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Note

Novel gel permeation chromatography packing composed of cross-linked and porous spherical particles of poly- γ -methyl-L-glutamate

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Porous beads composed of dextran and agar for gel permeation chromatography (GPC) in an aqueous system are commercially available and have been widely utilized (Sephadex and Sepharose, respectively)¹. However, they have the disadvantage that are so soft that they are susceptible to deformation and exhibit a high resistance to the flow-through of fluids. To overcome this, porous beads composed of cellulose, which show a high pressure-resistance superior to that of Sephadex and Sepharose, were prepared by a unique method^{2–4}. We consider that the excellent properties of cellulose are due to intermolecular hydrogen bonding.

In this paper, we describe poly- γ -methyl-L-glutamate (PMLG) as a readily available matrix material for GPC packings. PMLG beads can not only withstand a surprisingly high flow-rate, but also show typical GPC behaviour in both aqueous and organic systems. These properties are attributable to the inter- and intramolecular hydrogen bonding produced by the formation of specific conformations (β -structure and α -helix, respectively).

EXPERIMENTAL

Cross-linked and porous spherical particles of PMLG were prepared as follows: 2.5 wt.-% of PMLG (degree of polymerization *ca.* 2000) was dissolved in 1,2-dichloroethane containing 2.5–7.5 wt.-% of diluent (*e.g.*, decahydronaphthalene, diethylbenzene and 1-octanol). This solution was suspended at a concentration of 1.0–3.5 wt.-% in aqueous poly(vinyl alcohol) solution and the mixture was stirred at a fixed speed at 40°C for 12 h. After filtering, the spherical particles produced were washed with water, hot water, ethanol and diethyl ether. Spherical particles with an average diameter of 5–300 μm are obtained. These PMLG particles were placed in a three-necked flask fitted with a condenser for azeotropic distillation. Decahydronaphthalene–chloroform (3:1), 0.2 equiv. of sulphuric acid and 0.5 equiv. of triethylene glycol were added and the mixture was stirred slowly at 65°C with addition of fresh chloroform as necessary. Particles of PMLG cross-linked by triethylene glycol were obtained by filtering and washing with water and methanol. The total yield was 90–100%.

PMLG particles of average diameter 30 μm (range 25–44 μm) were packed in a glass column (30 cm \times 5 mm I.D.) and a stainless-steel column (15 cm \times 8 mm

I.D.) and GPC was carried out using polysaccharides and polystyrene, respectively, as standard samples. The porosity and the excluded molecular weight (M_{lim}) were determined from a calibration graph. The degree of pressure resistance was examined from the relationship between pressure drop and flow-rate.

RESULTS AND DISCUSSION

PMLG particles

Fig. 1 shows a typical electron micrograph of PMLG particles of diameter 15 μm . The shape of the particle is spherical and uneven. The average diameter can be regulated by the suspension conditions. For example, when 3 wt.-% of aqueous poly(vinyl alcohol) solution was used as a suspension medium, the diameter of the main products lay in the range 5–15 μm . Our procedure can provide spherical particles with an average diameter in the range 5–300 μm by adjusting the viscosities of the medium and the PMLG solution and the stirring speed.

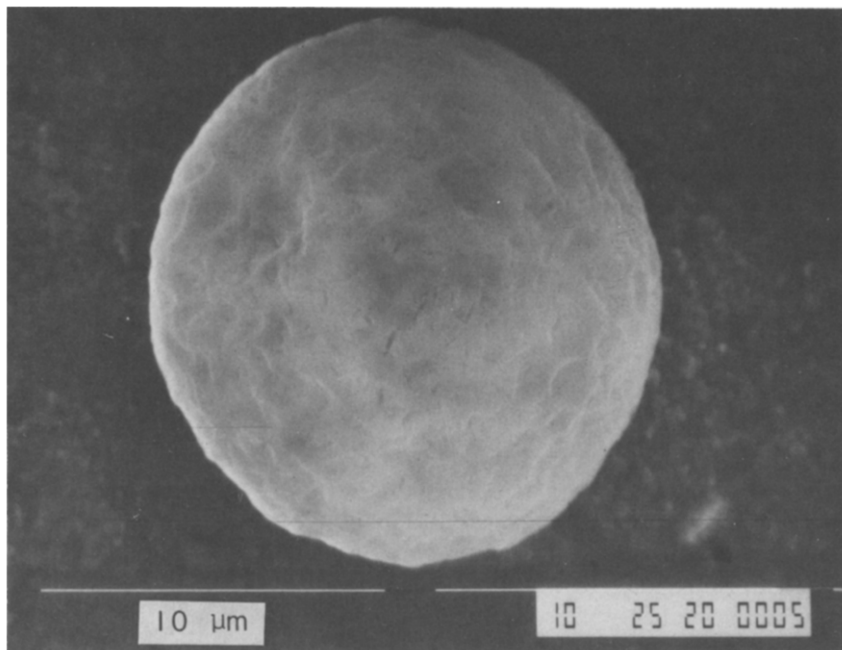


Fig. 1. Electron micrograph of the PMLG particle. Diluent, decahydronaphthalene (100 wt.-%); suspension, 2 wt.-%.

The PMLG particles obtained were insoluble and hardly swelled in the usual solvents used in chromatography, *e.g.*, water, methanol, ethanol, 2-propanol, acetonitrile, tetrahydrofuran, dioxane, benzene, chloroform and hexane. This insolubility must be due to the intermolecular hydrogen bonding with the β -structure of PMLG in addition to cross-linking. Non-cross-linked particles of PMLG are also

insoluble in the above-mentioned solvents, except chloroform. The IR spectra give absorption bands characteristic of the β -structure (1685 and 1630 cm^{-1}) and the α -helix (1650 cm^{-1})^{5,6}.

These properties produce a remarkably high flow-rate for a chromatographic process. Fig. 2 shows the relationship between the pressure drop and the flow-rate for the particles prepared using 100 wt.-% of decahydronaphthalene as a diluent. The PMLG particles easily reached $30\text{ ml/min} \cdot \text{cm}^2$ (corresponding to *ca.* 6 ml/min at 5 mm I.D.), but the flow-rate in the corresponding commercial packing reached a limit at 2 ml/min (at 5 mm I.D.).

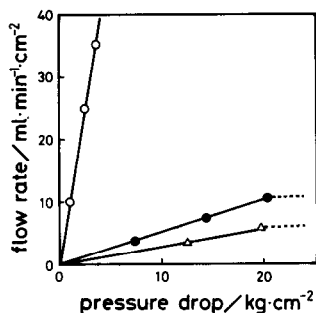


Fig. 2. Relationship between pressure drop and flow-rate in the aqueous system. \circ , PMLG particles (M_{lim} 10 000, size $25\text{--}44\ \mu\text{m}$); \bullet , Sephadex G-25 (M_{lim} 5000, size $44\text{--}105\ \mu\text{m}$); \triangle , Sephadex G-50 (M_{lim} 10 000, size $44\text{--}105\ \mu\text{m}$).

Gel permeation chromatography

The GPC behaviour was examined in aqueous and organic media, using polysaccharides and polystyrene, respectively, as standard samples. Calibration graphs characteristic of GPC were obtained in both mediums (Fig. 3). The excluded molecular weight (M_{lim}) is *ca.* 10 000 in both media. The similarity of the two systems supports the suggestion that cross-linked PMLG particles do not swell and are amphiphilic.

In addition, it was shown experimentally that M_{lim} could be adjusted up or down (from 10^3 to 10^5) by varying the concentration of decahydronaphthalene used as a diluent and by using diethylbenzene or 1-octanol instead of decahydronaphthalene. Typical results are given in Table I. It is very significant for GPC that packings with a series of M_{lim} values can be produced.

The amphiphilic properties of the PMLG particles as shown in Fig. 3 were followed by differential scanning calorimetry (DSC). The DSC thermogram of particles containing water (free water existing around the particles was removed by centrifugation) gives only the melting peak due to water. When this packing was washed with methanol in a column for 10 min, the water peak disappeared. Subsequently, after washing with benzene, the peak for benzene was observed. Even with a change of the medium from water to dioxane, a similar peak transfer was observed. Hence it is clear that the PMLG particles are amphiphilic and that the chromatographic replacement of the medium is very easy.

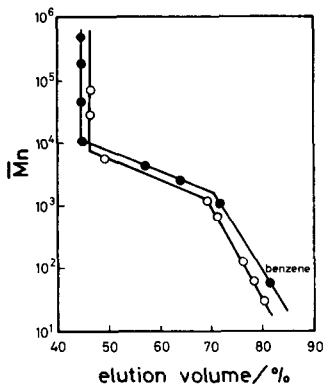


Fig. 3. Typical calibration graphs for porous PMLG particles prepared using decahydronaphthalene as the diluent for GPC. \circ , Water; \bullet , tetrahydrofuran.

In conclusion, PMLG particles constitute an excellent packing for GPC. This is attributed to the fact that PMLG possesses a hydrophilic moiety, $-\text{CONH}-$, and shows hydrophobic properties by forming specific conformations such as the β -structure and α -helix.

TABLE I

M_{lim} OF PMLG PARTICLES

Diluent	Amount (wt.-%)	M_{lim}
None	0	200
Decahydronaphthalene	100	8000
	200	40 000
	300	120 000
Diethylbenzene	100	7000
	200	7000
	300	8000
1-Octanol	100	1000
	300	10 000

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