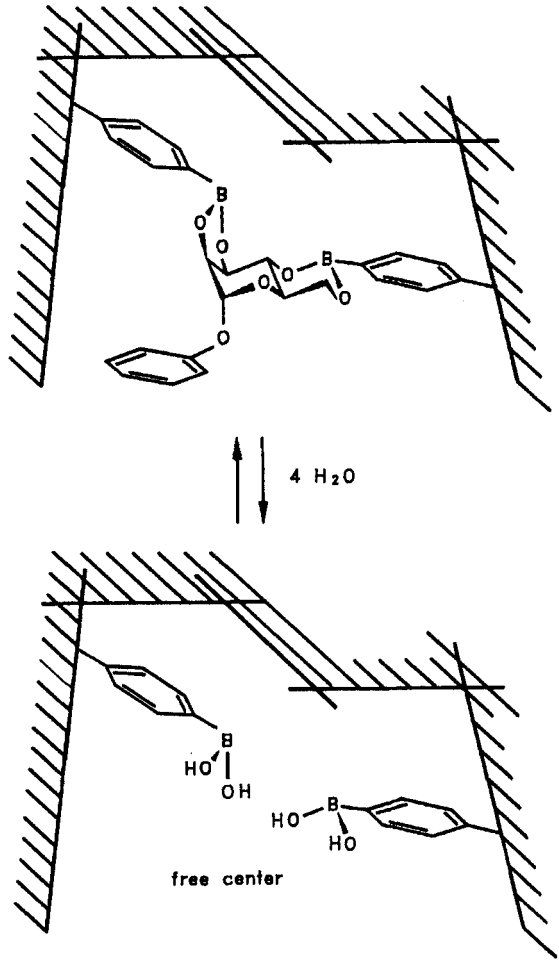
EXPERIMENTAL

Materials

D- and L-phenylmannopyranosides were synthesized by the procedure described elsewhere (11). The preparation of polymer sorbent E-3 was described previously (2). Polymerization was performed with 2.28 g of phenyl 2.3; 4.6-tetra-o-bis(4-vinylphenylboronyl-α-D-mannopyranoside, 15 g of ethyleneglycoldimethacrylate in presence of 15 g tetrahydrofurane / acetonitrile 1:1 with the addition of 50 mg of azobis(isobutyronitrile). Temperature 70°C for 20 hrs. The obtained polymer blocks were crushed, ground in a coffee mill and then milled in an Alpine mill (Contraplex 63 C). The polymer sample was then classified by a wind siever (Alpine Multiplex 100 MRZ). Fractions used for chromatography were 8-16 μm. The polymer sorbent P2 was prepared according to E-3 but instead of tetrahydrofurane/acetonitrile we used acetonitrile (ACN) with 5% NH_3 as inert solvent. Polymer sorbent P3 was prepared analogously to polymer sorbent E-3 but as the binding group we used p-(methacryloxy)-phenylboronic acid and as inert solvent ACN/toluene 1:1 (12). Solvents used as a mobile phase for the HPLC were ACN, (LiChrosolv) and NH_3 p.a. (25% aqueous solution), (Merck, Darmstadt, F.R.G.); water was prepared with Alpha-Q Water purification system (Millipore, Bedford, Massachusetts, U.S.A.).

Apparatus

The HPLC system consisted of the Waters M600 Multisolvent Delivery System and U6K injector (Waters div. of Millipore, Milford, Massachusetts, U.S.A.), thermostated column liquid jacket (home made), LCC-85/LC-Autocontrol spectrophotometric



Scheme I Schematic representation of uptake and release of α-phenylmannoside as a template in imprinted polymers

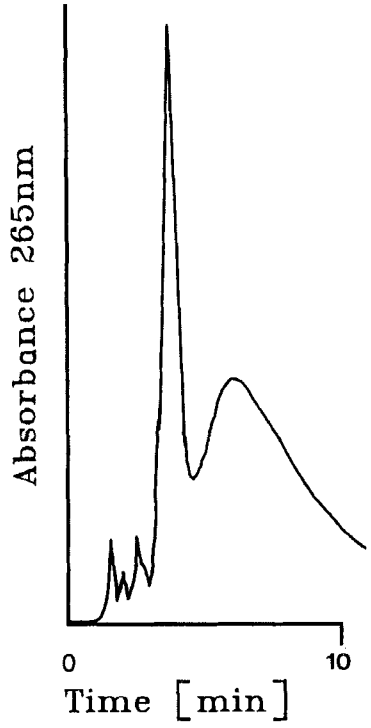


Fig.1 LC of rac. α-phenylmannoside on polymer E-3. Flow 1.2 ml/min, T = 60°C, mobile phase ACN/H_2O/NH_3 82.5/12.5/5

detector (Perkin-Elmer, Norwalk, Connecticut, U.S.A.), R401 refractive index detector (Waters div. of Millipore, Milford, Massachusetts, U.S.A.) and Back-Pressure Regulator (Supelco, Gland, Switzerland).

RESULTS AND DISCUSSION

In Scheme I the release und uptake of the template α-phenylmannoside in microcavities prepared by imprinting is

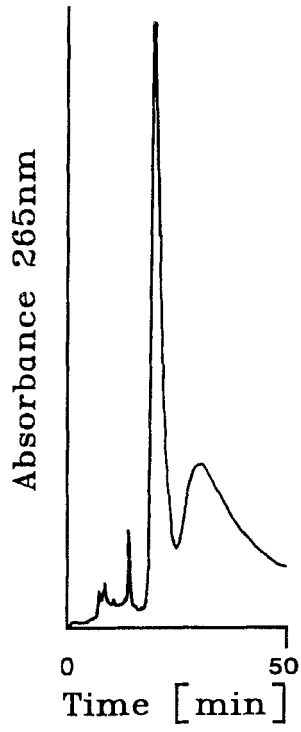


Fig.2 LC of rac. α-phenylmannoside on polymer P3. Flow 0.2 ml/min, T = 85°C, mobile phase ACN/NH_3 95/5

presented (2). Polymers containing this type of microcavities are used in liquid chromatography. Fig. 1 illustrates the chromatographic separation of the α-phenylmannoside enantiomers on a column packed with the ethylene glycol dimethacrylate based polymer E-3 imprinted with the D-enantiomer as the template. Spreading of the first peak indicates the moderate kinetics of interaction under the used experimental conditions, e.g. reversible esterification of one

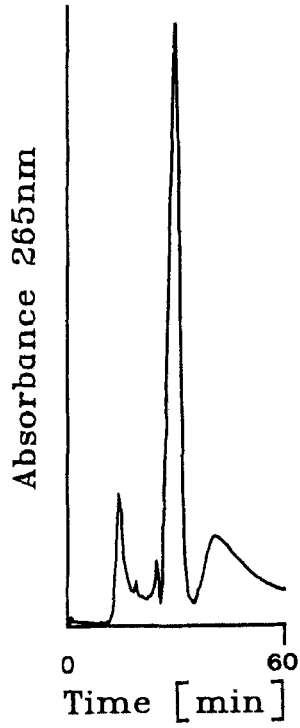


Fig.3 LC of rac. α-phenylmannoside on polymer P2. Flow 0.1 ml/min, T = 80°C, mobile phase ACN/H_2O/NH_3 82.5/12.5/5

hydroxyl pair with the functional boronic acid groups of the polymer. Increased spreading of the second peak can be interpreted as an effect of several phenomena. First, the template analogue is retarded by a two-point interaction whereas its enantiomer can only bind by a one-point interaction, a process which is considerably faster (10). For a two-point binding in the cavity the rate-constant of desorption is expected to be lower in the relatively narrow

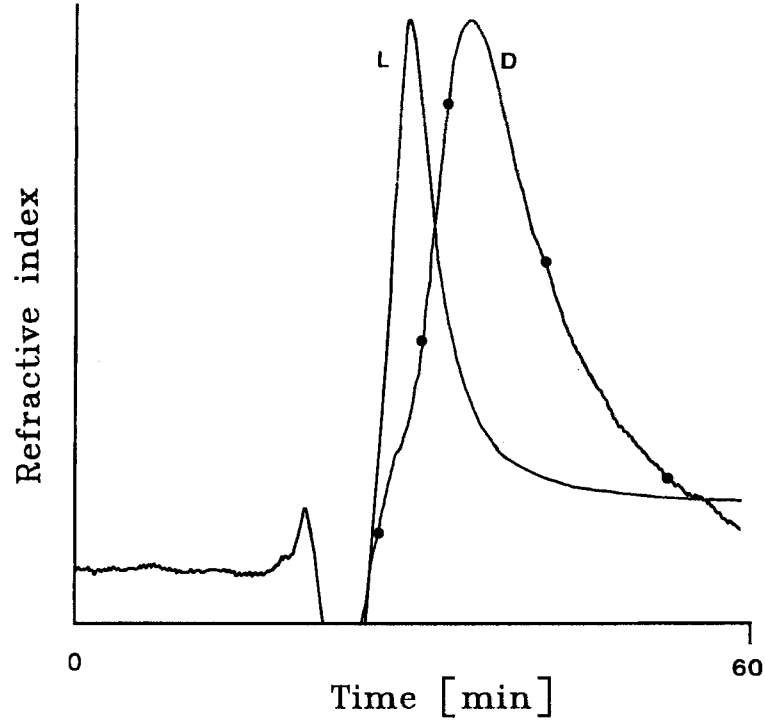


Fig.4 LC of L- and D-mannose on polymer E-3. Flow 0.1 ml/min, T = 80°C, mobile phase ACN/H_2O/NH_3 50/45/5

and rigid environment of the cavities and this can thus affect the kinetics of the binding reaction. A polymer which has more flexible functional groups is expected to have more favourable kinetics and therefore it was synthesized a binding site by introducing a spacer (methacrylic acid group) between the polymer body and the phenyl boronic acid group (polymer P3). As can be seen in Fig. 2 the chromatographic separation under optimized conditions was not improved after this modification.

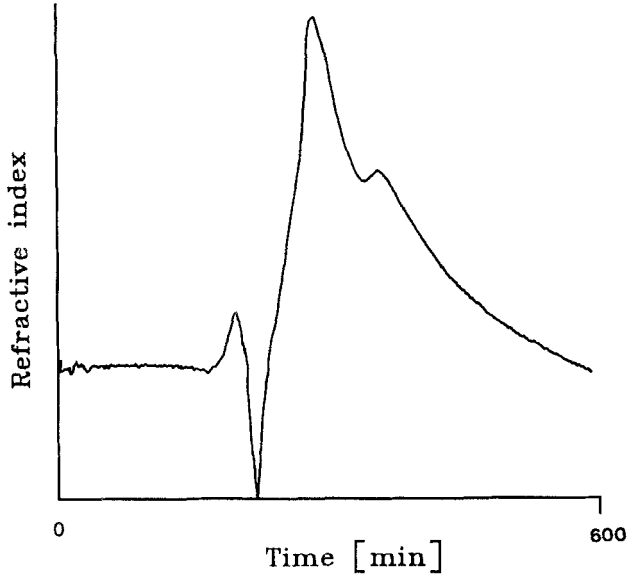


Fig.5 LC of rac. mannose on polymer E-3. Flow 0.01 ml/min, T = 80°C, mobile phase ACN/H_2O/NH_3 57.5/37.5/5

Though this separation is at higher temperature the kinetics seem to be very similar.

Both polymer sorbents, E-3 and P3, were polymerized under neutral conditions, e.g. the boron atom remained trigonal during the ester ring formation. During the HPLC we used ammonia in the mobile phase as a catalyst and this causes the boron atom to be tetrahedral. To test what effect the difference in coordination on the boron atom might have, the polymer P2 was polymerized in the presence of acetonitrile containing 5% aqueous solution of conc. NH_3 (25%), i.e. under conditions close to those during the chromatography. Fig. 3

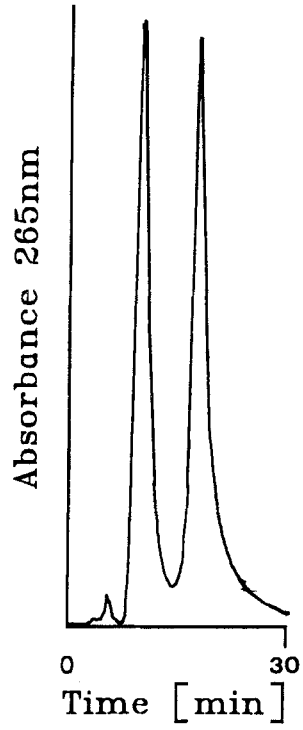


Fig.6 LC of rac. α-phenylmannoside on polymer E-3. Flow 0.5 ml/min, T = 60°C, gradient mobile phase, 92/8 - 50/50 ACN (5%NH_3)/ H_2O (5%NH_3) in 6 min, curve 8

illustrates the chromatographic property of the polymer and the similarity of this pattern indicates that neither the space requirement in the vicinity of the interacting center nor the spatial arrangement of the reacting boron atom affect to any marked extent the overall dynamics of the chromatographic process. The somewhat better separation in Fig. 3 with respect

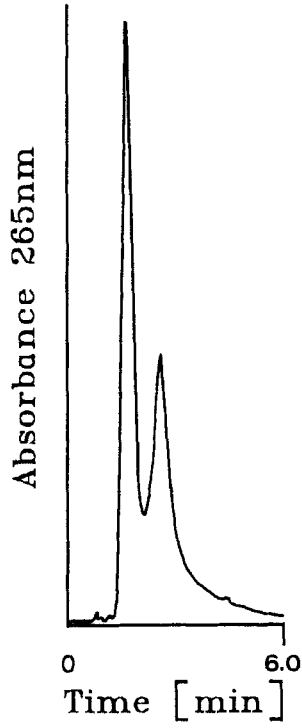


Fig.7 LC of rac. α-phenylmannoside on polymer E-3. Flow 2 ml/min, T = 80°C, gradient mobile phase, 85/15 - 30/70 ACN (5%NH_3)/ H_2O (5%NH_3) in 2 min, curve 8

to Fig. 1 is due to the higher temperature which has a strong positive effect on the separation efficiency (4).

In order to inspect the generality of the enantioselectivity of this chiral polymer, the retention of D-, and L-enantiomers of arabinose, glucose, galactose, lyxose, xylose, ribose and mannose was measured under different conditions on polymer E-3. Enantioselectivity was found only for mannose (Fig. 4 and 5)

and it is remarkable that the racemic resolution of mannose on this polymer is possible although the template is different. This finding offers the possibility of the racemic resolution of underivatized sugar which is not possible by other methods at the present time. It gives also an insight into the separation mechanism of template imprinted polymers. It encourages us to postulate that more than the precise shape of the cavities, the spatial arrangement of the interactive groups within the cavities plays a predominant role in the enantioselective separation mechanism of this sorbent type (13).

Further improvement of this chromatographic model can be achieved by gradient elution. Figs. 6-7 show the separation of Á-phenylmannoside enantiomers under different gradient conditions. Baseline separation of both the enantiomers with resolution 4.3 (calculated from band width at half-height) is reached in 25 min (Fig. 6) which is good enough for more than 99% enantiomeric purity. This result can serve as a base for highly efficient preparative applications if practicable examples suitable for this model are found. The chromatographic separation illustrated in Fig. 7 characterized by a resolution of 1.8 and analysis time of 4 min entitles us to classify the process as the "High Performance Liquid Chromatography" separation of enantiomers.

LITERATURE

1. G. Wulff, D. Oberkobusch, M. Minárik, Reactive Polymers 3, 261 (1985).

2. G. Wulff, H.-G. Poll, M. Minárik, J. Liqu. Chromatogr. 9, 385 (1986).

3. G. Wulff, M. Minárik in: M. Zief, L.J. Crane (Eds.) "Chromatographic Chiral Separations", Marcel Dekker, p.15, Marcel Dekker, New York, 1988.

4. G. Wulff, M. Minárik, J.H.R Chromatogr., Chromatogr. Commun. 9, 607 (1986).

5. G. Wulff, A. Sarhan, K. Zabrocki, Tetrahedron Lett. 44, 4329 (1973).

6. G. Wulff, W. Vesper, R. Grobe-Einsler, A. Sarhan, Makromol. Chem. 178, 2799 (1977).

7. Review see: G. Wulff in: W.T. Ford (Ed.) "Polymeric Reagents and Catalysts", ACS Symp. Ser. 308, 186 (1986).

8. L. Andersson, B. Sellergren, K. Mosbach, Tetrahedron Lett. 25, 5211 (1984).

9. B. Sellergren, M. Lepistö, K. Mosbach, J. Am. Chem. Soc. 110, 5853 (1988).

10. G. Wulff, D. Oberkobusch, M. Minárik in: G. v. Binst (Ed.) "Proceedings of the XVIIIth Solvay Conference on Chemistry", p.229, Springer Verlag, Berlin, 1986.

11. G. Wulff, J. Vietmeier, H.-G. Poll, Makromol. Chem. 188, 731 (1987).

12. G. Wulff, H.-G. Poll, Makromol. Chem. 188, 741 (1987).

13. G. Wulff, S. Kaess, J. Am. Chem. Soc. submitted.