Preparation of New Hydrophilic and Durable Porous Polymer Particles

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SYNOPSIS

A preparation method for new highly durable hydrophilic porous polymer particles consisting of acrylic monomers and copolymerizable cross-linking agents was studied. In the case of using only a two-functional acrylic monomer as the cross-linking agent, the obtained particles had a relatively low mechanical strength, but by the addition of a multifunctional acrylic monomer into the above system, it was found that the porous particles obtained became highly durable and showed excellent mechanical strength of more than 500 kg/cm^2. The effect of preparation conditions on the specific surface area of these particles consisting of three components was investigated from the following viewpoints: The first was aimed at the stabilized phase of droplets consisting of monomer/cross-linkers/porogen under various conditions of aqueous poly(vinyl alcohol) (PVA) media, and the second was the effect of composition changes of the monomer and cross-linkers on the inner porous structure of the particles obtained. Thus, the specific surface area of the particles was found to be significantly affected both by the degree of saponification (DS) of PVA and by the change of composition. On the other hand, the average pore radius of typical particles obtained in this way can be controlled in the range from 40 to 120 Å by the weight ratio of chlorobenzene as the porogen. Furthermore, the mechanical stability and durability of the column packed with the porous particles obtained were discussed. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

Organic porous polymer sorbents for high-performance size-exclusion chromatography (SEC) are widely used in the separation, purification, and analysis of proteins, polysaccharides, polynucleotides, and other natural polymers and, also, so are many types of synthetic polymers. Some of the best-known commercial products are Bio-Gel TSK, Fractogel TSK, and Sepharon HEMA, based on the hydrophilic polymers of the methacrylate or polyether type. Since it was first reported in 1959 that hydrophilic gels could be synthesized from dextran,[1] many gels for aqueous SEC have been prepared from a variety of hydrophilic polymers such as agar,[2] cellulose,[3] polyacrylamide,[4] and poly(vinyl alcohol).[5,6] However, they are still not free of defects: For example, most of the hydrophilic gels with a large ex-

clusion molecular weight (M_{lim}) cannot be used under high flow-rate conditions, because the larger the value of M_{lim}, the worse the pressure resistance. This is an unavoidable defect of gel swelling-type packings. On the other hand, one widely used organic porous polymer is polystyrene gel, which itself is an excellent packing for organic SEC. However, such organic porous polymer gels have decisive limitations, because the column efficiency is lower for compounds with relatively long retention times, owing to the strong selectivity due to the π-electrons of the aromatic rings.[7] Although other hydrophilic organic porous polymer sorbents, glycidyl methacrylate–ethylene glycol dimethacrylate gel[8] or copolymers of 2-hydroxyethyl methacrylate with ethylene dimethacrylate,[9] have been widely studied, they are generally used for reversed-phase chromatography characterized by strong interactions between the polar mobile phase and various sampie molecules.
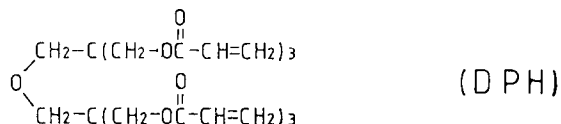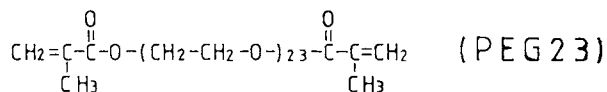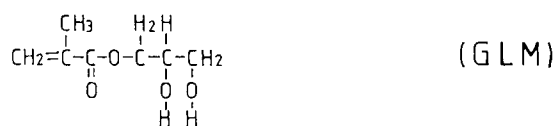
When one is considering the efficiency of the SEC chromatographic unit operation, perhaps the most

useful and flexible system would be the one capable of utilizing all common eluents from water to organic solvents. Several column packings have been described that go at least part way toward this ideal. For example, urea–formaldehyde polymer gel[10] and cross-linked glucomannan porous gel[11] seem to fall into this category. In addition, from recent trends of high-speed analysis or sample atomization for biological or clinical science, porous packings capable of use in high-performance liquid affinity chromatography (AFC) have now been expected. Therefore, we began to search for a novel type of porous polymer packing useful for both SEC and AFC. Thus, this article deals with the preparation and characterization of new durable porous polymer particles consisting of a three-component system.

EXPERIMENTAL

Materials

2,3-Dihydroxypropyl methacrylate (GLM) monomer was supplied by Nippon Oil & Fats Co., Tokyo, Japan. Two cross-linkers of poly(oxyethylene dimethacrylate) (PEG23) and dipentaerythritol hexaacrylate (DPH) were obtained from Shin-Nakamura Chemical Co., Wakayama, Japan. The initiator, benzoyl peroxide (BPO), used was a guaranteed reagent, which was obtained from Wako Pure Chemical Industries Co., Osaka, Japan. The material structures of the above three components used in this study are described below. The poly(vinyl alcohol)s (PVA) used in the polymerizations are summarized in Table I.

$$CH_2=\overset{CH_3}{\underset{\overset{\parallel}{O}}{C}}-\overset{H_2}{\underset{}{C}}-O-\overset{H}{\underset{\overset{|}{O}}{C}}-\overset{H}{\underset{\overset{|}{O}}{C}}-CH_2 \qquad (GLM)$$
$$\qquad\qquad\qquad\quad\; H \; H$$

$$CH_2=\overset{}{\underset{CH_3}{C}}-\overset{O}{\overset{\parallel}{C}}-O-(CH_2-CH_2-O-)_{23}-\overset{O}{\overset{\parallel}{C}}-\overset{}{\underset{CH_3}{C}}=CH_2 \qquad (PEG23)$$

$$O<\begin{matrix} CH_2-C(CH_2-O\overset{O}{\overset{\parallel}{C}}-CH=CH_2)_3 \\ \\ CH_2-C(CH_2-O\overset{O}{\overset{\parallel}{C}}-CH=CH_2)_3 \end{matrix} \qquad (DPH)$$

Preparation of Porous Particles

A series of three-component porous polymer particles was prepared by suspension polymerization using chlorobenzene as the inert solvent and various kinds of PVA as the stabilizer. The porous structure was formed by solvent extraction after polymerization. The standard polymerization was carried out in a 1000 cm³ three-neck cylindrical separable flask equipped with a reflux condenser, nitrogen inlet, and stirrer. The flask was immersed in a water bath thermostated at the reaction temperature. Weighed amounts of GLM, PEG23, DPH, BPO, and aqueous PVA solution were placed in a 1000 cm³ stainless steel beaker. The contents of the beaker were purged with nitrogen through the glass tube and then dispersed by a homomixer M-type (Tokusyukiko Industries Co., Tokyo, Japan) at 2000 rpm for 10 min. After this treatment, the dispersed solution was

Table I   PVA Used in This Study

Grade	Degree of Saponification (mol %)	Degree of Polymerization	Supplier
PVA-500	89.0	500	Wako Pure Chemical Co.
X-0965-1	52.0	300	Kuraray Co.
HM-05	62.0	590	Kuraray Co.
X-0965-2	66.0	300	Kuraray Co.
L-7	70.5	680	Kuraray Co.
X-0982	72.0	500	Kuraray Co.
X-0983	74.0	1000	Kuraray Co.
505	77.0	500	Kuraray Co.
405	81.0	500	Kuraray Co.
205	88.0	500	Kuraray Co.
X-0965-4	92.0	300	Kuraray Co.

poured into the separable flask and stirred. The stirrer speed was maintained at 200 rpm. The particles obtained after polymerization were removed by a centrifugal sedimentation (2500 rpm, 5 min) and filtered through a glass filter. The particles were then thoroughly cleaned with water, acetone, and water again.

Characterization of the Porous Particles

The particle size and size distribution were determined by a Lasermicron sizer Pro-7000 (Seishin Kigyo Co., Tokyo, Japan). A few milligrams of the dried particles was circulated through the sample cell, followed by dispersion in water with ultrasonic equipment for 3 min, and then measured. The surface area of the particles was measured by nitrogen adsorption isotherms. Nitrogen adsorption at 77 K was determined volumetrically by Autosorb-1 (Yuasa Ionics Co., Takatsuki, Japan). The BET equation was used to analyze the low relative pressure region of the isotherm to determine the surface area of the particles.

Average pore radii and pore volume of the particles were also calculated as regards the equivalent hollow cylinders using the following equation[12]:

$$r = 2V/S$$

where the terms V and S are the total pore volume and the specific surface area, respectively.

Morphology observation of the porous particles was made by a scanning electron microscopy (SEM) Model S-510 (Hitachi Industries Co., Hitachi, Japan) and cross-sectional observation was carried out by a electron microscope DS-130 (Akashi Industries Co., Tokyo, Japan) after cutting off the particles wrapped with epoxy resin Quetol 812 (Nisshin EM Co., Tokyo, Japan) using a cutting machine Ultratome NOVA (LKB Produkter AB, Sweden).

Mechanical strength of the porous particles was investigated using a column-packing experiment. A 15 mL Packer was connected at the top of the stainless steel column (4 × 150 mm) packed with the porous particles prepared in this study and filled with a mixture of glycerin/methanol (35/65, v/v). Methanol, used as a packing solvent, was purged into the column at a flow rate of 2 mL/min. The back pressure was raised gradually until particle destruction occurred. Another method for testing mechanical strength was studied using the packed column. In these cases, the relationship between back pressure and flow rate were measured.

A durability test of the column packed with the porous particles obtained in this study was conducted with a high-performance liquid chromatograph (Shimadzu, LC-6A) equipped with a system controller (Shimadzu, SCL-6B) at a column oven temperature of 35°C. The mobile phase system and the elution program used in the experiment will be described in detail below.

RESULTS AND DISCUSSION

Effect of PVA as Stabilizer

Porous particles having good mechanical strength could not be produced from suspension polymerization of a two-component system using 2,3-dihydroxypropyl methacrylate (GLM) and poly-(oxyethylene dimethacrylate) (PEG23). However, the addition of a multifunctional cross-linker such as dipentaerythrytol hexaacrylate (DPH) into this system was found to give porous particles much improved in their mechanical strength. However, the physical properties of the particles prepared under usual suspension polymerization conditions of these three components were contrary to expectation, i.e., the obtained data of both specific surface area and total pore volume of the particles prepared in these cases were very small and poorly reproducible.

To investigate the origin of these phenomena, scanning electron micrography (SEM) of the surface of the particles prepared was carried out. A typical SEM photograph of the particles is shown in Figure 1. This photograph clearly shows that a thin film
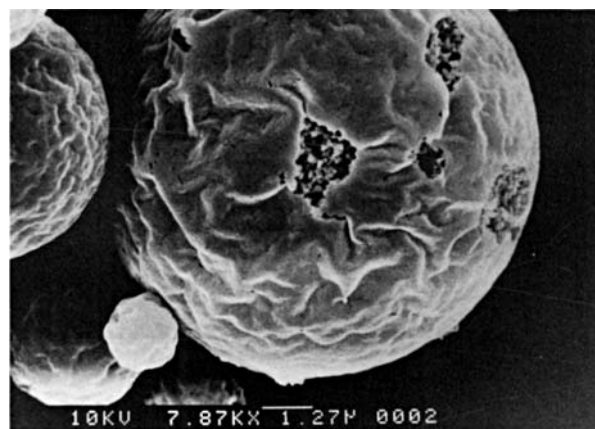
Figure 1 Scanning electron micrograph of typical porous GLM–PEG23–DPH polymer particles prepared using PVA with DS over 85 mol %.
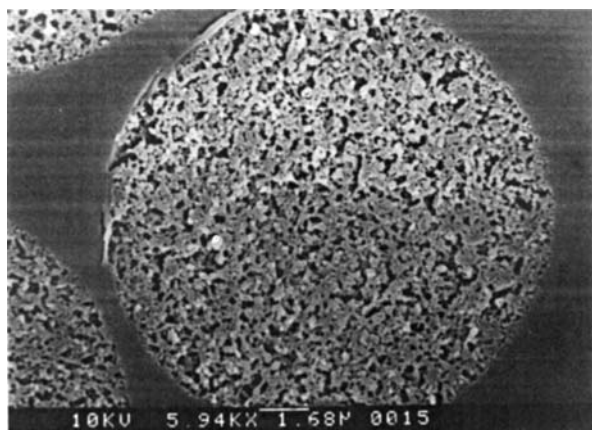
Figure 2 Scanning electron micrograph of the cross section of porous GLM–PEG23–DPH polymer particles prepared using PVA with DS over 85 mol %.

has been covered on the surface of the particles and stripped off from the surface to form "craters." An inner structure of the particles could be seen through most parts of these craters. To make clearer the inner structure of the particles, they were wrapped with a commercial epoxy resin as a binder matrix, followed by cutting using a microtome to prepare the cross-sectional samples of the particles. Figure 2 shows one of the typical SEM photographs of a cross section of the particles. In the figure it is clear that the inner structure of the particles was thoroughly porous. In view of these observations, the most reasonable consideration about the particle structure could be assumed: that although the inside

of the particles consisted of hard constituents with porous structure, the surface of these particles was covered, such as by a microcapcel, by the different constituents of the polymers. Such phenomena are most likely caused by the state of the stabilization of liquid droplet dispersion in the aqueous PVA solution containing the three polymerizable components and porogen.
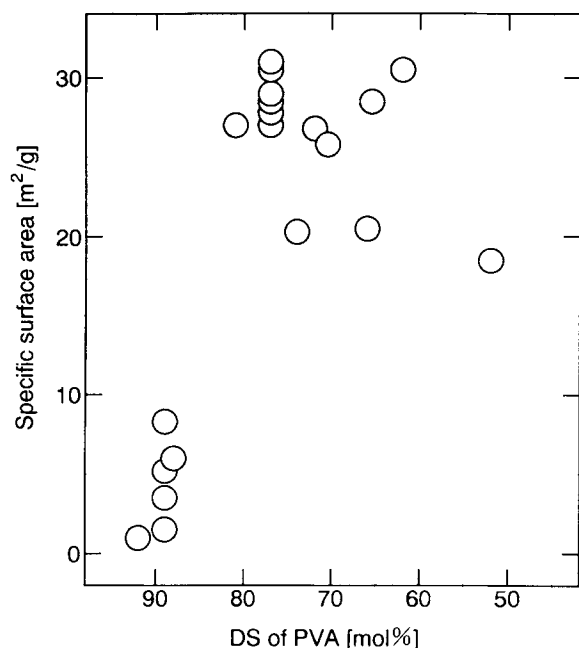
On the basis of these assumptions, the effect of PVA on physical properties such as specific surface area of the porous particles prepared under the conditions of various degrees of saponification (DS) of PVA in the same ratio of monomer and cross-linkers was studied. The typical runs of experimental results are summarized in Table II. The roughness factor in Table II is a qualitative parameter of the unevenness of the surface of the particles, and these factors have been calculated from the specific surface area of each particle divided by its calculated surface area regarded as a nonporous hard sphere. Thus, the larger value of this factor indicates the more uneven surface of the particles.

It was found that all the physical properties of the particles drastically changed in the region near 85 mol % of DS of the PVA used in the polymerization. Furthermore, in Figure 3, all the experimental results that the DSs of the PVA used are plotted as the abscissa and the specific surface areas of the particles are obtained as the ordinate. The degree of polymerization of PVA, by the way, had almost no effect on the specific surface area of the porous particles obtained but did have a slight effect on the average size of the particles. Needless to say, it be-

Table II   Effect of the Degree of Saponification of PVA on Physical Properties of Porous Particles

DS of PVA (mol %)	Average Particle Diameter (μm)	BET Specific Surface Area (m²/g)	Average Pore Radius (Å)	Total Pore Volume (mL/g)	Roughness Factor
52	12.3	18.6	205.8	0.19	12.9
62	12.1	30.3	78.2	0.12	23.3
66	11.4	20.2	160.4	0.16	16.8
71	11.0	25.9	89.9	0.12	19.5
72	11.8	26.8	201.4	0.27	23.5
74	14.1	20.1	94.8	0.10	19.9
77	11.3	25.2	77.6	0.29	24.8
81	11.8	27.0	200.3	0.27	24.3
88	13.4	6.0	229.3	0.07	5.9
92	15.6	1.1	n.d.	n.d.	1.2

ᵃ n.d.: not detected.

Figure 3   Influence of degree of saponification of PVA used in polymerization on the specific surface area of porous polymer particles obtained.

came clear from Figure 3 that the surface morphology of these porous particles had been affected by the DS of the PVA used as a stabilizer; i.e., in other words, such a change of DS affects the hydrophilic and hydrophobic balance in each stabilized layer of the droplets in the suspended solutions.

The raw materials GLM and PEG23 used in this study are both water-soluble even at room temperature, whereas DPH and chlorobenzene can be only slightly dissolved in warm water. Hence, it is possible to say that it may change the composition in the PVA-stabilized layer of each droplet. This is supported experimentally by the observations described below. For example, a typical SEM photograph of particles prepared using DS = 77 mol % of PVA is shown in Figure 4. The most striking aspect in Figure 4 is that particles having a completely naked porous surface structure without a thin-covered film (as shown in Fig. 1) have been produced. Similar photographs can be also observed from particles prepared using DS = 81 mol % of PVA as the stabilizer during polymerization. Such a phenomenon could be explained as follows: The droplets of chlorobenzene solution containing GLM, PEG23, and DPH were stabilized by forming the protected colloidal layer of PVA in aqueous medium in the usual manner. It seems reasonable to consider that the

protected layer stabilized by using the higher DS of PVA will be induced to dissolve the hydrophilic molecules such as GLM or PEG23 owing to their hydrophilic character. It can be presumed that the PVA protective layer of these droplets could be stabilized by the formation of an outer layer having a little different composition from the inner ones. Therefore, it is obvious that this composed layer may consist of a little higher concentration of hydrophilic monomers such as GLM or PEG23 and may give rise to a phase separation at the beginning of polymerization, followed by the formation of thin-film-like microcapcels, and, then, finally producing some "craters" due to particle collision under vigorous stirring conditions. On the other hand, in the case of the experiments using a lower DS of PVA, it can be presumed that such a layer as described above cannot be formed because the hydrophobic character in the PVA protected layer of the droplets does prohibit the hydrophilic monomers from dissolving. Thus, it could be concluded that the suspension polymerization of the present system using a lower DS of PVA proceeded without any formation of the thin film caused by phase separation at the initial stage of polymerization.

Effects of Monomer and Cross-linker Contents

Physical properties of the particles prepared in this three-component system were found to be significantly affected by the content of the monomers. The experimental results in the present system for investigating the effect of the monomer ratio to the
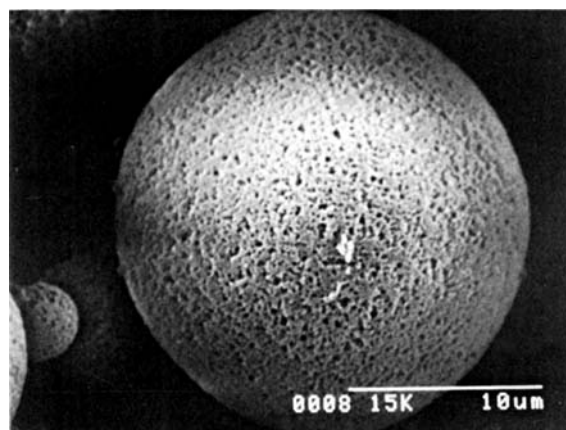
Figure 4   Scanning electron micrograph of typical porous GLM–PEG23–DPH polymer particles prepared using PVA with DS = 77 mol %.
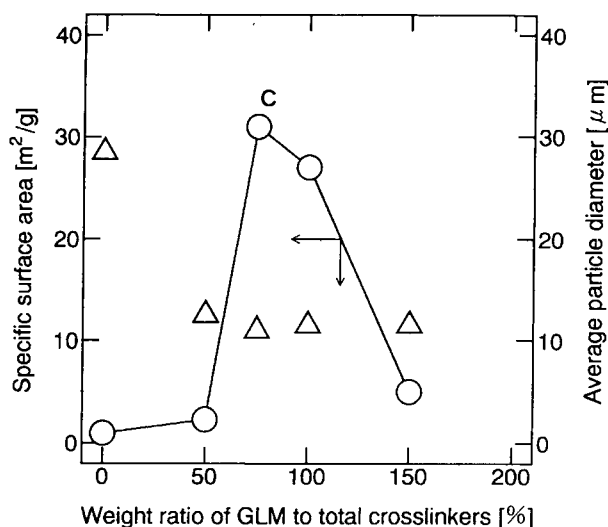
Figure 5 Effects of weight ratio of GLM monomer to total cross-linkers on the specific surface area of the porous particles obtained.

cross-linkers on the specific surface area of the particles prepared under each condition are depicted in Figure 5. These results suggest that the physical values such as total pore volume and specific surface area (BET) of porous particles cannot be determined in the usual way in that the volume ratio of porogen against total amounts of nonvolatile gel-forming materials almost enables us to control the physical properties of the particles. As seen in Figure 5, it is clear that the specific surface area of these porous particles has been drastically altered by the change of the monomer/cross-linkers ratio even though all were made under the same preparation conditions except for the ratio noted above. This seems to indicate that the specific surface area may be definitely correlated to the internal cross-linking structure of the porous particles.

Furthermore, the effect of two kinds of cross-linkers, i.e., the PEG23/DPH ratio on the specific surface area, was also examined. The experimental results are shown in Figure 6. In this experiment, the weight ratio of monomer/cross-linkers was chosen to be a constant of $\frac{3}{4}$ at point C in Figure 5. A specific feature can be pointed out from Figure 6: that the specific surface area increases with an increase of DPH content in the cross-linker's composition and the maximum surface area has increased to 120 m²/dry g. Similarly, the total pore volume of the particles was found to increase with an increase of DPH content such as 0.01, 0.16, 0.42, 0.53, and 0.43 mL/dry g, respectively. These findings suggest that the greater the content of the six-func-

tional cross-linker involved in the system the larger the porosity of the particles achieved. Therefore, it is clear that in this three-component system the content of the monomer or cross-linkers plays a decisive role on the physical properties of the particles.

Control of Pore Radius

Control of the pore radius of the particles produced from this system was investigated by the method of the volume change of porogen. These experiments were carried out based on the data described above. To obtain better reliability of the data, duplicate systems were adopted, i.e., the following two systems were chosen as the monomer compositions from the consideration of the data described above: Systems A and B were in weight ratios of 30/5/35 and 30/15/25 for GLM/PEG23/DPH, respectively. Both systems are typical compositions that contain particles that can possess a relatively large specific surface area. In fact, under polymerization conditions using chlorobenzene as a porogen with eight steps from 0.5 to 2.5 as P values, which indicate the weight ratio of porogen against total amount of gel-forming materials, experiments were carried out. The experimental results on the physical properties of these porous particles obtained are summarized in Table III.

The relationship between these P values and average pore radius measured from each particle is also shown in Figure 7. As is obvious from these data, a certain upper and lower value may exist for the average pore radius controlled in both systems, and
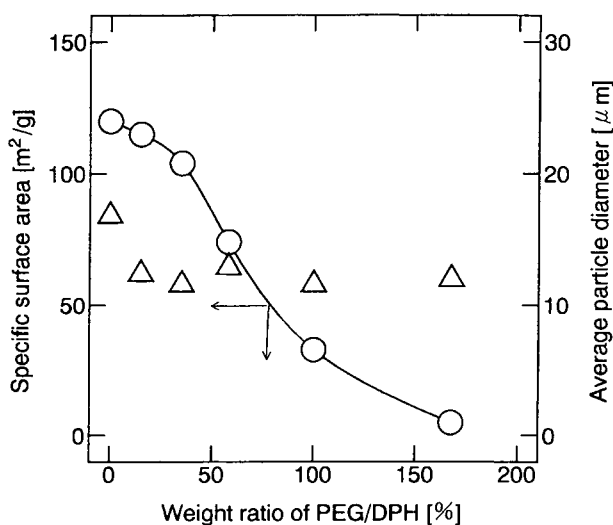
Figure 6 Effects of weight ratio of PEG23/DPH on the specific surface area of the porous particles obtained.

they are definitely dependent on the P values. Existence of such limitations for pore radius could be interpreted as follows: The occurrence of maximum or minimum pore radius can be attributed to the issue of the pore formation process under the conditions in either a diluted or concentrated solution of chlorobenzene involving monomer and cross-linkers, respectively. Moreover, assuming that pore formation would have resulted from the production of macromolecules by a cross-linking reaction between polymer segments in organic media, it seems reasonable to postulate that in the case of concentrated solution of monomer and cross-linkers the cross-linking reaction may occur in many places and that many polymer chains tend to pack together as closely as their geometry allows. From these results, the inner solvent may be presumed to be squeezed out from the porogen space and to lead to certain minimum pore radius formation. In the case of the dilute solution, on the other hand, it would be very difficult for such a cross-linking reaction between polymer segments to occur. Thus, polymer phases possessing unstabilized functional groups and still remaining unchanged may be presumed to form the particles with a maximum pore radius in a random fashion. In such a sense, it seems to be an interesting subject as to how the cross-linking reaction between polymer segments occurs in different kinds of inert solvent like porogen, because the pore formation would be initiated by phase separation based on solubility between the porogen solvent and the polymer segments produced in the initial stage of polymerization. The duplicate experiments as shown in Figure 7 have given relatively good reliability on the data. Thus, it could be concluded that the average pore radius may be controlled in the range from 0.5 to 1.5 of P values in both systems A and B and that their pore radius can be controlled in the region from ca. 45 to 120 Å in system A and from 60 to 114 Å in system B. However, these values would not be considered to indicate a maximum range in pore radius of the particles prepared in this three-component system, since they may change to a different porogen as mentioned above.

Column Durability

Evaluation of the durability of a packed column of prepared particles is very important in a practical sense. Thus, a study was carried out by the measurement of a flow-rate/back-pressure relationship and by the microscopic observations of the packings after 500 cycles of repeated passes of mobile phases selected as one of the typical affinity high-perfor-

Table III Experimental Results on Physical Properties of Typical Porous Particles Prepared under Different P Value Conditions
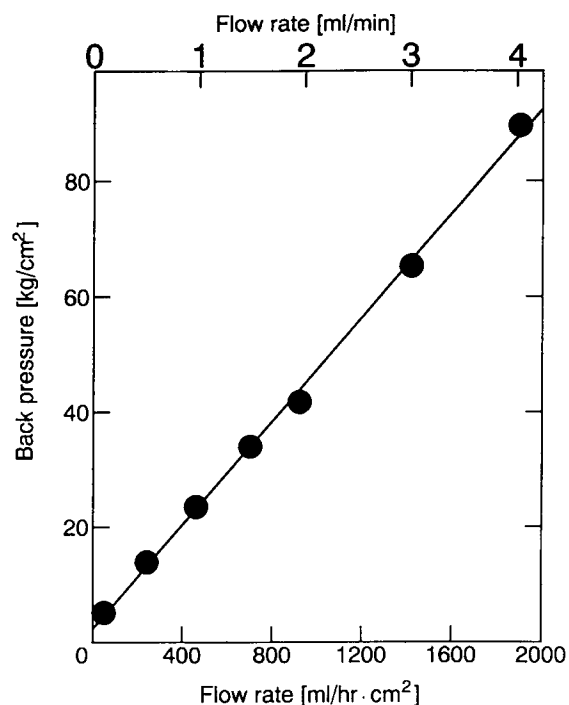
P Value	System A				System B			
	Average Particle Diameter (μm)	BET Specific Surface Area (m²/g)	Average Pore Radius (Å)	Total Pore Volume (mL/g)	Average Particle Diameter (μm)	BET Specific Surface Area (m²/g)	Average Pore Radius (Å)	Total Pore Volume (mL/g)
0.5	16.0	18.8	39.6	0.037	10.7	0.82	n.d.	n.d.
0.7	14.7	92.8	45.5	0.21	9.7	2.08	42.1	0.01
0.8	15.5	101.1	53.0	0.27	10.2	27.5	58.9	0.09
0.9	14.7	107.5	64.3	0.35	14.7	37.2	63.0	0.12
1.0	16.3	112.1	75.8	0.42	10.5	50.6	70.3	0.18
1.5	17.8	110.3	120.5	0.66	15.0	69.0	114.1	0.40
2.0	19.0	105.6	116.3	0.61	13.8	73.6	91.9	0.34
2.5	20.4	96.0	81.6	0.39	15.0	70.3	100.1	0.35

Dispersion conditions: Kuraray PVA505, concn 0.33 wt %. Homomixer used at 3000 rpm for 10 min.
n.d.: not detected.
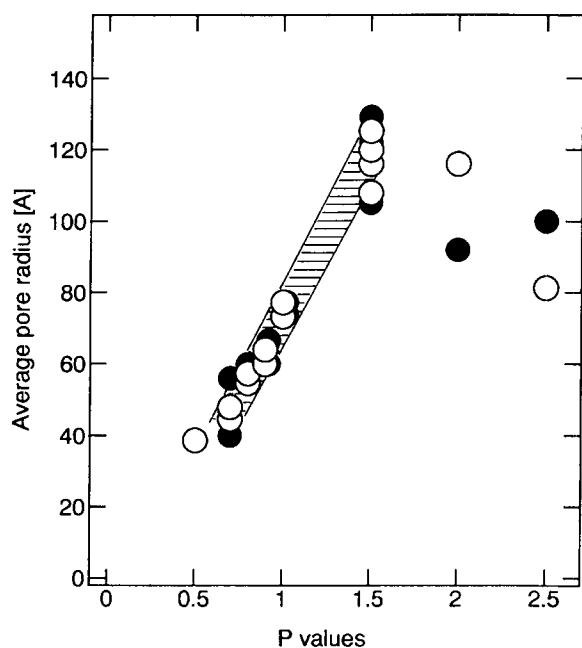
Figure 7   Experimental results for average pore radius control of porous GLM–PEG23–DPH polymer particles by means of P values. The shaded portion of the figure seems to be controllable. (○) System A; (●) system B.

Figure 8   Experimental results on correlation between flow rate and the back pressure of the column packed with porous GLM–PEG23–DPH particles.

mance liquid chromatography systems described in Table IV. A study on the relationship measurements between flow rate and back pressure were undertaken using a 4 × 150 mm stainless steel column, packed with typical particles with 17.8 µm as the average particle diameter and 120 Å as the average pore radii prepared in system A, and using purified and degassed water as the mobile phase. The results obtained are shown in Figure 8. A good relationship for mechanical stability was obtained in this column. In the same way, the particles prepared in system B as well as in other conditions of system A were

found to show a similar behavior easily accessible for analytical HPLC purposes. In addition, the durability of the packed column using the same particles of system A was evaluated under the HPLC condition using acidic and alkali eluents as is shown in Table IV. Figure 9 is a schematic diagram of repeated passes of these mobile phases in this experiment. As the result, even after the passing of 500 cycles of various types of eluents, no defected or fractured particles could be found by the microscopic observations. From these findings, the porous poly-

Table IV   Mobile Phase System Used in Durability Experiment

Mobile Phase	Composition	pH	
I	0.2M Ammonium acetate 0.1M Magnesium chloride	9.0	Adjusted with 1M NaOH
II	0.2M Ammonium acetate	5.5	Adjusted with 1M HCl
III	0.2M Ammonium acetate 0.3M Sorbitol	9.0	Adjusted with 1M NaOH
IV	0.1M HCl	1–2	
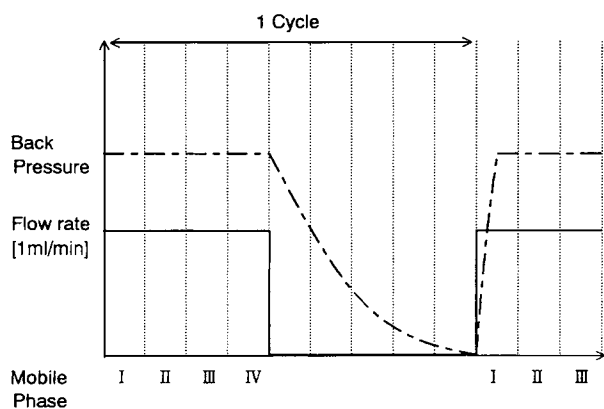
Figure 9 Schematic diagram for column durability experiments using a 4 × 150 mm column packed with porous particles prepared in this study. All experiments were carried out automatically using an HPLC apparatus equipped with an elution program controller. Each mobile phase was eluted at intervals of 1 min with constant rate of 1 mL/min.

mer particles obtained in this study seem to have enough durability to be available for the HPLC packings.

CONCLUSIONS

New hydrophilic and high durable porous polymer particles were successfully prepared using a three-component system. Morphology of these particles was significantly affected by the degree of saponification of PVA used as a stabilizer in suspension polymerization. Their specific surface areas and total pore volumes were also found to be dependent on

weight ratios of constituents. The pore radius of the particles could be well controlled by the P values under their specific conditions. Furthermore, packed columns with the porous particles obtained in this study have a good mechanical stability and adequate durability easily accessible for analytical HPLC purposes.

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