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STUDIES OF MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

II. APPLICATION TO GEL PERMEATION CHROMATOGRAPHY OF TECHNIQUES DEVELOPED FOR MICRO HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Various micro high-performance liquid chromatographic techniques have been successfully applied to gel permeation chromatography by reducing detector cell volume, injection volume and void volume in ports and joints. Phthalic esters, various oligomers and polymers were separated on appropriate micro columns, as well as on ordinary wide-bore columns. Use of these micro columns means that smaller amounts of expensive packing materials and carrier solvents are required, and that the chromatographic operations and instruments can be simplified.

INTRODUCTION

Gel permeation chromatography (GPC) has a unique separation mechanism based on differences in the molecular size of the sample components. It is a powerful method, especially for the separation and identification of high-molecular-weight substances and the determination of molecular-weight distribution[1–6]. Column dimensions of *ca.* 8 mm I.D. and 30–100 cm length have been generally adopted[7–9], except in a few instances[6,10]. The diameter of these columns is appreciably larger than those of the columns used in other high-performance liquid chromatographic (HPLC) methods, for the following reason. The partition coefficient (K_D) in GPC is 0–1 because of the chromatographic mechanism, so that a solute component gives a relatively small dispersion. Therefore, if HPLC columns (2–3 mm I.D.) are used for GPC, the dispersion in the columns becomes so small that the dispersion in void volume in other parts of the apparatus (mainly joints and injection ports) becomes significant.

However, use of wide-bore columns requires large amounts of expensive packing materials and carrier solvents. Furthermore, the solute components may disperse widely, resulting in a decrease in the sensitivity.

We have developed micro high-performance liquid chromatography (MHPLC) for various columns by using the many available techniques[11]. If MHPLC techniques

could be applied to GPC, then the consumption of packing materials, carrier solvents and sample solutions would be decreased and the sensitivity increased. The dispersion of sample components would be smaller, and miniaturization of GPC columns would make it smaller still, perhaps as little as 1–5 μl. We have investigated these possibilities, using a detector cell system and injection system suited to GPC micro columns, and achieved the separation of phthalic esters, oligomers and polymers.

EXPERIMENTAL

Packing materials and preparation of micro columns

The packing materials used are listed in Table I, by their commercial names. Fluororesin was used as the main column material. GPC micro columns were prepared as described for MHPLC[11]. The dispersion of a solute component in GPC micro column is so small that the detector cell volume and the injection volume must be smaller than that for ordinary MHPLC, and "on-column" and "on-cell" systems must be adopted in order to minimize the dispersion in other parts of the apparatus. These problems were solved by the application of MHPLC techniques.

TABLE I

PHYSICAL CHARACTERISTICS OF PACKING MATERIALS

*Packing materials**	*Particle size (μm)*	*Excluded mol.wt.***
TSK G1000H	15	$1 \cdot 10^3$
TSK G2000H	—	$1 \cdot 10^4$
TSK G4000H	5	$4 \cdot 10^5$
Shodex A-801	10	$1 \cdot 10^3$
Shodex A-802	10	$5 \cdot 10^3$
Shodex A-804	10	$5 \cdot 10^5$
HSG-10	8 ~ 10	$3 \cdot 10^2$
HSG-15	7 ~ 9	$3 \cdot 10^3$
HSG-50	8 ~ 10	$1 \cdot 10^6$

* These are the commercial names.

** Based on polystyrene mol.wt.

Chromatographic technique

Methods similar to those used in ordinary MHPLC were employed for the feed of carrier liquid and the injection of sample solution. The method of injection was slightly different from that described previously[11]. The sample solution was drawn into a stainless steel tube, with *ca.* 0.1 μl of air, and the tip tube mopped with gauze before being connected to the micro column. The air disappeared in the micro-column owing to the pressurized passage of the carrier liquid, and it did not influence the chromatographic operation. A UV spectrophotometer equipped with a micro flow-cell was used as the detector system.

RESULT AND DISCUSSION

Relationship between flow-rate and column efficiency

Fig. 1 shows the relationship between flow-rate and column efficiency on the GPC micro columns packed with various stationary phases, in the region of low

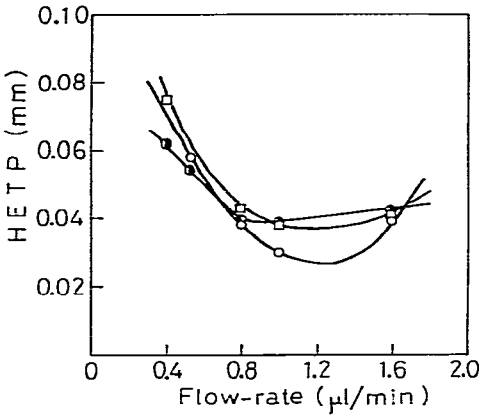

Fig. 1. Relationship between HETP and flow-rate. Columns: ○, 25 cm × 0.5 mm I.D. Shodex A-801; □, 16 cm × 0.5 mm I.D. TSK G2000H; ◑, 16 cm × 0.5 mm I.D. HSG-10. Sample, 1% benzene in tetrahydrofuran; injection volume, 0.01 μl; eluent, THF; detection wavelength, 254 nm.

flow-rate. In the region of high flow-rate, the relationship was similar to that in the ordinary GPC columns. However, column efficiency was reduced at extremely low flow-rates, which does not occur with ordinary GPC columns. The increase of HETP in flow-rates below 1 μl/min may result from the fact that the contribution to column efficiency by longitudinal diffusion of molecules, corresponding to the second term in van Deemter equation, is generally negligible in HPLC, but, at the low flow-rates in GPC micro columns, this diffusion in the mobile phase is no longer negligible and may contribute to the column efficiency. The minimum HETP values in Fig. 1 (0.03–0.04 mm) are not inferior to those obtaining in ordinary GPC.

The influence of sample size on the column efficiency and the separation of sample components

The sample size must be reduced in proportion to the column size and the column capacity. Fig. 2 shows the effects of varying the sample size on the resolution: the smaller the sample, the better the resolution. The injection volume must therefore be as small as possible, preferably 0.02 μl or less. Such very small amounts of sample solution can be injected by using the method developed during the investigation of MHPLC[11].

The influence of flow-cell volume on the resolution

Use of a GPC micro column may make the dispersion of solute components very small (less than a few microlitres), and the volume of the micro flow-cell must be suitably reduced. The influence of flow-cell volume on the separation of sample components was investigated and the results are shown in Fig. 3. With a flow-cell of volume 0.63 μl, the separation of dioctyl phthalate (DOP) and dibutyl phthalate (DBP) was incomplete. However, DOP and DBP were satisfactorily separated using a micro flow-cell of volume 0.13 μl. These results show that the cell volume should preferably be 0.1 μl or less.

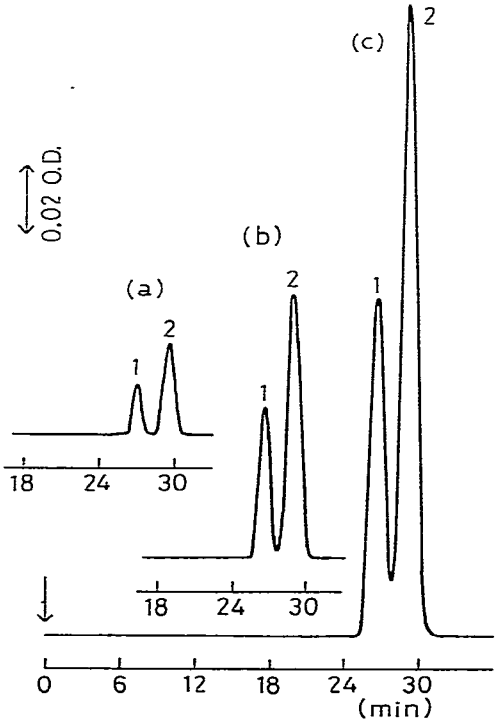

Fig. 2. Effect of sample size on resolution. Column, 16 cm × 0.5 mm I.D. Shodex A-801. Peaks: 1 = dioctyl phthalate; 2 = dibutyl phthalate. Sample, 0.49% DOP and 0.51% DBP in tetrahydrofuran. Sample size: (a) 0.006 μl; (b) 0.02 μl; (c) 0.06 μl. Eluent, tetrahydrofutan; flow-rate, 0.67 μl/min; detection wavelength, 244 nm.

Examples of GPC separation

Separation of phthalic esters. Fig. 4 shows a chromatogram of dilauryl phthalate (DLP), DOP, DBP and diethyl phthalate (DEP) on a 25 cm × 0.5 mm I.D. Teflon column packed with Shodex A-801. They were satisfactorily separated, and the dispersion of each peak was only 1.5 μl. The GPC micro column gave HETP values of 0.04–0.08 mm, which are not inferior to those obtaining in ordinary GPC using relatively wide-bore columns.

The separation of the phthalic esters was carried out on a 50 cm × 0.25 mm I.D. column packed with TSK G1000H, and on a 31 cm × 1.0 mm I.D. column packed with Shodex A-801. As the cross-sectional area of the former column is only one-thousandth that of an ordinary GPC column, chromatographic operating conditions may be reduced by a factor of 10^3. Carrier flow-rate was reduced to 0.4 μl/min and sample amounts to 50 ng, and consequently the dispersion of chromatogram to 0.7 μl. The total amount of carrier liquid required to complete chromatographic run was 16 μl. Under these conditions, good separations were achieved within 40 min. The separation of phthalic esters on the 1.0 mm column was somewhat better than on the 0.5 mm column, and was achieved within 18 min. A typical chromatogram of dinonyl phthalate (DNP), DLP, diheptyl phthalate (DHP), DBP, DEP and dimethyl phthalate (DMP) on the 1.0 mm column is shown in Fig. 5.

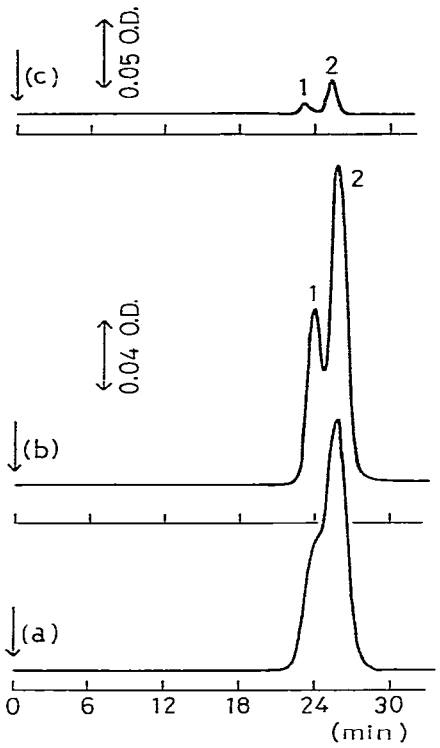

Fig. 3. Effect of cell volume and sample size on resolution. Column, 12 cm × 0.5 mm I.D. Shodex A-801. Sample: 0.47% DOP and 0.53% DBP. Sample size: (a) and (b) 0.06 μl; (c) very small, Cell volume: (a) 0.64 μl; (b) and (c) 0.13 μl. Peaks: 1, DOP; 2, DBP. Eluent, tetrahydrofuran; flow-rate, 0.67 μl/min.

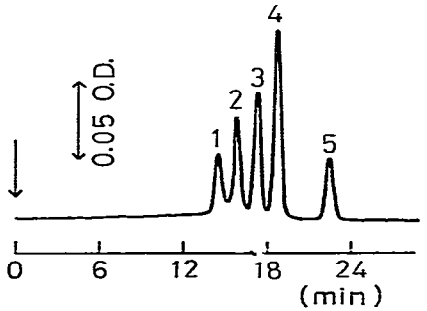

Fig. 4. Separation of a mixture of DLP, DOP, DBP, DEP, and benzene. Peaks: 1 = DLP; 2 = DOP; 3 = DBP; 4 = DEP; 5 = benzene. Column, 25 cm × 0.5 mm I.D. Shodex A-801. Sample: 0.8% DLP, 0.8% DOP, 0.8% DBP, 1.0% DEP and 1.3% benzene in tetrahydrofuran. Sample size, 0.01 μl; eluent, tetrahydrofuran; flow-rate, 1.6 μl/min.

These results show that the micro columns are applicable to GPC, as the column efficiency and separation are as good as the commercially available wide-bore columns can achieve.

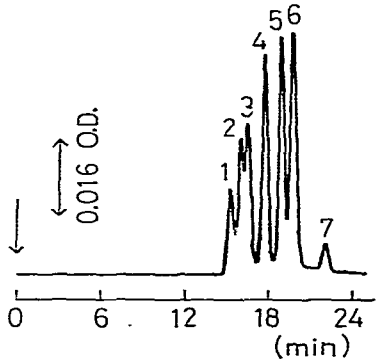

Fig. 5. Separation of a mixture of DLP, DNP, DHP, DBP, DEP, DMP and benzene. Peaks: 1 = DLP; 2 = DNP; 3 = DHP; 4 = DBP; 5 = DEP; 6 = DMP; 7 = benzene. Column, 32 cm × 1 mm I.D. TSK G1000H. Sample: 0.60% DLP, 0.62% DNP, 0.62% DHP, 0.57% DBP, 0.63% DEP, 0.61% DMP and 0.97% benzene in tetrahydrofuran. Sample size. 0.006 μl; eluent, tetrahydrofuran; flow-rate, 8 μl/min.

Separations of oligomers. It was relatively difficult to separate oligomers satisfactorily in a short time when ordinary equipment and column sets were used. Therefore, the separation was achieved with very long columns[12], recycle systems[12,13] or soft gels[14]. However, the analysis time was long in all cases. Recently, it was shown that high resolution is attainable using columns packed with very small gel particles, and the application of high-resolution GPC to oligomers and plasticizers was reported[8].

GPC of oligomers on the 0.5-mm GPC micro column did not give successful results, apparently because of the insufficient column capacity. The problem was solved by using the 1.0-mm GPC micro column. A chromatogram of polystyrene 600 is shown in Fig. 6. The sample components are well separated, but a faster and better separation may be possible using smaller diameter particles as packing material (*ca.* 2–3 μm).

Typical gel permeation chromatograms of oligomers of epoxy resin utilized in adhesives are shown in Fig. 7. In the separation of Epicoat 828, peaks corresponding

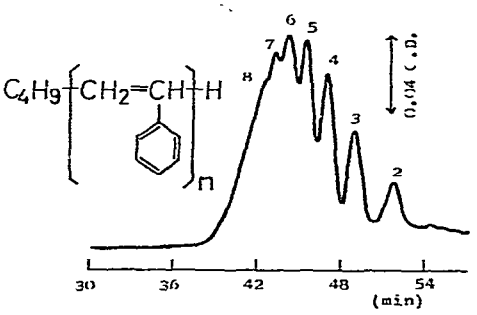

Fig. 6. Separation of polystyrene oligomers. Numbers at peaks are the degree of polymerization. Column, 31 cm × 1 mm I.D. TSK G2000H. Sample, 1.2% polystyrene 600 in tetrahydrofuran; sample size, 0.12 μl; eluent, tetrahydrofuran; flow-rate, 2.67 μl/min.

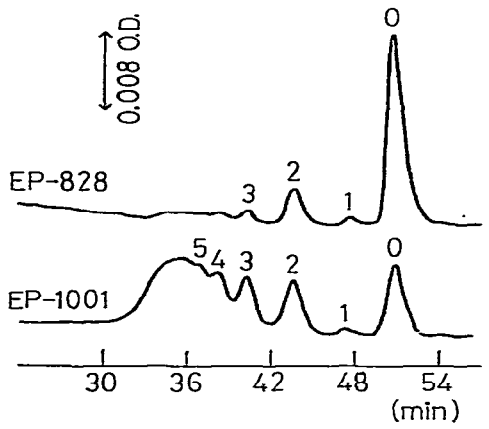

Fig. 7. Separation of polyepoxide oligomers. Column, 31 cm × 1 mm I.D. TSK G2000H. Sample, 1% Epicoat 828 and 1% Epicoat 1001 in tetrahydrofuran; Sample size, 0.12 μl; eluent, tetrahydrofuran; flow-rate, 2.67 μl/min.

to degrees of polymerization 0, 1, 2 and 3 were observed and in the separation of Epicoat 1001, peaks corresponding to degrees of polymerization 0, 1, 2, 3, 4, 5 and 6 were observed. These results show that oligomers can be well separated on appropriate GPC micro columns in a very short time. The separation may be improved by varying the chromatographic conditions.

The separation of standard polystyrene. A mixture of high-molecular-weight polymers could be separated by micro columns packed with polystyrene-gel particles with pore sizes corresponding to 400,000–500,000 excluded-molecular-weight. A typical separation of standard narrow-distribution polystyrenes on a 34 cm × 0.5 mm I.D. Teflon column packed with Shodex A-804 is shown in Fig. 8. The separation by GPC micro column was not inferior to that by the ordinary wide-bore column[7,10]. The separations were also carried out at flow-rates varying in the range 1–8 μl/min. The analysis time for one complete separation run was 14 min at a flow-rate of 4 μl/min, and only 7 min at a flow-rate of 8 μl/min. Thus, the separative analysis of polymers can be achieved in a short time using GPC micro columns.

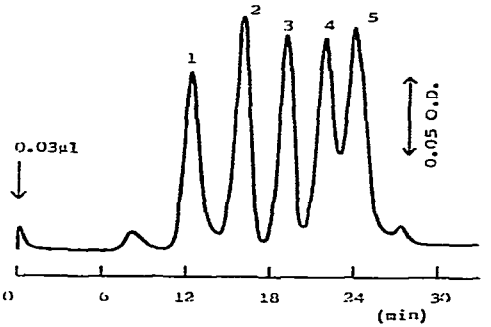

Fig. 8. Separation of standard polystyrenes. Column, 34 cm × 0.5 mm I.D. Shodex A-804. Sample size, 0.03 μl; eluent, tetrahydrofuran; flow-rate, 2 μl/min. Peaks: 1 = 498,000 (0.9%); 2 = 110,000 (0.9%); 3 = 37,000 (0.8%); 4 = 10,000 (1.0%); 5 = 2100 (1.1%).

CONCLUSION

Micro GPC was developed by application of the various techniques of MHPLC to GPC, and the required amounts of sample, packing material and carrier solvent are decreased. Micro GPC can be easily performed by simple instruments and operations, and is useful for the analysis of various polymers and oligomers. It would be applicable to preparatory experiments to select the operating conditions for fractionation by GPC.

REFERENCES

1 J. C. Moore, *J. Polym. Sci., Part A-2*, 2 (1964) 835.
2 F. Kamiyama, H. Matsuda and H. Inagaki, *Polym. J.*, 1 (1970) 518.
3 H. Inagaki, F. Kamiyama and T. Yagi, *Polym. J.*, 4 (1971) 133.
4 E. P. Otocka, *Macromolecules*, 3 (1970) 691.
5 N. Catsimpoolas and J. Kenney, *J. Chromatogr.*, 64 (1972) 77.
6 B. J. Gudzinowicz and K. Alden, *J. Chromatogr. Sci.*, 9 (1971) 65.
7 J. N. Little, J. L. Waters, K. J. Bombaugh and W. L. Pauplis, *J. Polym. Sci., Part A-2*, 7 (1969) 1775.
8 Y. Kato, S. Kido, H. Watanabe, M. Yamamoto and T. Hashimoto, *J. Appl. Polym. Sci.*, 19 (1975) 629.
9 J. N. Little, J. L. Waters, K. J. Bombaugh and W. Pauplis, *J. Chromatogr. Sci.*, 9 (1971) 341.
10 E. P. Otocka, *J. Chromatogr.*, 76 (1973) 149.
11 D. Ishii, K. Asai, K. Hibi, T. Jonokuchi and M. Nagaya, *J. Chromatogr.*, 144 (1977) 157.
12 K. J. Bombaugh, W. A. Dark and R. F. Levangie, *J. Chromatogr. Sci.*, 7 (1969) 42.
13 W. Heitz and H. Ullner, *Macromol. Chem.*, 120 (1968) 58.
14 W. Heitz, B. Bomer and H. Ullner, *Macromol. Chem.*, 121 (1969) 102.