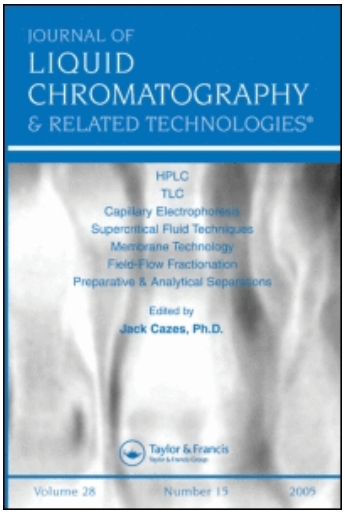

Journal of Liquid Chromatography & Related Technologies

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

High Performance Size Exclusion Chromatography Using Porous Glass Packing Materials

Sadao Mori[a]
[a] Department of Industrial Chemistry, Faculty of Engineering Mie University, Tsu, Mie, Japan

To cite this Article Mori, Sadao(1986) 'High Performance Size Exclusion Chromatography Using Porous Glass Packing Materials', Journal of Liquid Chromatography & Related Technologies, 9: 12, 2623 — 2632
To link to this Article: DOI: 10.1080/01483918608076889
URL: http://dx.doi.org/10.1080/01483918608076889

JOURNAL OF LIQUID CHROMATOGRAPHY, 9(12), 2623-2632 (1986)

HIGH PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY USING POROUS GLASS PACKING MATERIALS

Sadao Mori

Department of Industrial Chemistry
Faculty of Engineering
Mie University
Tsu, Mie 514, Japan

ABSTRACT

Porous glass packing materials of average particle diameter 5 μm have been packed into a 7.2 mm i.d. x 25 cm column by viscous-slurry packing parocedure. Average pore diameters of porous glasses were 170 Å, 500 Å, 1000 A, and 2000 A. The numbers of theoretical plates were between 7000 and 8000 per a column for porous glasses of pore diameters of 170, 500, and 1000 A, and 5000 for that of 2000 A. The retention volumes of narrow molecular weight-distribution polystyrene standards have been determined using tetrahydrofuran as mobile phase for the construction of calibration curves. Separations of polystyrene over molecular weight ranges of 1000 and 4,000,000 have been obtained by combining all four porous glass columns in series. Molecular weight averages of NBS 706 polystyrene have been measured and compared with the values determined with polystyrene gel columns. Both results were equivalent to the manufacturer's data. Porous glasses thus appear to be a useful packing materials for HPSEC.

INTRODUCTION

There are a number of materials which are used as column packings in high performance size exclusion chromatography

 0148-3919/86/0912-2623$3.50/0

(HPSEC). Most successfully used packing material is polystyrene gel. Inactivated silica gel is applied in some cases. Porous glass is rigid, mechanically stable, and easy for controlling pore sizes and pore uniformities. It can also be used with polar and non-polar solvents which are not applied to polystyrene gel.

There have been several application reports for porous glass packing materials so far: separation of polystyrene using tetrahydrofuran as mobile phase(1), comparison with columns packed with polystyrene gels (2), effects of pore structure or pore size distribution(3 - 6), inactivation of the surface of porous glasses by silanization to prevent adsorption of solutes onto the glasses (7 - 9). However, these works were made with porous glasses of larger particle diameter such as 35 to 75 μm. Commercially available porous glasses have these particle sizes. By sieving these porous glasses, only the particles between 44 and 50 μm were packed and applied for high-speed SEC, though the column efficiency was not satisfactory (10).

Recently, porous glass materials having average particle diameter of 5 or 10 μm and relatively narrow distribution of particle size have been developed, though it is still on the stage of trial manufacture. Packing procedure for these materials has been reported (11). This paper describes the performance of these materials for HPSEC of polystyrenes.

EXPERIMENTAL

Packing Materials and Packing Procedure

Porous glasses were FPG 170, FPG 500, FPG 1000, and FPG 2000 which were obtained from Fuji Photo Film Co., Ltd., Japan. These materials have averge pore diameters 170 Å, 500 Å, 1000 Å, and 2000 Å, respectively and are irregular shaped particles obtained by crushing the original larger materials. Average particle diameter of the porous glasses used in this experiment was 5 μm (±

2 μm). Pore volumes and surface areas of the porous glasses were 0.96 ml/g and 145 m^2/g for FPG 170, 1.01 and 57 for FPG 500, 1.03 and 29 for FPG 1000, and 0.91 and 15 for FPG 2000.

Column packing was carried out on a Chemco high-pressure slurry-packing apparatus with an air-driven pneumatic pump, Model 124PP + 124 A (Chemco Co., Kita-ku, Osaka 530, Japan). A slurry reservoir (a packer) was an empty tubing of 16 mm i.d. x 40 cm length and had an inner volume of 80 ml. An empty column of 7.2 mm i.d. x 25 cm length was connected to the reservior through an extension tubing of the same size to the column blank.

A slurry solvent was a mixture of methanol and ethylene glycol (70/30, v/v) for FPG-170, (25/75, v/v) for FPG-500 and FPG 1000, and methanol for FPG 2000. A 8-g portion of porous glass was slurried in 70 ml of the slurry sovlent and degassed. Carbon tetrachloride, 25 ml, and the degassed slurry were poured into the column-reservoir assembly from the top of the reservoir in this order, and then the rest of assembly was filled with n-heptane which was used as a packing solvent.

Packing pressure was increased gradually so as to maintain packing flow rate between 15 and 20 ml/min and finally raised to 500 Kg/cm^2. After about 80 ml of n-heptane passed through the column-reservoir assembly, packing pressure was decreased and maintained the packing flow rate to 30 ml/min. After another 200 ml of n-heptane passed through the assembly, the reservoir was disconnected. The equilibration for columns was performed by pumping chloroform for 5 minutes, methanol for 5 minutes, and finally teterhydrofurane for 30 minutes at 3.0 ml/min each in succession. The column was disconnected from the extension tubing.

Measurements of SEC

SEC was performed on a Jasco TRIROTAR-V high-performance liquid chromatograph (Japan Spectroscopic Co., Ltd., Hachioji,

Tokyo 192, Japan) with a differential refractometer Model SE-11 (Showa Denko Co., Ltd., Minato-ku, Tokyo 105, Japan) and a sample loop injector, Model VL-611. Mobile phase was tetrahydrofuran and flow rate was 1.0 ml/min. Four columns of FPG 170, FPG 500, FPG 1000, and FPG 2000 were connected in series in reverse order. Sample concentration was 0.1% for polystyrene standards of narrow molecular weight distribution below $1x10^6$ molecular weight, 0.05% for those over $1x10^6$ molecular weight, and 0.2% for polystyrene NBS 706. Sample injection volume was 0.1 ml.

The number of theoretical plates (N) for each column was determined by injecting 0.05 ml of a 0.5% benzene solution at flow rate of 0.5 ml/min. The value of N was calculated by measuring the width of the peak at half height. Similarly, construction of a calibration curve for each column was performed by injecting 0.05 ml of polystyrene standard solutions at the same flow rate.

For comparison purpose, two Shodex A80M columns (each 8 mm i.d. x 50 cm, Showa Denko, Co.) packed with polystyrene gels were used and a calibration curve and molecular weight distribution of NBS 706 polystyrene were determined. The value of N for this column was 40000 plates per meter by injecting 0.2 ml of a 0.5% benzene solution.

RESULTS and DISCUSSION

The values of N for columns packed with porous glasses were as follows: 8000 plates per a 25 cm column for FPG 170, 7600 for FPG 500, 7000 for FPG 1000, and 5000 for FPG 2000. Viscous solvent such as a mixture of ethylene glycol and methanol was used as slurry sovlent in order to prevent sedimentation of particles during packings. Methanol was used as slurry solvent for FPG 2000, because a mixture of methanol and ethylene glycol was too viscous to pack it in reasonable time (e.g., usually, in five minutes for other porous glasses). With increasing pore size of

porous glasses, viscosity of slurry solvent had to be decreased. Prevention of intermixing of slurry with packing solvent in the slurry reservoir or in the column blank was preferable (12) and therefore, n-heptane was used as packing solvent. Viscous solurry solvent and non-soluble packing solvent in the slurry are characteristic of our packing procedure (11).

Calibration curves for columns packed with different porous glasses are shown in Figure 1. From these curves the exclusion limits as molecular weights for polystyrenes can be determined as follows:

Substrate	Exclusion limit
FPG 170	1.0×10^5
FPG 500	6.0×10^5
FPG 1000	2.0×10^6
FPG 2000	over 4.5×10^6

Though porous glass FPG 2000 has enough pore volume as gross characteristic, inner volume, V_i, in the column calculated from the calibration curve was very small comparing with other porous glasses and this reason was attributed to crush during packing.

Figure 2 shows calibration curves for all four porous glass columns combined in series and for two Shodex A80M columns. It is seen that the resolution of the two system is not equivalent (slope of calibration curves are different). Resolution over 2×10^6 molecular weight and below 6×10^3 molecular weight is better for Shodex A80M columns and that between these two molecular weights is better for a set of porous glass columns. The slope and linearity of a calibration curve for porous glass columns can be improved by changing a combination of porous glass columns (strictly speaking, by mixing porous glasses at an appropriate ratio and by packing them together), and therefore , it is possi-

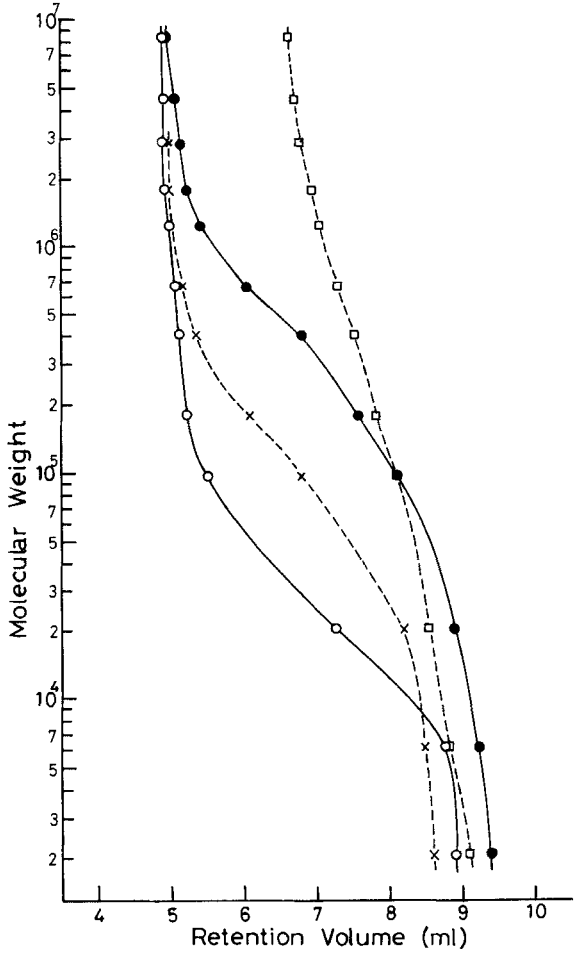

Figure 1. Calibration curves for each porous glass (FPG) column using polystyrenes: (o——o) FPG 170; (x---x) FPG 500; (●——●) FPG 1000; and (□---□) FPG 2000.

Column: 7.2 mm i.d. x 25 cm length; mobile phase: tetrahydrofuran at 0.5 ml/min flow rate; sample: 0.1%, 0.05 ml injection.

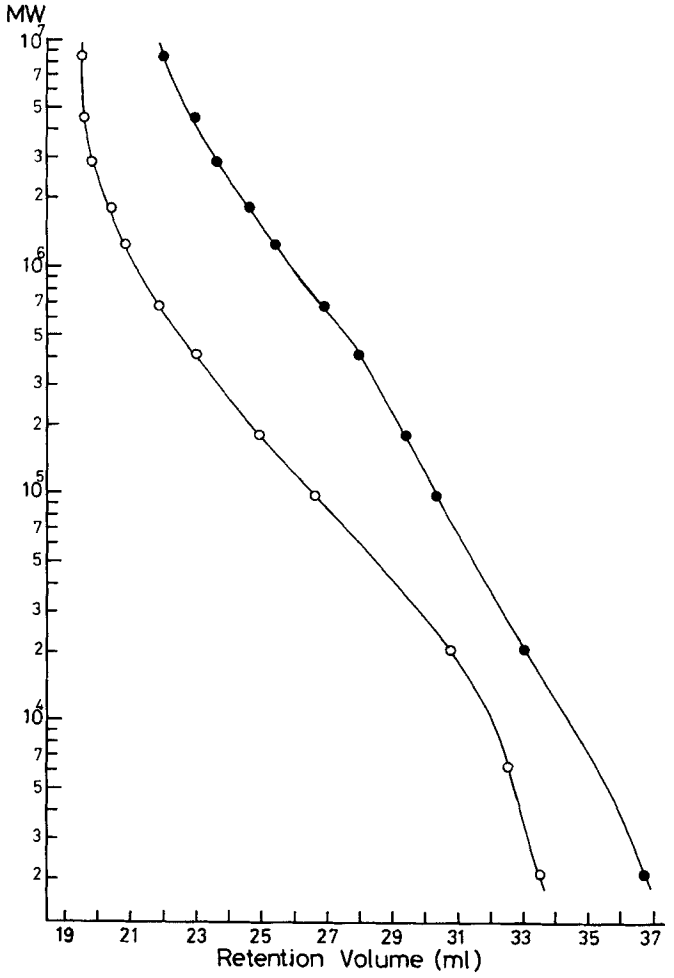

Figure 2. Calibration curves for the two different column systems: (o——o) four porous glass columns; (●– –●) two polystyrene gel columns.

Mobile phase: tetrahydrofuran at 1.0 ml/min flow rate; sample: 0.1%, 0.1 ml injection.

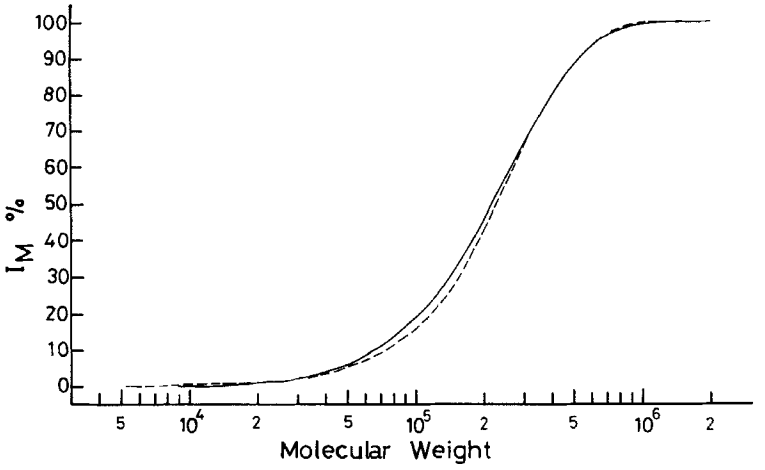

Figure 3. Normalized integral molecular weight distribution curves for NBS 706 polystyrene: (——) four porous glass columns; (– – –) two polystyrene gel columns.

ble to say that both column systems have the equivalent resolution. The ratio of inner volume and interstitial volume in columns is somewhat higher for porous glasses.

The performance of the combined porous glass columns was estimated by measuring the chromatogram of NBS 706 polystyrene and calculating molecular weight distribution and compared with Shodex A80M columns. Average molecular weights obtained from both systems are as follows:

Column system	$\bar{M}_w$	$\bar{M}_n$
porous glass columns	2.63×10^5	1.37×10^5
polystyrene gel columns	2.68×10^5	1.37×10^5

Both column systems gave almost equivalent values to the manufacturer's data: $\bar{M}_w = 2.58 \times 10^5$ and $\bar{M}_n = 1.37 \times 10^5$. Though the difference between average molecular weight calculated from the chro-

matograms obtained on both column systems are small, their integral molecullar weight distribution curves differ slightly. Figure 3 shows the results which are probably due to higher resolution between $2x10^4$ and $1x10^6$ molecular weights for porous glass columns. Practically, this difference is negligible comparing with other types of column sets (13).

REFERENCES

1. Cantow, M. J. R. and Johnson, J. F., The Use of Porous Glass as a Column Packing for Gel Permeation Chromatography, J. Appl. Polym. Sci., 11, 1851, 1967.

2. Spatorico, A. L., Exclusion Chromatography Using Controlled Porosity Glass. I. Comparison With Styrene Gels, J. Appl. Polym. Sci., 19, 1601, 1975.

3. Zhdanov, S. P., Koromaldi, E. V., Vinogradova, R. G., Ganetskii, M. B., Golynko, O. M., Zhilzova, N. E., Belenky, B. G., Vilenchik, L. Z., and Nefedov, P. P., Application of Porous Glasses for Gel Permeation Chromatography of Polymers, J. Chromatogr., 53, 77, 1970.

4. Cooper, A. R., Bruzzone, A. R., Cain, J. H., and Barrall, E. M., II, Gel Permeation Chromatography: Physical Characterization and Chromatographic Properties of Corning Porous Glasses, J. Appl. Polym. Sci., 15, 571, 1971.

5. Cooper, A. R. and Johnson, J. F., Gel Permeation Chromatography: The Effect of Pore Size Distribution on Separation Efficiency, J. Appl. Polym. Sci., 15, 2293, 1971.

6. Zhdanov, S. P., Belenkii, B. G., Nefedov, P. P., Koromaldi, E. V., and Kuznetsova, M. A., Influence of the Structure of Macroporous Glasses on the Separation of Polymers by Gel Permeation Chromatography, J. Chromatogr., 77, 149, 1973.

7. Cooper, A. R and Johnson, J. F., Gel Permeation Chromatography: The Effect of Treatment with Hexamethyldisilazane on Porous Glass Packings, J. Appl. Polym. Sci., 13, 1487, 1969.

8. Cooper, A. R., Gel Permeation Chromatography: Evaluation of Porous Glass-ColumnPacking Materials for LowMolecular Weight Solutes, J. Appl. Polym. Sci., 17, 2707, 1973.

9. Dawidowicz, A., Waksmundzki, A. and Sokołowski, S., Gel Permeation Chromatography Using Porous Glass. Investigations of the Influence of Silanization on Exclusion and Adsorption, Sep. Sci., 12, 573, 1977.

10. Gudzinowicz, B. J. and Alden, K., High-Speed Gel Permeation Chromatography, J. Chromatogr. Sci., 9, 65, 1971.

11. Mori, S., Kato, M. and Suzuki, M., Column Packing Procedure of Porous Glass Packing Materials for High Performance Aqueous Size Exclusion Chromatography and Evaluation of the Columns, Bunseki Kagaku, 34, 492, 1985.

12. Siemon, C. C., Preparation of High Efficiency Columns for High Performance Liquid Chromatography, J. Liq. Chromatogr., 6, 765, 1983.

13. Mori, S., Effects of Column Combinations on the Measurement of Average Molecular Wegiths and the Molecular-Weight Distribution in High-Performance Size-Exclusion Chromatography, J. Chromatogr., 174, 23, 1979.