Journal of Chromatography, 208 (1981) 74–77
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,553

Note

Suitability of TSK-gel Toyopearl packing for the gel permeation chromatographic analysis of dextran

PHILIP E. BARKER*, BRIAN W. HATT and GEORGE J. VLACHOGIANNIS
Chemical Engineering Department, University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Great Britain)
(Received November 28th, 1980)

The molecular weight characterisation of dextran was one of the first applications of gel permeation chromatography (GPC). Several GPC packings suitable for the analysis of molecular weight distribution (MWD) of dextran are available[1]. In our laboratory several of these packings have been tried with dextran and the results of these trials were reported[2,3].

The TSK-gel PW-type (AMTO, Amsterdam, The Netherlands) has been used successfully for dextran analysis by Fisons Pharmaceutical[4] for fifteen months. This type of packing shows high efficiency (*ca.* 13,000 plates/m), it has been used with aqueous solutions with no significant loss in efficiency during this period. However, analysis times are typically around 40 min.

Recently samples of the TSK-gel Toyopearl (Toyo Soda, Tokyo, Japan) packing material, very similar to the TSK-gel PW type but of larger size, were obtained to be tried as an analytical packing for dextran.

PROPERTIES OF THE PACKING

TSK-gel Toyopearl[5], which is produced by the polymerisation of hydrophilic vinyl monomers, is a completely new packing material for GPC. It is a semi-rigid, mechanically and chemically stable, spherical gel and it can be used under low pressure drop (less than 10 kg/cm^2) conditions.

Toyopearl is produced in seven types[6], each with a different pore size distribution, covering a wide range of molecular weights. Each type is produced in three sizes, ranging from 20 to 100 μm.

The samples of packing examined were: (1) Toyopearl HW 55S, having, according to the manufacturer[6], a fractionation range for dextran from $1 \cdot 10^3$ to $2 \cdot 10^5$ daltons. The size of the particles was between 20–40 μm, and (2) Toyopearl HW 65S, having a reported fractionating range from $1 \cdot 10^4$ to $1 \cdot 10^6$ daltons and the same particle size as the HW 55S packing.

EXPERIMENTAL

A relatively simple chromatography system was used, consisting of a pump,

0021-9673/81/0000–0000/$02.50

sample introduction device, column, detector and recorder. All the results were manually measured and calculated from the chromatographs.

The eluent was pumped with a positive displacement pump (MPL, Metering Pumps, London, Great Britain). The samples were applied with a 100-μl syringe (Field Instruments). The eluate was monitored with a Model 1107 LJ differential refractometer (Laboratory Data Control) and the chromatograph displayed on a Type 2 flat-bed recorder (Smiths, Venture Servoscribe).

The packing technique used two glass columns of 1 m × 4 mm I.D. each, (Corning, Corning, NY, U.S.A.) connected directly together with a funnel on the top of the upper column. The columns were filled with water to remove the air and a slurry of the packing was poured into the funnel; it was left for *ca.* 12 h to settle into the columns. The eluent was then pumped through the columns to compress the packing into the lower column. The eluent rate was controlled so that a pressure drop of around 7 kg/cm^2 was generated.

The column filled with the packing was then connected to the chromatographic system. Throughout the work, the eluent used was a solution (0.02%, w/v) of potassium hydrogen phthalate in distilled water.

The efficiency of each column was measured with glucose as the solute by the equation: $N = 8 \cdot (t_R/W_{h/e})^2$ where N is the number of theoretical plates, t_R is the peak retention time and $W_{h/e}$ is the peak width at the peak height (h) divided by e, the base of the natural logarithm.

The dextran used had a wide molecular weight range of between $2 \cdot 10^2$ and $2 \cdot 10^6$ daltons, to test the packing's performance over this range which is of interest to our work. This dextran had a weight average molecular weight, $\bar{M}_w$, of 70,000 daltons (batch 161 D40, Fisons Pharmaceuticals (Holmes Chapel, Great Britain). Dextran T2000 (Pharmacia, Uppsala, Sweden) and glucose were used to determine the void volume, V_0, and pore volume, V_i, respectively. When t_0 and t_i are the times taken for totally excluded and totally included molecules to be eluted from the column, respectively, and F is the flow-rate of the mobile phase, then $V_0 = t_0 \cdot F$ and $V_i = t_i \cdot F$.

RESULTS

HW 55S

This packing has a satisfactory efficiency of *ca.* 5500 plates/m for glucose and a good resolution for the dextran used (see Fig. 1).

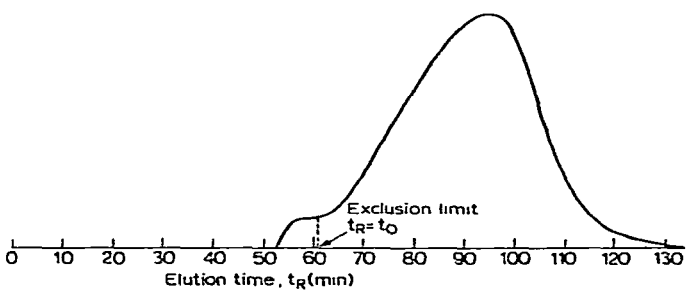

Fig. 1. Gel permeation chromatogram of Dextran B161 D40 on TSK-gel Toyopearl HW 55S (20–40 μm). Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

The disadvantages are: (a) it has a low exclusion limit, although higher than that reported by the manufacturer, and (b) the analysis time is too long (*ca.* $2\frac{1}{2}$ h) (Fig. 1).

HW 65S

The HW 65S packing, although it has a high exclusion limit, gave poor resolution. It does not separate the chromatographic peaks of dextran ($\bar{M}_w \approx 70{,}000$ daltons) and of glucose (180 daltons) (see Fig. 2). It also has a low efficiency of approximately 575 plates/m for glucose.

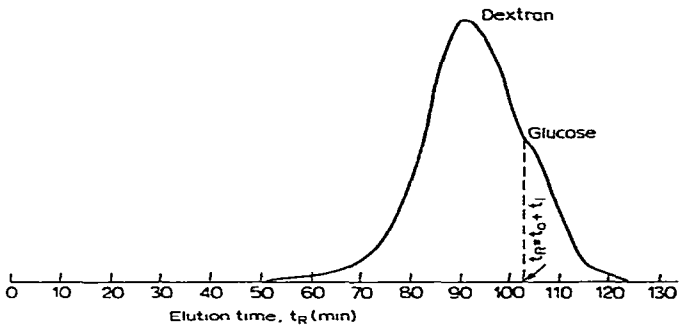

Fig. 2. Gel permeation chromatogram of Dextran B161 D40 and glucose on TSK-gel Toyopearl HW 65S (20–40 μm). Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

Mixtures of the HW 55S and HW 65S Packings

Since both packings have advantages and disadvantages we thought it worthwhile to investigate the effect of combining the high efficiency and good resolution of the HW 55S packing with the high exclusion limit of the 65S packing.

The following mixture combinations have been tried:

Mixture A: 26 cm^3 of HW 55S and 2 cm^3 of HW 65S.

Mixture B: 23.4 cm^3 of HW 55S and 10.6 cm^3 of HW 65S.

Mixture C: 24.3 cm^3 of HW 55S and 24.7 cm^3 of HW 65S.

Mixture D: 19.65 cm^3 of HW 55S and 25.3 cm^3 of HW 65S.

A shoulder appeared on the chromatographic peaks of dextran 70,000 for the first three mixtures. This may be due to the low exclusion limit, but as the percentage of HW 65S packing in the mixture was increased, the shoulder increased instead of decreasing, until it became a second peak (Fig. 3).

Mixture D has a suitable exclusion limit since no shoulder or exclusion peak appeared on the chromatographic peak of the dextran (Fig. 4). It also has good resolution for the dextran and its efficiency is satisfactory, *ca.* 1500 plates/m. The only disadvantage is the long analysis time ($\approx 2\frac{1}{2}$ h).

CONCLUSIONS

Although TSK-gel Toyopearl is mainly a laboratory preparative packing it is also suitable for analytical work on dextrans as it has a satisfactory efficiency, good resolution, and a suitable fractionation range.

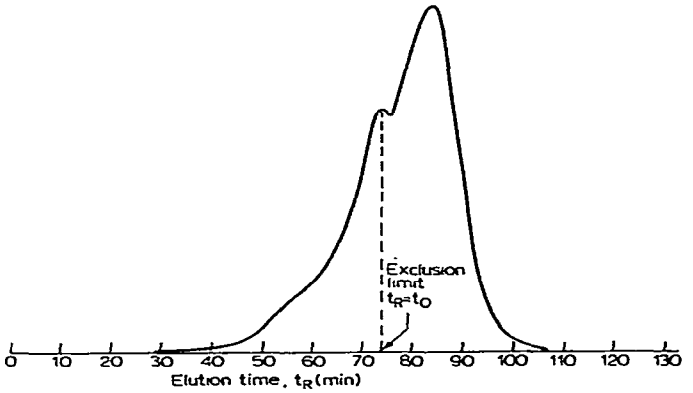

Fig. 3. Gel permeation chromatogram of Dextran B161 D40 on packing mixture C. Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

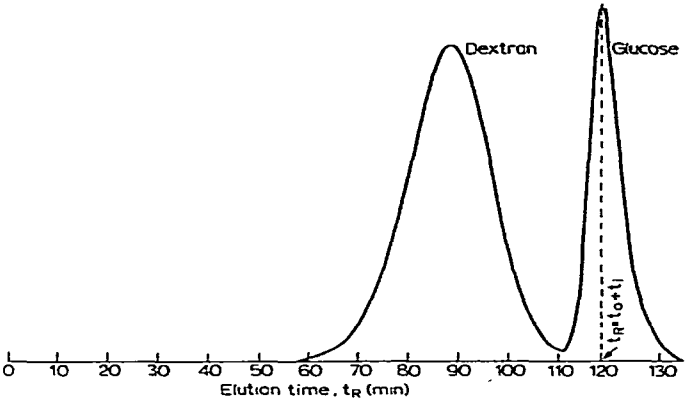

Fig. 4. Gel permeation chromatogram of Dextran B161 D40 and glucose on packing mixture D. Eluent 0.02% potassium hydrogen phthalate aqueous solution. Column: 100 cm × 4 mm I.D.

The main disadvantage with this packing is that the flow-rates are very low (3–6 cm^3/h) because of the low working pressure of the packing (<7 kg/cm^2), and therefore the analysis time is long (2–3 h).

ACKNOWLEDGEMENT

The authors would like to thank the Toyo Soda Manufacturing Co. Ltd., Japan, for supplying the packings, and Mr. R. M. Alsop of Fisons Pharmaceutical Ltd., for his helpful discussions during the course of this work.

REFERENCES

1 A. R. Cooper and D. P. Matzinger, *Amer. Lab.*, 13 (1977).
2 P. E. Barker, B. W. Hatt and S. R. Holding, *J. Chromatogr.*, 174 (1979) 143–151.
3 P. E. Barker, B. W. Hatt and S. R. Holding, *J. Chromatogr.*, 206 (1981) 27–34.
4 R. M. Alsop and I. Earl, Fisons Pharmaceuticals, Holmes Chapel, Great Britain, personal communication.
5 *TSK-gel for HPLC Packing Material and Packed Columns*, Toyo Soda Manufacturing Co. Ltd., Tokyo, 1980.
6 *TSK-gel Toyopearl*, Toyo Soda Manufacturing Co. Ltd., Tokyo, 1980.