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Note

High-speed aqueous gel permeation chromatography using a poly(vinyl alcohol) hollow fibre

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An important property in high-speed gel permeation chromatography (GPC) in aqueous systems is the pressure resistance of the packing. Most hydrophilic gels available for GPC cannot be used at high flow-rates, because the larger is the value of the exclusion molecular weight, M_{lim}, the lower is the resistance to pressure. This is an unavoidable defect of gel (swelling) type packings. To overcome this, organic beads of permanent porous resins or of less extensively swelling types have been made from cellulose[1,2], poly(γ-methyl L-glutamate)[3–5] and pullulan[6,7] by our unique procedures. These packings are very rigid and allow higher flow-rates than any other available products.

In this communication, we report the application of a porous poly(vinyl alcohol) (PVA) hollow fibre to aqueous GPC for high-speed analysis. Permeable substances are separated by sieving within the reticulated matrix of the PVA wall. The preparation of the fibre and its separation ability are described.

EXPERIMENTAL AND RESULTS

The new capillary column was prepared from a porous PVA hollow fibre (0.37 mm I.D., Kuraray Co.), which is utilized as an artificial kidney. A typical procedure was as follows: 3 m of non-treated PVA hollow fibre was soaked for 2 h in 1000 ml of water–methanol (1:1) containing 80 g of sodium hydroxide at room temperature. The wet fibre was immediately placed into 800 ml of dimethyl sulphoxide (DMSO)–acetone and was kept at 60°C for 2 h. Epichlorohydrin (20–100 ml) was added to this solution and gently stirred at 60°C for 18 h. The resulting cross-linked PVA hollow fibre was washed with water and methanol and dried in air. The outside of the PVA hollow fibre was coated with poly(vinyl chloride)–dioctyl phthalate (8:2).

Fig. 1 shows a scanning electron micrograph of a section of the hollow fibre column prepared by this procedure. The double layer structure (inner cross-linked hydrophilic PVA and outer hydrophobic coating materials) is obvious.

The aqueous GPC properties were examined at a flow-rate of 0.05 ml/min, employing a Waters Assoc. 6000 p.s.i. pump (Model 510) controlled by a Type 680 automated gradient controller. The permeable substances used were homologues of poly(oxyethylene), alcohols and heavy water. A calibration curve was constructed

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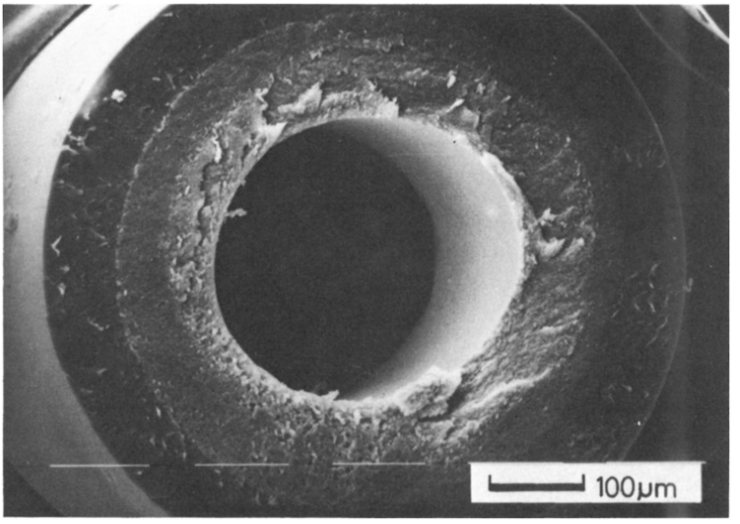

Fig. 1. Scanning electron micrograph of a section of the PVA hollow fibre column.

from the molecular weights and the elution times of the permeable substances after subtraction of the dead volume. Figs. 2 and 3 show the elution pattern and the calibration curve, respectively, obtained with a PVA hollow fibre column cross-linked by use of 40 ml of epichlorohydrin in DMSO–acetone (7:3). Substances with high number-average molecular weights (M_n > 40000) are eluted in about 4 min. This corresponds to an elution volume of 0.2 ml, *i.e.*, approximately the same as the inner (void) volume of the column (1.5 m × 0.37 mm I.D.), 0.16 ml. Consequently, this molecular weight, 40000, corresponds to the exclusion molecular weight, M_{lim}, in conventional GPC. For substances with molecular weights less than M_{lim}, the large

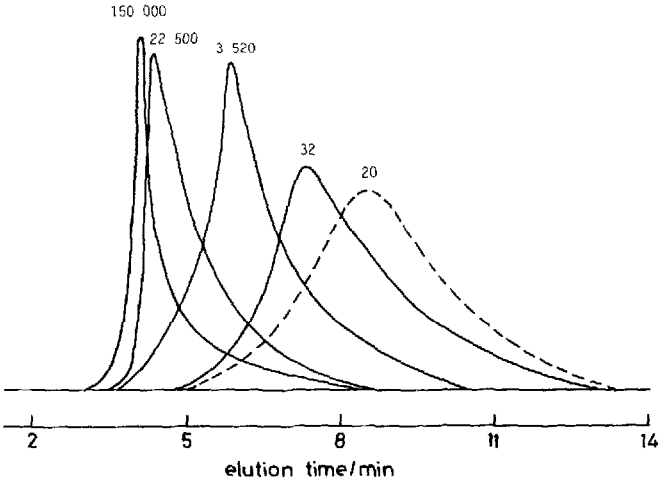

Fig. 2. Chromatogram of some hydrophilic substances. Column: cross-linked by use of 40 ml of epichlorohydrin in DMSO–acetone (7:3); 150 cm. Eluent: water. Flow-rate: 0.05 ml/min. Temperature: 25°C. Detector: differential refractometer. The molecular weights of the substances are indicated.

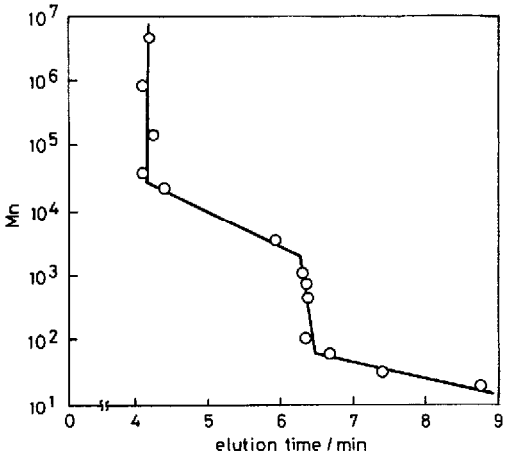

Fig. 3. Calibration curve obtained from Fig. 2 for the PVA hollow fibre column.

molecules are eluted faster than the small ones according to the principle of GPC. It is estimated that the small molecules are retarded by temporary diffusion into the stationary PVA matrix.

The PVA hollow fibre applied in this study shows unique properties. The calibration curve in Fig. 3 indicates that the PVA hollow fibre possesses two kinds of reticular structure: pore sizes corresponding to molecular weights of 40 000–3000 and of less than 100. Therefore, a large separation factor, α, is obtained for low-molecular-weight substances. For example, the value for diethylene glycol and heavy water is 1.4, which is difficult to achieve in conventional GPC.

It was shown that the form of the calibration curve was dependent on the cross-linking conditions (*cf.*, Fig. 4). This result indicates that the composition of

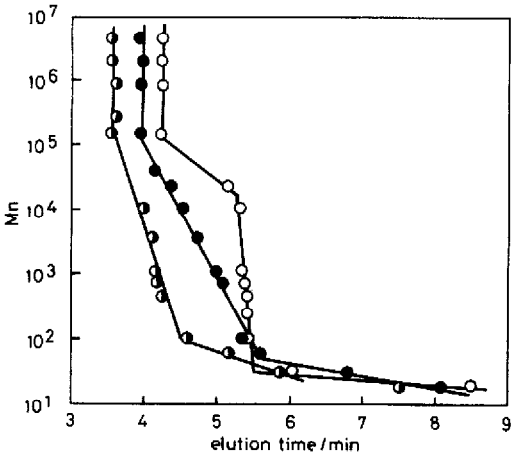

Fig. 4. Calibration curves for PVA hollow fibre columns cross-linked under different conditions. ○ = DMSO–acetone (7:3), 20 ml epichlorohydrin; ● = DMSO–acetone (1:9), 20 ml epichlorohydrin; ◑ = DMSO–acetone (3:7), 100 ml epichlorohydrin.

medium and the amount of epichlorohydrin in the cross-linking process affect the reticular structure of the PVA matrix. DMSO and acetone are good and poor solvents for PVA, making it swell and shrink, respectively.

High-speed micro-analysis was achieved by the application of the hydrophilic PVA hollow fibre, which is difficult by conventional GPC.

The PVA hollow fibre adopted in the present study is a material suitable to chromatography of low-molecular-weight substances. The theoretical plate number is low because of the large inner diameter, as shown in Fig. 2. However, it has been observed in other capillary column systems that the theoretical plate number increases with decreasing inner diameter[8].

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