

SHORT COMMUNICATIONS

Porous Poly(γ -methyl L-glutamate) Spherical Particles as a Novel GPC Packing Material

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Some natural polysaccharides and polystyrene are available as packing materials for gel permeation chromatography (GPC) in aqueous and organic media, respectively.¹ In the former, porous cellulose spherical particles were prepared by our unique method² and industrialized by Chisso Co.^{3,4} This packing is excellent for its pressure- and temperature-resisting properties. In the latter, spherical macroreticular resin is utilized. However, the study on amphiphilic GPC packing applicable to both aqueous and organic systems is restricted and not practical.

We paid attention to the properties of polypeptide, especially, poly(γ -methyl L-glutamate) (PMLG); this material possesses a hydrophilic part, $-\text{CONH}-$, in the chemical structure and is also hydrophobic by forming a specific conformation such as α -helix or β -structure. In this communication, we wish to report the first example of the production of porous and spherical particles composed of PMLG alone. PMLG particles showed amphiphilic and pressure-resisting properties useful for GPC.

Porous and spherical particles of PMLG were produced as follows: PMLG (P_n , ca. 2000) was dissolved in 1,2-dichloroethane containing 2.5 wt% of decahydronaphthalene (*cis*-

and *trans*-decalin mixture) as a diluent to give a 2.5 wt% solution. This solution was suspended in a 2.0 wt% aqueous poly(vinyl alcohol) (viscosity: 40—46 cP, degree of saponification: 86.5—89 mol%) solution. The mixture was stirred at fixed speed (500 ± 20 rpm) at 40°C for 12 h. After filtrating the spherical particles produced were washed with water, hot water, ethanol, and ether in order. By this process, the spherical particles of diameter 25—44 μm are obtained in a yield of 50—90%. It was confirmed experimentally that the average diameter of PMLG particles could be adjusted from 5 to 300 μm by varying the concentration of poly(vinyl alcohol) in suspension and the stirring speed. In this study, some properties were investigated for particles of diameter about 30 μm .

PMLG particles were insoluble in usual solvents for chromatography, *e.g.*, water, methanol, ethanol, 2-propanol, acetonitrile, tetrahydrofuran, benzene, hexane and hexane mixtures (but swelled remarkably in chloroform and 1,2-dichloroethane), without further treatment such as crosslinking. The IR spectra of the PMLG particles has absorption bands characteristic of carbonyl group at 1685 and 1630 cm^{-1} as well as 1650 cm^{-1} . The former two peaks belong to those of β -struc-

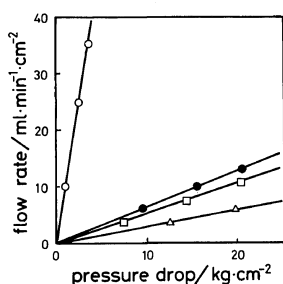


Figure 1. Relationship between flow-rate and pressure drop: —○—, PMLG particles (water); —●—, PMLG particles (THF); —□—, Sephadex G-25, $M_{1\text{im}}=5000$ (water), size 44–105 μm ; —△—, Sephadex G-50, $M_{1\text{im}}=10000$ (water), size 44–105 μm .

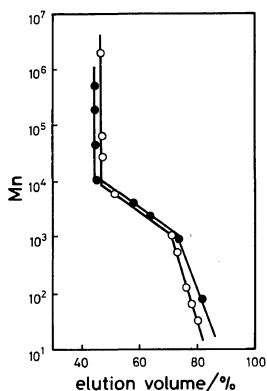


Figure 2. Calibration graphs for porous PMLG spherical particles: —○—, water; —●—, THF.

ture and the latter one of α -helix.⁵ Consequently, the insolubility of PMLG particles is considered to be due to the intermolecular hydrogen bonding in the β -structure. As a result of this property, the PMLG particles show remarkable high-pressure-resistance in the chromatographical process. PMLG particles were packed in a glass column (30 cm \times 5 mm i.d.) and a stainless column (15 cm \times 8 mm i.d.), and the relationship between the flow rate and the pressure drop of column was examined in both water and tetrahydrofuran (THF). As shown in Figure 1, the PMLG particles are quite superior to packings on the market in flow rate.

GPC behavior was then examined in aque-

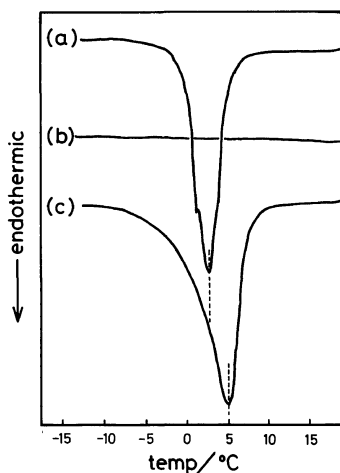


Figure 3. DSC thermograms of PMLG particles⁶ containing the medium for GPC; obtained at a heating rate of 2°C min⁻¹ with a SSC-580/DSC-10 (Seiko I & E). (a) water; (b) methanol; (c) benzene.

ous and organic media. Polysaccharides and polystyrene were used as standard samples, respectively. Calibration graphs characteristic of GPC were obtained in both media (Figure 2). The excluded molecular-weight ($M_{1\text{im}}$) was 10000 in both systems. In addition, it was experimentally checked that the value of $M_{1\text{im}}$ could be adjusted from 10³ to 10⁵ by varying the concentration of decalin used as the diluent and by using diethylbenzene or 1-octanol instead of decalin. It is very significant for GPC that packings with a series of $M_{1\text{im}}$ can be produced.

The amphiphilic property of the PMLG particles as shown in Figure 2 was examined by differential scanning calorimetry (DSC). The DSC thermogram of particles⁶ containing water gives only a melting peak due to water (Figure 3a). When this packing was washed with methanol in a column for 10 min, the peak of water disappeared (Figure 3b). After being washed with benzene, the peak due to benzene was observed (Figure 3c). Even in the case of replacement of medium from water to dioxane, same peak-transfer was observed. Thus it is clear that the PMLG particles are

amphiphilic and replacement of medium is very easy in chromatography.

In conclusion, PMLG particles are excellent packing material for GPC. This is attributed to the specific conformation of PMLG. We are now studying the crosslinking and partial modification of PMLG particles.

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6. Wet-Particles: free water (or medium) existing around the particles is removed by centrifuging.