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# Semi-preparative high-performance liquid chromatographic resolution of brompheniramine enantiomers using β-cyclodextrin in the mobile phase

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ABSTRACT

The semi-preparative reversed-phase HPLC separation of brompheniramine enantiomers using β-cyclodextrin as a chiral mobile phase additive is described. Enantiomers were recovered free from mobile phase components on-line by a novel column switching procedure. On a 250 mm × 10 mm I.D. cyano column, a throughput of 8 mg per hour (0.65 mg per gram column packing per hour) was achieved at product optical purities of 88% and above. This was achieved using two passes through the system.

Comparison is made with other reported semi-preparative chiral separations using β-cyclodextrin, and the potential utility of the described method discussed.

## INTRODUCTION

β-Cyclodextrin has found much use in recent years as a chiral resolving agent in HPLC, either as a mobile phase additive [1–3] or incorporated into stationary phases [4–6]. The cyclodextrin imparts enantioselectivity to the chromatographic system by formation of diastereomeric inclusion complexes with the analyte enantiomers [7].

The majority of reported enantiomer separations using cyclodextrins have been carried out only on an analytical scale. However, there is a need in the pharmaceutical industry for semi-preparative separation of enantiomers in order to provide milligram quantities of pure enantiomers for pharmacological investigation.

Preparative chiral separations on cyclodextrin stationary phases have been reported. Thus, β-cyclodextrin polymer gels have been used for the mg-scale resolution of racemates such as methyl mandelate [8] and indole alkaloids [9]. In these cases, low throughputs were obtained owing to the poor efficiency of these gels and the low flow-rates employed.

Vigh *et al.* [10–12] have recently shown that β-cyclodextrin-silica HPLC stationary phases may be employed to effect semi-preparative separation of isomeric compounds in displacement mode. Using two 250 × 4.6 mm I.D. Cyclobond I columns in series, up to 6 mg of various racemates were resolved in high yield and optical purity in run times of 3–6 hours [12].

Work in this laboratory has focused on the possibility of using β-cyclodextrin as a mobile phase additive for semi-preparative chiral separations in elution mode, taking advantage of the distinct selectivity obtained in this way. Hitherto, the use of mobile phase additives has largely been limited to analytical scale chiral separa-

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tions, owing to the difficulty of separating the resolved enantiomers from the chiral additive. A novel solution to this problem has recently been presented from this laboratory, involving on-line recovery of the resolved enantiomers from the chiral eluent via column switching [13]. This was initially applied to the resolution of trimeprazine enantiomers, a racemate that is easily resolved using β-cyclodextrin (selectivity, $\alpha = 1.24$, $R_s = 2.1$ at low column loading). A throughput in excess of 1 mg per hour was obtained, using only a small column (100 mm length × 4.6 mm I.D.).

In this paper, the semi-preparative resolution of brompheniramine enantiomers by a similar procedure is presented. The separation of brompheniramine enantiomers using β-cyclodextrin-containing eluents on analytical scale has previously been described by Mularz [14], who reported selectivity, $\alpha$, of only 1.13 ($R_s = 1.7$) in this case. The semi-preparative resolution of this racemate therefore provides an illustration of the application of β-cyclodextrin as an eluent additive with on-line recovery of enantiomers to racemates where only moderate selectivity is observed.

## EXPERIMENTAL

### *Equipment and materials*

The chromatograph consisted of an LDC Constametric $III_G$ pump and LDC Spectromonitor III and Pye-Unicam LC3 UV detectors. A valve switching unit (purpose-built) was provided by ICI Research Engineering Laboratory, Alderley Park, Macclesfield, UK, and consisted of two Rheodyne 7010 valves controlled by a timer unit.

Spherisorb S5CN columns were obtained from Hichrom (Theale, Berks., UK). A Spherisorb S5C6 column was obtained from Capital HPLC (Edinburgh, UK). Lichroprep RP18 25–40 μm material was obtained from BDH (Poole, Dorset, UK). Hamilton PRP-1 12–20 μm material was donated by Hamilton (Reno, NV, USA). Recovery columns (100 × 10 mm cartridges and holders) were donated and packed by SGE (Milton Keynes, UK).

Acetonitrile (MeCN), methanol (MeOH), ethanol (EtOH) and triethylamine (TEA) (all HPLC grade), glacial acetic acid (HOAc), maleic acid (both SLR grade) and dimethylsulphoxide ($Me_2SO$) (AR grade) were obtained from Fisons (Loughborough, UK). β-cyclodextrin (β-CD) hydrate was donated by Wacker Chemie (Munich, Germany). Brompheniramine and pheniramine (both as racemic maleate salts) were donated by A.H. Robins (Horsham, Sussex).

### *Resolution of brompheniramine enantiomers*

Brompheniramine enantiomers were separated on a 250 mm × 10 mm I.D. column containing 5-μm cyanopropyl-silica (Spherisorb S5CN), using a mobile phase consisting of methanol–aqueous triethylammonium acetate [0.85% TEA (v/v), acetic acid to pH 4] (5:95, v/v) containing 12 mg $ml^{-1}$ β-cyclodextrin hydrate, at a flow-rate of 3.5 ml $min^{-1}$.

The instrumentation employed to effect resolution and recovery of enantiomers is shown in Fig. 1. The pump was an LDC Constametric $III_G$. Detector 1 was an LDC Spectromonitor III UV detector operated at 285 nm, 2.0 AUFS, and was used to monitor the separation of enantiomers. Detector 2 was a Pye-Unicam LC–UV detector operated at 290 nm, 1.28 AUFS, and

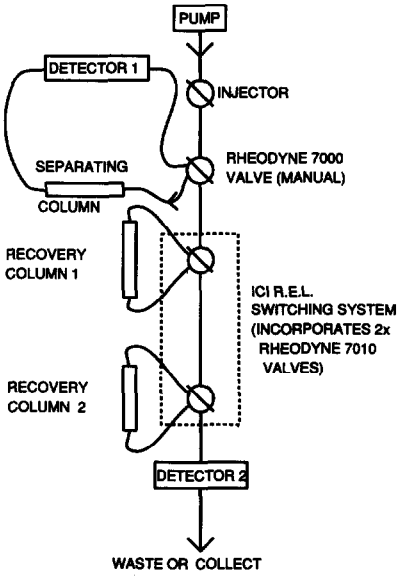


Fig. 1. Column switching system for on-line recovery of enantiomers following chiral separation.

was used to monitor the effluent from the on-line recovery system.

Injections of ±-brompheniramine maleate (5 mg) were made onto the separating column using a Rheodyne 7125 injector equipped with a 100-$\mu$l loop. Following separation, the enantiomers were switched onto two recovery columns (100 mm × 10 mm I.D.) packed with 12–20 $\mu$m polystyrene-divinylbenzene (Hamilton PRP-1) using the purpose-built valve switching system. A total of 22–24 repeat injections of racemate were made, giving a total recovery column loading of about 60 mg per enantiomer. The recovery columns were then each flushed with 180 ml of methanol–water (5:95, v/v), and the brompheniramine fractions were eluted with methanol. The separating column was by-passed during the flushing and elution stages of the procedure so as to avoid the need to re-equilibrate with mobile phase between runs.

A sample of each fraction was retained for subsequent analysis. The remainder of each fraction was evaporated to dryness, re-dissolved in mobile phase, and further purified by a second pass through the system as described above.

*HPLC assay of fractions*

The fractions were assayed for recovery and optical purity using a Spherisorb C6 column (150 mm × 3 mm I.D.) with a mobile phase consisting of acetonitrile–aqueous triethylammonium acetate [0.89% TEA (v/v), acetic acid to pH 4] (10:90, v/v) containing 21 mg ml$^{-1}$ $\beta$-cyclodextrin hydrate, at a flow-rate of 1 ml min$^{-1}$.

Calibration standards of ±-brompheniramine maleate at 0.01, 0.05 and 0.25 mg ml$^{-1}$ in mobile phase were prepared and injected in triplicate to produce a calibration graph for each enantiomer using peak height ratio brompheniramine : internal standard (±-pheniramine maleate).

*NMR assay of fractions*

The chemical purity of the fractions was assessed by comparison of their 270-MHz $^1$H-NMR spectra in $^2H_2O$ with that of the unresolved standard. Recovery was also checked by NMR, by addition of one mole equivalent of maleic acid and comparison of the resulting proton signal integrals.

*Optimisation of enantiomer separation*

Initial optimisation was carried out on an analytical (250 mm × 4.6 mm I.D.) column packed with Spherisorb S5CN, with mobile phase flow-rate 1 ml min$^{-1}$.

*Optimisation of recovery procedure*

The recovery procedure was optimised using "off-line" experiments in which recovery columns (packed with either Lichroprep RP18 25–40 $\mu$m or Hamilton PRP-1 12–20 $\mu$m) were loaded with brompheniramine dissolved in mobile phase, and flushed with various solvents before the brompheniramine was eluted with methanol, and the recovery and optical purity assessed by NMR. In this way, the performances of the two above materials were compared, and the procedure optimised.

## RESULTS

*Optimisation of brompheniramine enantiomer separation*

The effect of mobile phase composition on resolution of brompheniramine enantiomers at low column loading is illustrated in Table I.

It is well established that the presence of organic modifiers reduces enantioselectivity induced by $\beta$-cyclodextrin in the mobile phase [1]. Low eluent modifier levels were therefore em-

TABLE I

EFFECT OF MOBILE PHASE COMPOSITION ON RESOLUTION OF BROMPHENIRAMINE ENANTIOMERS (2 $\mu$g ON COLUMN) ON A SPHERISORB S5CN (250 × 4.6 mm I.D.) COLUMN

All eluents made up to 100% with buffer [0.8% (v/v) triethylamine, acetic acid to pH 4].

| Modifier | % (v/v) | [$\beta$-CD] (mg ml$^{-1}$) | $k'_1$ | $\alpha$ | $R_s$ |
|---|---|---|---|---|---|
| MeCN | 5 | 16 | 0.9 | 1.14 | 1.0 |
| | 10 | 20 | 0.8 | 1.12 | 0.7 |
| MeOH | 3 | 11 | 1.5 | 1.14 | 0.9 |
| | 5 | 11 | 1.7 | 1.14 | 0.8 |
| $Me_2SO$ | 5 | 11 | 1.6 | 1.12 | 1.1 |
| | | 20 | 1.1 | 1.15 | 0.9 |
| EtOH | 5 | 20 | 0.9 | 1.12 | 0.8 |

ployed. It was necessary then to use a column packing of low hydrophobicity, such as cyanopropyl-silica, in order to give reasonable solute retention values. Another significant factor was cyclodextrin solubility. This was found to be reduced on addition of methanol, but enhanced by the presence of the other modifiers employed. Addition of triethylamine to the mobile phase improved peak shape but had little effect on selectivity. Selectivity was found to be maximised at pH values less than 6, with the solute molecules in cationic form.

Highest selectivity was obtained using a mobile phase containing 5% (v/v) $Me_2SO$ and 16 mg $ml^{-1}$ $\beta$-cyclodextrin. Highest resolution at 2 $\mu$g loading was observed with a mobile phase containing the same level of modifier but lower cyclodextrin content, due to increased retention. Differences between the resolution values obtained with the eluents investigated were generally small, as Table I shows. Highest resolution at 1 mg on column was obtained using a mobile phase containing 5% (v/v) MeOH and 11 mg $ml^{-1}$ $\beta$-cyclodextrin. The resolution obtained using this mobile phase on a semi-preparative (10 mm I.D.) column is illustrated in Fig. 2. An amount of 5 mg was deemed to be the optimum loading on this column, on the basis of using two passes through the semi-preparative system to obtain high optical purity with minimum wastage of material.

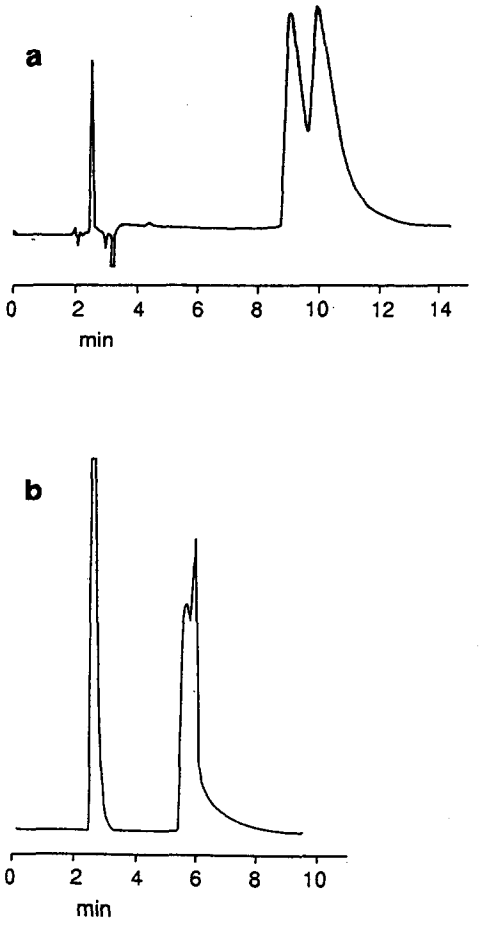


Fig. 2. Resolution of brompheniramine enantiomers on an S5CN column (250 × 10 mm I.D.). Conditions as text. (a) 10 $\mu$g ±-brompheniramine maleate on column. Detection: UV 254 nm, 0.1 AUFS; (b) 5 mg ±-brompheniramine maleate on column. Detection: UV 285 nm, 2.0 AUFS.

*Optimisation of recovery procedure*

The capacities of Hamilton PRP-1 and Lichroprep RP18 for brompheniramine, loaded at 1 mg $ml^{-1}$ in the chosen eluent, were measured in off-line "breakthrough" experiments and found to be 3.8 mg per gram of packing material (PRP-1) and 2.8 mg per gram packing material (RP18). The higher capacity of the polymeric material was found to allow a much more thorough flushing step, resulting in a product free from cyclodextrin or buffer components. Hamilton PRP-1 was therefore the material used to pack the recovery columns for on-line use.

The length of the flushing step required to yield chemically pure product was found to be substantial —about 40 column volumes. However, use of a switching valve to by-pass the analytical column during flushing of the recovery columns allowed a high flow-rate to be used during flushing. This, together with the fact that a number of injections of racemate could be made before recovery column flushing, ensured that this part of the procedure made up less than 20% of the overall time involved.

*Semi-preparative resolution of brompheniramine enantiomers with on-line recovery*

The purity and recovery of the fractions obtained after each pass through the system are summarised in Table II. The HPLC analysis of the fractions is illustrated in Fig. 3. The more

TABLE II

OPTICAL PURITY (ENANTIOMERIC EXCESS, e.e.) AND RECOVERY OF BROMPHENIRAMINE ENANTIOMERS AFTER SEMI-PREPARATIVE RESOLUTION ON A 250 mm × 10 mm I.D. COLUMN (CONTAINING 11.8 g PACKING MATERIAL), DETERMINED BY HPLC ASSAY OF FRACTIONS

Conditions as in text.

| | After 1st pass | | After 2nd pass | |
|---|---|---|---|---|
| | Peak 1 | Peak 2 | Peak 1 | Peak 2 |
| Recovery (%) | 68.0 | 72.0 | 49.7 | 35.1 |
| e.e. (%) | 82.2 | 75.8 | 95.4 | 88.0 |
| Throughput (mg/h) | 11.9 | 12.8 | 4.5 | 3.2 |

efficient and retentive C6 column gave near-baseline resolution of the enantiomers under analytical conditions (although was too retentive to give high throughput in semi-preparative mode).

After one pass through the system, the fractions showed enantiomeric excess values less than the 90% required for pharmacological use. The second pass resulted in a substantial improvement in optical purity.

Recoveries were 35–50% overall. No brompheniramine was detected in the eluate from the recovery columns on flushing with methanol–water. The loss of brompheniramine observed must therefore reflect the diversion of optically impure material to waste between the two collected fractions. In this respect, there is a "trade-off" between throughput, recovery and optical purity.

Fig. 4 illustrates the chemical purity of the fractions. They were found to contain up to 0.4 mol% of β-cyclodextrin, but no triethylamine or other buffer components were detected by NMR.

The optical rotations of the products as free base in dimethylformamide were determined. The first eluting peak gave a positive rotation and the second peak a negative rotation. As might be anticipated, this elution order is the reverse of that reported on a Cyclobond I column [15].

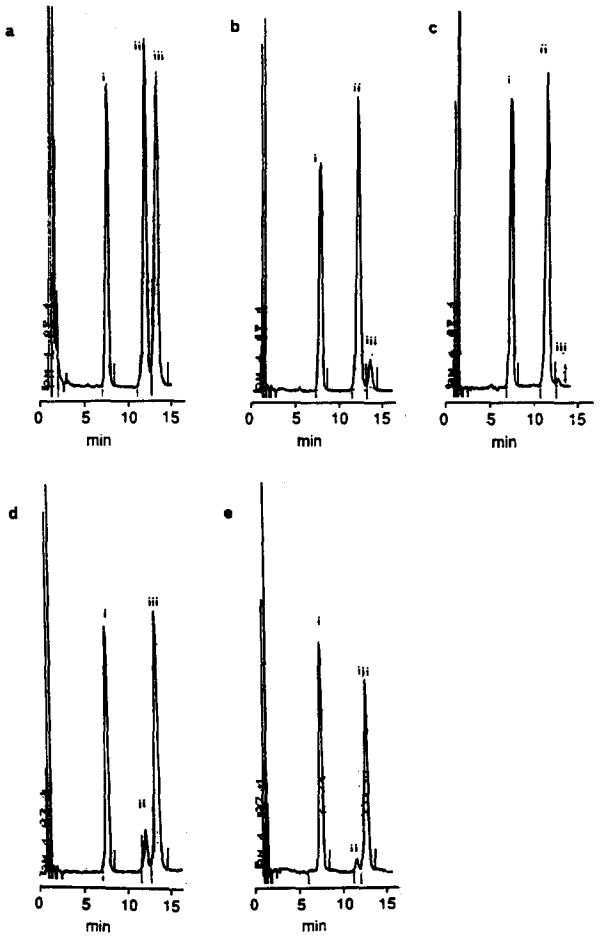


Fig. 3. Analytical resolution of brompheniramine enantiomers on S5C6 column (150 × 3 mm I.D.). Conditions as text. Peak identities: i = ±-pheniramine (I.S., 1 μg on column); ii and iii = brompheniramine enantiomers. (a) ±-Brompheniramine maleate standard, 3.5 μg on column; (b) peak 1 fraction after 1st pass; (c) peak 1 fraction after 2nd pass; (d) peak 2 fraction after 1st pass; (e) peak 2 fraction after 2nd pass.

Further confirmation of the antipodal nature of the products was provided by their NMR spectra in the presence of β-cyclodextrin. As reported by Casy and Mercer [16], the addition of β-cyclodextrin to racemic brompheniramine results in duplication of proton NMR signals. On addition of β-cyclodextrin to the resolved products, no duplication of signals was observed (resolution being too low to allow detection of the minor antipode in each fraction). On mixing

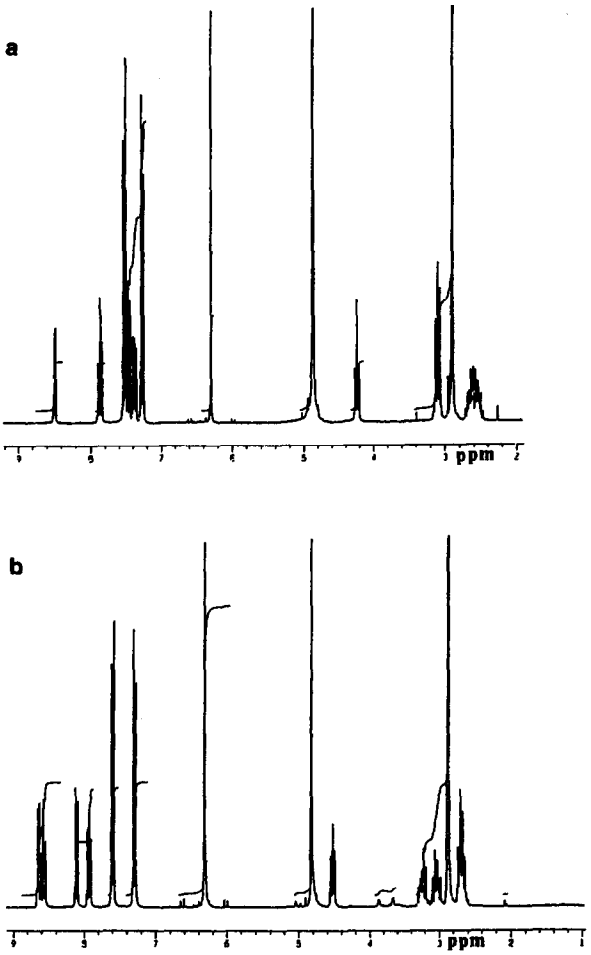


Fig. 4. 270-MHz $^1$H-NMR spectra of brompheniramine maleate in $^2H_2O$. (a) Racemate (standard). (b) HPLC peak 1 fraction after 2nd pass.

the fractions to re-form a racemate, duplication of some signals in the presence of the cyclodextrin was again observed.

## DISCUSSION

Table III summarises the semi-preparative enantiomer separations carried out in this laboratory using β-cyclodextrin. It can be seen that semi-preparative product purity and throughput depend to a large extent on the selectivity obtained under analytical conditions. The work reported herein is of value in demonstrating that useful separation can be carried even when analytical selectivity is only moderate. This is particularly important, as it is rare for β-cyclodextrin to impart selectivity greater than that seen for brompheniramine. The majority of semi-preparative separations using this resolving agent are therefore likely to need the kind of approach developed here.

The throughput obtained here using β-cyclodextrin as a mobile phase additive was 0.65 mg racemate per gram column packing per hour. This is similar to the throughputs achieved by Vigh *et al.* [10–12], using a β-cyclodextrin stationary phase in displacement mode. However, the latter technique was applied to somewhat more difficult separations in elution mode than brompheniramine, and resulted in high yields of products at higher optical purities (>99%). This is not unexpected, since displace-

TABLE III

SUMMARY OF SEMI-PREPARATIVE CHIRAL SEPARATIONS CARRIED OUT USING β-CYCLODEXTRIN-CONTAINING ELUENTS

| Racemate | α | e.e. (%) | | Throughput [mg/(hg)] | Ref. |
|---|---|---|---|---|---|
| | | Peak 1 | Peak 2 | | |
| TA1 | 1.61 | 99.1 | 98.4 | 3.5 | [18] |
| Trimeprazine | 1.33 | 99 | 92 | 4.4 | [13] |
| TA12 | 1.22 | 96.3 | 87.6 | 2.1 | [18] |
| Brompheniramine | 1.14 | 95.4 | 88.0 | 0.7 | |

ment mode is widely reported to be more efficient than elution mode for preparative scale separations [17].

However, the use of β-cyclodextrin as a mobile phase additive in elution mode may still be advantageous. Optimisation of semi-preparative separation in elution mode is more facile, since it is based closely on an analytical-scale separation. Use of β-cyclodextrin as an eluent additive allows more flexibility in optimisation than when a Cyclobond stationary phase is used, since column type and cyclodextrin type may be varied with ease. Furthermore, this technique will be particularly applicable to solutes where better inherent enantioselectivity is obtained using β-cyclodextrin as an eluent additive. Differences in this respect result from the modification of the cyclodextrin on bonding to the silica support to form a stationary phase.

The on-line recovery technique applied here overcomes the most obvious disadvantage of the use of chiral mobile phase additives, *i.e.* the potential difficulty in separating the resolved enantiomers from the additive. The approach used also produced products free from other buffer components. This is of particular importance in resolution of pharmaceutical racemates, which are often basic compounds requiring the addition of mobile phase components such as triethylamine to achieve efficient chromatography.

## ACKNOWLEDGEMENTS

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## REFERENCES

1 D. Sybilska, *Ordered Media in Chemical Separations* (*ACS Symposium Series*, No. 326), American Chemical Society, Washington, DC, 1987 p. 218.
2 M. Gazdag, G. Szepesi and L. Huszár, *J. Chromatogr.*, 351 (1986) 128.
3 D.W. Armstrong, L.A. Spino, S.M. Han, J.I. Seeman and H.V. Secor, *J. Chromatogr.*, 411 (1987) 490.
4 K.G. Feitsma, J. Bosman, B.F.H. Drenth and R.A. de Zeeuw, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 7 (1984) 147.
5 T.J. Ward and D.W. Armstrong, *J. Liq. Chromatogr.*, 9 (1986) 407.
6 S.M. Han, Y.I. Han and D.W. Armstrong, *J. Chromatogr.*, 441 (1988) 376.
7. A. Cooper and D.D. MacNicol, *J. Chem. Soc. Perk. Trans. II*, (1978) 760.
8 A. Harada, M. Furue and S. Nozakura, *J. Polym. Sci: Polym. Chem. Ed.*, 16 (1978) 189.
9 B. Zsadon, L. Décsei, M. Szilasi, F. Tüdôs and J. Szejtli, *J. Chromatogr.*, 270 (1983) 127.
10 G. Vigh, G. Quintero and G. Farkas, *J. Chromatogr.*, 484 (1989) 237.
11 G. Vigh, G. Quintero and G. Farkas, *J. Chromatogr.*, 484 (1989) 251.
12 G. Vigh, G. Quintero and G. Farkas, *J. Chromatogr.*, 506 (1990) 481.
13 A.D. Cooper and T.M. Jefferies, *J. Pharm. Biomed. Anal.*, 8 (1990) 847.
14 E.A. Mularz, *Ph.D. Dissertation*, Seton Hall University, NJ, 1988.
15 A.D. Mercer, *Ph.D. Dissertation*, University of Bath, Bath, 1989.
16 A.F. Casy and A.D. Mercer, *Mag. Reson. Chem.*, 26 (1988) 765–774.
17 Cs. Horváth, in F. Bruner (Editor), *The Science of Chromatography* (*Journal of Chromatography Library*, Vol. 32), Elsevier, Amsterdam, 1985, pp. 179–205.
18 A.D. Cooper, *Ph.D. Dissertation*, University of Bath, Bath, 1991.