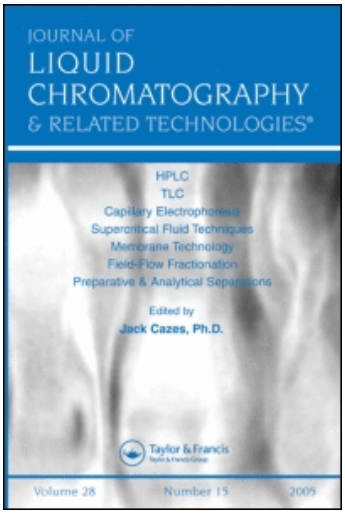

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NOVEL PACKING FOR HIGH PRESSURE LIQUID CHROMATOGRAPHY. PARTIALLY ALKYLATED AND CROSS-LINKED PMLG SPHERICAL PARTICLES

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Abstract

Novel spherical and macroporous particles for the high pressure liquid chromatography were prepared by our unique method. The packing was composed of poly-γ-methyl-L-glutamate, and were functionalyzed by the partial cross-linking and long-chain alkylation. The packings are very rigid and show satisfactorily chromatographic properties. They are attributable to the suitable polarity and hydrophobicity introduced by polypeptide-units and long-chain alkyl groups, respectively.

Introduction

High-performance(-pressure)liquid chromatography(HPLC) has developed rapidly in the past decade.[1] This is due to the

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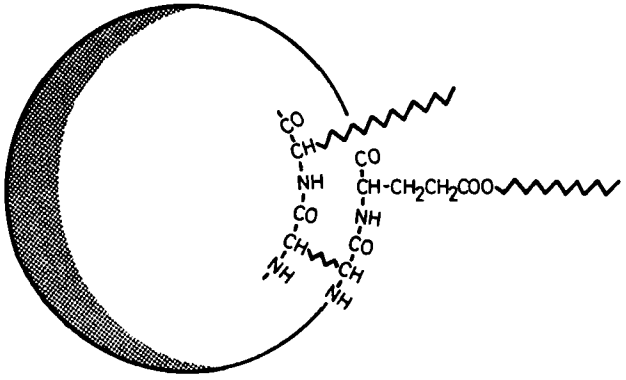

Figure 1

Schematic illustration of the packing.

development of the pressure-resisting high-performance packings: especially, octadecylated silica gel (ODS) which has made many contributions to the reverse phase chromatography. ODS is applied very frequently with remarkable improvements in the chromatographic technique.[2] However, ODS is not free of defects. For example, the residual silanol group of ODS results in acidic properties which give rise to abnormaladsorptions,and ODS is susceptible to hydrolyze in an alkaline and an acidic solution. On the other hand, macroporous styrene resins (or gels) which are used in place of ODS are too hydrophobic. We consider that the development of new packing materials is one of means of solving for these problems.

In this communication, we would like to describe a new packing, constructed using a unique material which has not been

previously applied as packing material for reverse phase chromatography. The packing is spherical and macroporous. The skeleton is composed of partially cross-liked poly (γ-methyl-L-glutamate), PMLG, and long-chain alkylated branches (imaged as shown in Figure 1). The preparation and some chromatographic properties are reported in this paper.

Experimental

Preparation

Spherical and porous particles composed of PMLG only were prepared by our previous procedure.[3] The diameter of the particles was adjusted to 25 - 44 μm. PMLG particles were suspended in a decalin / chloroform (3/1) mixture containing 0.5 eq. of dodecanol, 0.3 eq. of 1,6-hexanediol and 0.2 eq. of sulfuric acid. The mixture was stirred at 65°C for 24 h under azeotropicconditions removing methanol produced by the trans-esterification. Partially cross-linked and dodecylated PMLG particles were obtained by filtrating and washing with chloroform.

Chromatography

The PMLG particles prepared were packed in a glass column (30 cm x 5.0 mm I.D.). The chromatograph included a Waters

Associates 6000 psi pump (Model 510) and LC Spectrophotometer (Model 481).

Results and Discussion

PMLG particles

Figure 2 shows a typical photograph of PMLG particles cross-linked and partially dodecylated. The diameter of the particles is ca. 25 - 35 μm, and the shape is spherical and somewhat uneven.

Particles composed of PMLG only are insoluble in usual solvents used in chromatography even without the cross-linking.[4]

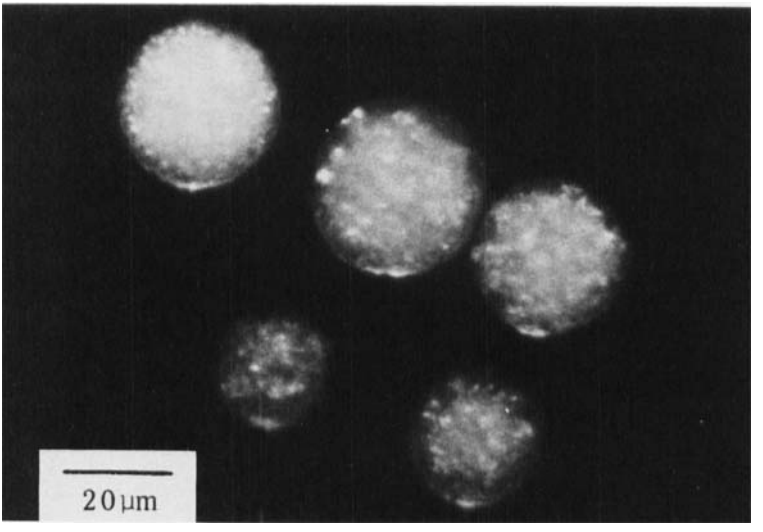

Figure 2

Photograph of partially dodecylated and cross-linked PMLG particles.

This insolubility is increased by the cross-linking with 1,6-hexanediol. The cross-linked particles swelled little even in chloroform which is a good solvent for PMLG. It was observed that none cross-linked particles swelled greatly and dodecylated particles without the cross-linking were soluble in chloroform.

The degree of dodecylation and cross-linking were determined chromatographically and confirmed by elemental analyses. PMLG particles were refluxed in alkaline methanol solution for 24 h to saponify completely. Dodecanol and 1,6-hexanediol produced were analyzed chromatographically, using μBondapak C_{18} (Waters Associates) and water / acetonitrile as an eluent. The amount of dodecanol and 1,6-hexanediol were 20 % and 15 %, respectively, for the unit-mol of PMLG. These values satisfied the elemental analyses as shown in Table 1.

Table 1. Elemental analyses of the packing

particle		C%	H%	N%	C/N
A	calcd	53.75	6.77	10.44	5.14
	found	53.74	6.97	10.20	5.27
B	calcd	57.34	7.84	7.87	7.29
	found	57.25	7.67	7.98	7.17

particle **A**: PMLG only.
calcd for $(C_6H_9NO_3)_n$.

particle **B**: partially dodecylated and cross-linked PMLG.
calcd for $(C_6H_9NO_3)_{65}$-$(C_{17}H_{31}NO_3)_{20}$-$(C_8H_{12}NO_3)_{15}$.

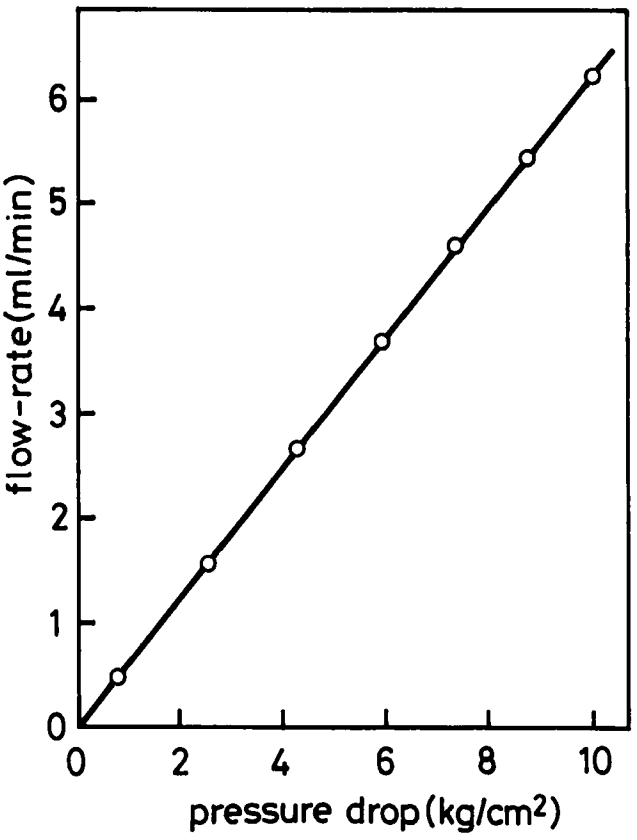

Figure 3

Pressure-flow property of PMLG particles. Data were obtained using a 5.0 mm x 30 cm column of PMLG particles in water / acetonitrile (9 / 1).

Reverse phase chromatography

The relationship between the flow-rate and the pressure drop of a column was examined. As shown in Figure 3, a good straight line was obtained up to more than 6 ml/min. This result supports that the PMLG packing is very hard and swollen very little.

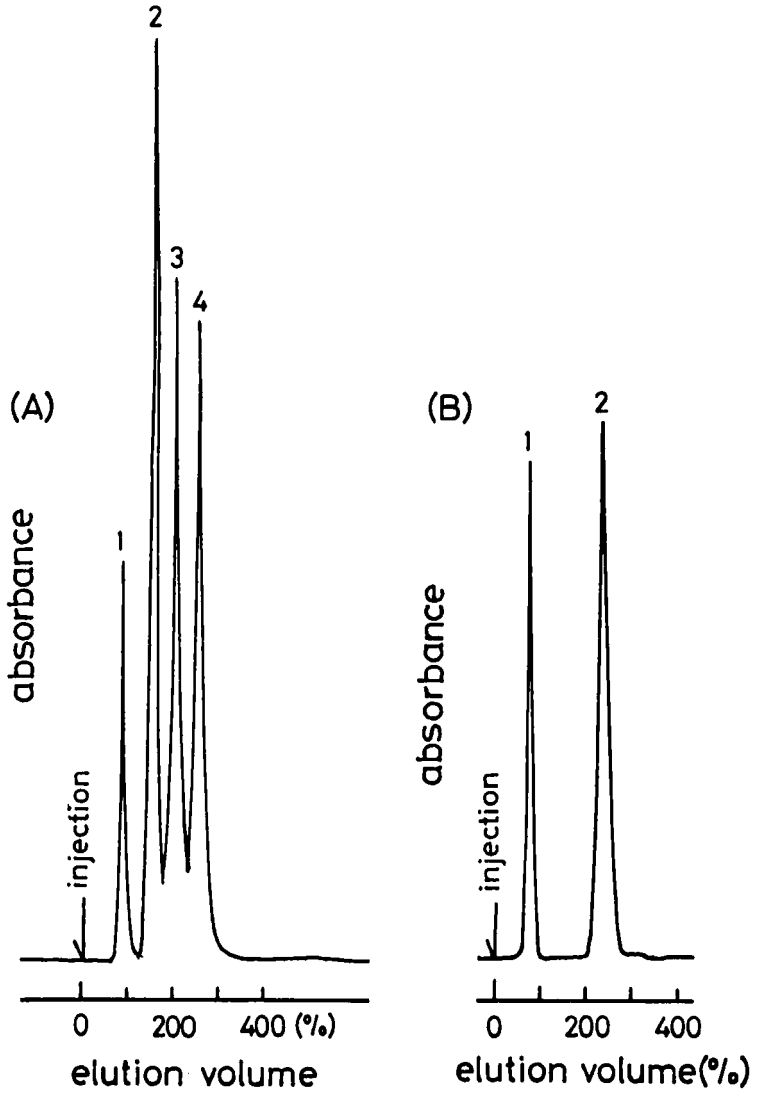

Figure 4

Separation on alkyl benzoates (A) and salicylic acid derivatives (B). Flow-rate: 0.5 ml/min. Detector: UV spectrophotometer. Sample: 0.5 wt% acetonitrile (A) and ethanol (B) solution. Eluent: water / acetonitrile (7/3) (A) and water / ethanol (9/1) (B). Elution order: 1. benzoic acid, 2. ethyl benzoate, 3. butyl benzoate,4. hexyl benzoate (A), and 1. acetyl salicylic acid, 2. salicylic acid methyl ester(B).

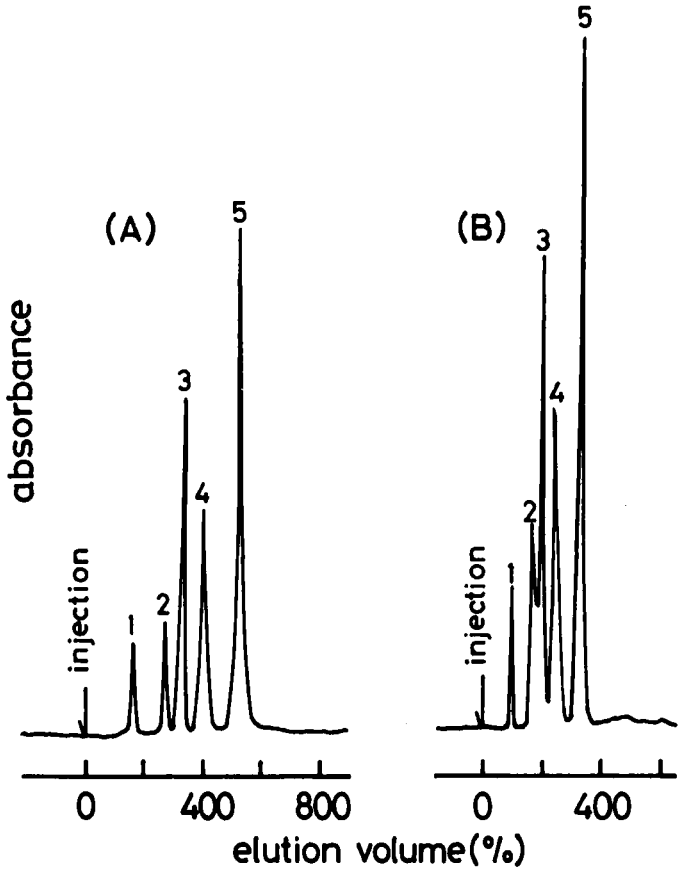

Figure 5

Separation on benzene (1), naphthalene (2), biphenyl (3), fluorene(4) and anthracene (5). Flow-rate: 0.5 ml/min. Detector: UV spectrophotometer. Sample: 0.1 - 0.5 wt% acetonitrile solution. Eluent: water / acetonitrile (6 / 4) (A), and pH 10.0, 0.01 M-borate buffer / acetonitrile (6 / 4) (B).

Using this column, the retention volume of typical samples was investigated. Figure 4 and 5 show the chromatograms for alkyl benzoates, salicylic acid derivatives and aromatic compounds. The retention volume is expressed as the percentage that the elution volume is of the column volume. The elution behaviors shown in Figure 4 and 5 are similar to those of other commonly used reverse phase column packings. It indicates that the PMLG packing has the proper hydrophobicity and polarity.

It is well-known that ODS is susceptible to hydrolysis in alkaline solution (more than pH 8). Thus, the chromatographical stability in an alkaline solution is an important property which should be examined. Figure 5-B is a chromatogram in the medium of 0.1 M-borate buffer (pH 10.0) / acetonitrile (6 / 4). No abnormal symptom was observed in a chromatogram. It indicates that the aliphatic ester bonding is difficult to hydrolyze in a chromatograph process. However, the use at higher pH may be dangerous as ester bonding exists.

Conclusion

In this paper, we described the preparation and some properties of a new packing composed of synthetic polypeptide for reverse phase chromatography. Poly (*r*-methyl-L-glutamate) used in this study is low-priced and is easily prepared as spherical particles. The spherical particles of PMLG prepared are very

hard and tolerate a high flow-rate. The hydrophobicity of the packing can be adjusted by the transesterification of ester residues (and other modifications also are possible).

The theoretical plate was not described in this paper. A high theoretical plate number may be realized by the preparation of particles with the diameter range of 5 to 10 μm and their packing under a high pressure.

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