Journal of Chromatography, 355 (1986) 434–437
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 18 392

Note

Preparation and gel permeation chromatographic properties of pullulan spheres

YOSHIAKI MOTOZATO, HIROTAKA IHARA, TAKASHI TOMODA and CHUICHI HIRAYAMA*

Department of Synthetic Chemistry, Faculty of Engineering, Kumamoto University, Kumamoto 860 (Japan)

(Received December 5th, 1985)

Gel permeation chromatography (GPC) in aqueous media has been achieved with the use of porous gels prepared from water-soluble natural polysaccharides, *e.g.*, dextran and agar, and synthetic polymers, *e.g.*, poly(vinyl alcohol) and poly(acrylamide)[1]. However, at high molecular weights, M_{lim}, the pressure resistance becomes a problem. For the purpose of resolving this problem, we have synthesized new packings from cellulose[2,3] and poly(γ-methyl L-glutamate)[4,5]. These gels allow remarkably high flow-rates in GPC.

Here we report the application of a new material, pullulan (Fig. 1) to GPC packings. Pullulan is a water-soluble polysaccharide first used in industry in Japan in 1978. It has been shown to be a linear polymer of maltotriose containing maltotetraose and shows no abnormality such as crystallization and gelation[6–11]. The preparation and the excellent GPC properties of pullulan spheres are also described.

EXPERIMENTAL AND RESULTS

Pullulan spheres were prepared as follows: 60 g of pullulan were suspended in 600 ml of formamide and dissolved by vigorous stirring at 540°C. A 180-ml volume of pyridine and 400 ml of acetic anhydride were added and the mixture was stirred at 50°C for 48 h. Pullulan acetate was obtained by reprecipitation from 7 l of water. It was then dissolved in dichloromethane to give a 1.1–3.7% (w/w) solution. This solution was poured into 2.0% (w/w) poly(vinyl alcohol) aqueous solution at 30°C

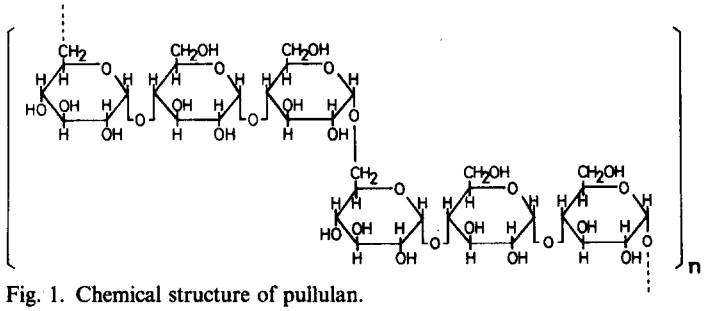

Fig. 1. Chemical structure of pullulan.

0021-9673/86/$03.50

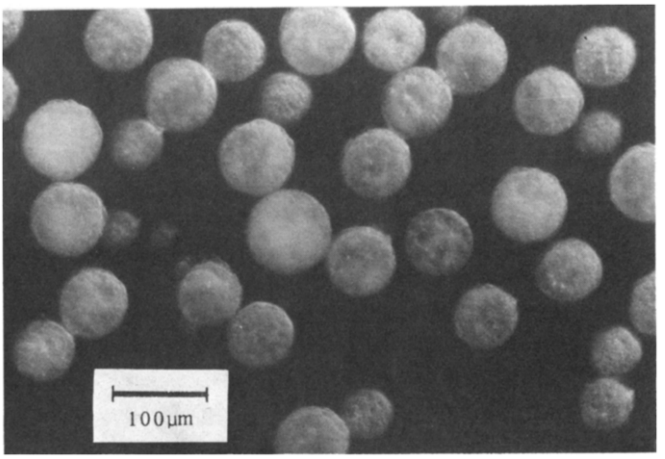

Fig. 2. Optical micrograph of pullulan spheres.

and stirred vigorously. Spheres of pullulan acetate were produced upon gradual evaporation of dichloromethane (sphering process). These beads were filtered off and then suspended in 300 ml of 10 *M* sodium hydroxide–methanol solution and stirred at room temperature for 24 h (saponification process). The saponified beads were cross-linked by epichlorohydrin in 165 ml of acetone–dimethyl sulphoxide at 60°C (cross-linking process). The total yield of the cross-linked pullulan spheres was 70–90%. In this study, the GPC properties of spheres of diameter from 44 to 105 μm were investigated. Fig. 2 shows a typical optical micrograph of the pullulan spheres prepared.

The pullulan spheres were examined in aqueous systems, packed in a glass

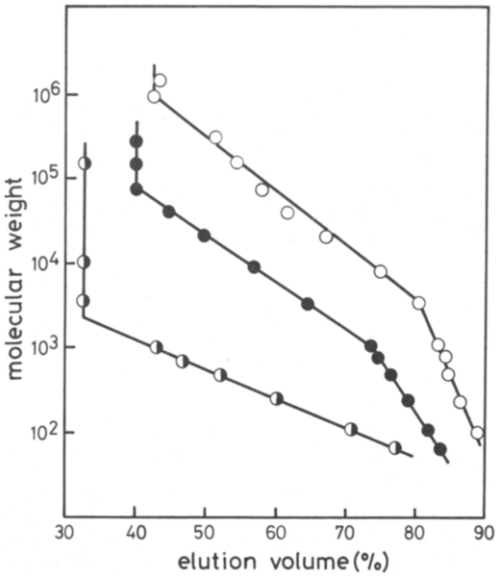

Fig. 3. Calibration graphs for pullulan spheres: ○—○, P-5; ●—●, P-3; ◑—◑, P-1.

TABLE I

PREPARATION CONDITIONS AND M_{lim} VALUES FOR PULLULAN SPHERES

Gel No.	*Polymer* concentration*	*Acetone/DMSO ratio***	M_{lim}***
P-1	3.7	1.0	2000
P-2	2.2	1.0	20 000
P-3	1.5	1.0	70 000
P-4	1.1	0.67	1 000 000
P-5	1.1	1.0	850 000
P-6	1.1	2.3	170 000
P-7	1.1	4.0	50 000
P-8	1.1	9.0	35 000

* The concentration of pullulan acetate (%, w/w) in the sphering process.
** The position of the suspension medium in the cross-linking process.
*** Excluded molecular weight.

column (30 cm × 5 mm I.D.). Poly(ethylene glycol) was used as a standard sample. As shown by the calibration curves in Fig. 3, pullulans show typical GPC behaviours. The exclusion limits, M_{lim}, are summarized in Table I. The value of M_{lim} varied according to the preparation conditions, for example, the concentration of pullulan acetate and the composition of suspension media in the sphering and the cross-linking processes, respectively; it is also related to the pore size of the network of pullulan spheres. Consequently, it is believed that the pullulan content in the media has direct effects upon the formation of the pullulan network.

By adjusting the preparation conditions, pullulan spheres with M_{lim} from 10^3

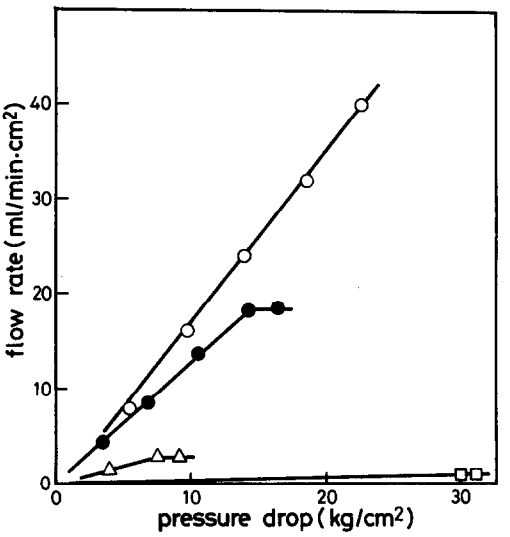

Fig. 4. Relationship between the flow-rate and the pressure drop. ○—○, Pullulan spheres P-7 (M_{lim}, $5 \cdot 10^4$); ●—●, Sephadex G-50m (M_{lim}, $1 \cdot 10^4$); △—△, Sephadex G-150m (M_{lim}, $15 \cdot 10^4$); □—□, Bio-Gel P-30 (M_{lim}, $5 \cdot 10^4$).

to 10^6 can be produced. Usually, macroporous gels with large M_{lim} such as 10^6 are manufactured by using a diluent in the sphering process[2-5]. However, for pullulan spheres, values of M_{lim} up to 10^6 can be obtained even in the absence of a diluent. This is a noteworthy characteristic of pullulan material.

High-speed GPC is desired in aqueous systems. In this context, the pressure resistance is an important property. Fig. 4 shows the relationship between the flow-rate and the pressure drop, compared with the behaviour observed for Sephadex gels and Bio-Gel. All measurements were made by use of a 15 cm × 4 mm I.D. metal column packed with spheres ranging from 44 to 105 μm in diameter. The straight line in Fig. 4 indicates that these pullulan spheres form rigid and stable packing materials for high-pressure chromatography.

It was confirmed that there are no problems in using pullulan spheres for GPC. For example, the packing exhibits almost no interactions (hydrophobic interactions) with sample substrates and can be used over a wide range of pH because it is not ionic.

In conclusion, this study is the first example of the sphering of pullulan and its application to GPC as a packing material. Pullulan spheres give a wide range of M_{lim} and have good properties, such as high flow-rates and no interaction with substrates, for chromatography.

ACKNOWLEDGEMENTS

We thank Mr. Kanji Sugihara for his capable experimental assistance. This work was partially supported by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education.

REFERENCES

1 L. Fischer, *An Introduction to Gel Chromatography*, North-Holland, Amsterdam, 1969.
2 Y. Motozato, K. Matsumoto and C. Hirayama, *Nippon Kagaku Kaishi*, (1981) 183.
3 Y. Motozato and C. Hirayama, *J. Chromatogr.*, 298 (1984) 499.
4 H. Ihara, T. Yoshinaga, Y. Motozato and C. Hirayama, *Polym. J.*, 17 (1985) 1301.
5 C. Hirayama and H. Ihara, *J. Chromatogr.*, 347 (1985) 357.
6 R. Bauer, *Zentralbl. Bakt. Parasitenk. Infektionskr. Hyg. Abt. 2 Naturwiss. Mikrobiol. Landwirtsch. Technol. Umweltschutzes*, 98 (1938) 133.
7 H. Bender, J. Lehmann and K. Wallenfels, *Biochim. Biophys. Acta*, 36 (1959) 309.
8 S. Ueda, K. Fujota, K. Komatsu and Z. Nakashima, *Appl. Microbiol.*, 11 (1963) 211.
9 K. Wallenfels, G. Keilich, G. Bechtler and D. Freudenberger, *Biochem. Z.*, 341 (1965) 433.
10 R. Taguchi, Y. Sakano, Y. Kikucji, M. Sakuma and T. Kobayashi, *Agric. Biol. Chem.*, 37 (1973) 1635.
11 B. J. Catley, *FEBS Lett.*, 10 (1970) 190.