The Modification of Porous Chromato-Gel Surface for Liquid Chromatography by Poly(4-vinylpyridine) Microgels

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SYNOPSIS

A porous chromato-gel for liquid chromatography was modified by fixing poly(4-vinylpyridine) (P4VP) microgels of homogeneous size on its surface, and the microgels were further quarternized by using iodomethane or bromoacetic acid, in order to vary the chromatographic characters of the original chromato-gel. The character of the porous chromato-gel before and after modification was investigated by the retention volume (Ve) of dextran and pullulan standards with various molecular weights (MW: 2×10–2×10^6 g/mol). It was found that a single layer of microgels had been fixed compactly on the surface of the chromato-gel, and the retention volume (Ve) and the limit exclusion molecular weight (LEMW) [the molecular weight where the $d(Ve)/d(\mathrm{MW})$ became maximum] were affected remarkably by the sizes and chemical characters of the fixed microgels. The retention volume and LEMW decreased when the pore size formed by microgels was smaller than the hole size of the chromato-gel, while the reverse result was obtained when the pore size formed by the microgels became larger than the hole size of the chromato-gel. After the microgels were quarternized, the retention volume decreased further. Furthermore, the chromato-gel quarternized by bromoacetic acid showed excellent chromatographic character. This study provided an advantage in that the chromatographic characters of the chromato-gel can be varied and improved easily. © 1993 John Wiley & Sons, Inc.

INTRODUCTION

The applications[1-9] of polymer microgels or microspheres with homogeneous size (narrow-size distribution) have become more and more attractive because of the microgels' special, interesting characters. Especially, the character of large surface area of microgel has been utilized most extensively for the longest time. Examples of some such uses are as a coating agent[1-4] and as an adhesive agent.[5] In recent years, the applications in medical science have come to prominence. Examples of this use include drugs,[6-8] fixed chemically or physically on the surface of the microgels and then carried to a particular part of the body and released with the appropriate speed (polymer drug); and diagnostic medicine,[9] where antibodies (or antigens) are fixed on a microgel and the antibody–antigen reaction can be confirmed by the naked eye by observing the aggregation of microgels. The most interesting and simplest character, that is, the homogeneity of the size of the microgel itself, however, has been utilized rarely in the application areas.

In this study, we used poly(4-vinyl-pyridine) (P4VP) microgels of homogeneous size to modify the surface of the porous chromato-gel for liquid chromatography in order to vary its character as a new application of microgels. This study was stimulated by the simple and interesting character of microgels, that is, the sizes of pores formed by microgels not only have very narrow distribution but also were very small (about only 15% of the diameter of the microgels) if the arrangement of microgels of homogeneous size on the surface of the chromato-gel was ordered hexagonal packing. Thus, it can be expected that the difficulties for production of the chromato-gel with homogeneous hole sizes and for adjustment of hole sizes can be overcome easily only

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by fixing the microgels of homogeneous size on its surface and by changing the sizes of microgels, and the lower limit of the hole size and exclusion size of the chromato-gel can be lowered further by using the smaller microgels. In addition, the characters of the chromato-gel can be changed further by modifying the microgels with various agents.

P4VP microgel was selected in this study because its chemical properties, such as acidity, basicity, and hydrophilic and hydrophobic properties, can be modified. P4VP also can be cross-linked both within and among microgels easily. The characters of the modified chromato-gel can thus be further changed easily only by quarternizing the P4VP microgels on the surface of the chromato-gel with various quarternizing agents.

In previous studies, we established the preparative methods of monoingredient (not stained by surfactant) P4VP microgels of 70–700 nm diameter of homogeneous size.[10] We also have confirmed that these P4VP microgels can form ordered hexagonal packing easily[11] due to their homogeneous size. In this study, we first modified the surface of porous chromatogel by fixing the P4VP microgels on it and investigated the effects of microgel sizes on the chromatographic character. Further, we studied the effects of characters of microgels by quarternizing the microgels with iodomethane and bromoacetic acid. The chromatographic character was studied by the retention volume of dextran and pullulan standards.

EXPERIMENTAL

Materials

Porous chromato-gels for liquid chromatography were obtained by aqueous suspension polymerization (80°C, 5 h) of methyl methacrylate (MMA), glycidyl methacrylate (GMA), and divinyl benzene (DVB) monomers, where buthyl peroxide was used as an initiator; poly(vinyl alcohol) aqueous solution (2 wt %), as a suspension stabilizer; and the mixture of diethyl benzene and iso-octanol, as a diluent for preparing macroreticular gel. The hole size can be adjusted by varying the mixing ratio of diethyl benzene and isooctanol. Two kinds of chromato-gels with different hole sizes and diameters were used in this study. Their composition and characters are shown in Table I.

Table I Composition[a] and Characters of Chromatogels

Sample Code	Characters		
	Diameter (μm)	Hole (nm)	Br/Unit[b] (mol %)
MGV-7Br	140–180	18	13.2
MGV-8Br	104–150	100	

[a] Composition: methyl methacrylate, 64.5 (mol %); glycidyl methacrylate, 20.1 (mol %); divinylbenzene, 15.4 (mol %).
[b] The fraction of —Br groups introduced was calculated from Br/(MMA + GMA) (unit/unit × 100%).

To fix chemically the P4VP microgels on the chromato-gel, the chromato-gel was treated with a concentrated hydrogen bromide (HBr) solution (chromato-gel, 20 g; acetone 30 mL; HBr aqueous solution 20 mL) at 55°C for 8 h to introduce the bromo groups on glycidyl groups. The chemical reaction is shown schematically in Figure 1 and the fraction of introduced bromo groups is shown in Table I.

P4VP microgels were prepared with a special emulsion polymerization method where the partially quarternized reactive polymer emulsifier was used. The detailed methods for preparation and characterization of P4VP microgels were described elsewhere.[10] Three kinds of P4VP microgels with different diameters were employed (Table II). Before use, each microgel solution was dialyzed by closing

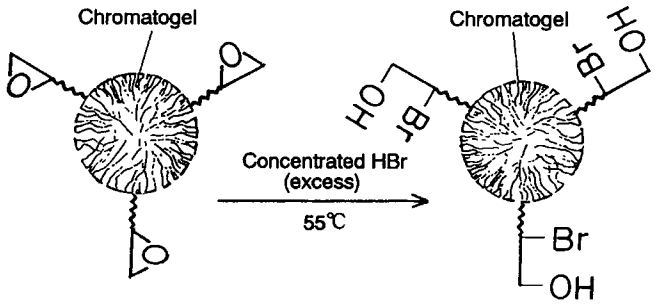

Figure 1 The reaction scheme for the introduction of the bromo groups on glycidyl groups.

Table II The Character of P4VP Microgels

Sample Code	Divinylbenzene/4VP (unit mol/mol)	Diameter (nm)
VCM5-1	0.05	700
VCM2-2	0.05	300
VCM2-7	0.05	70

V50 [2,2'-azobis(2-methylpropionamidine)dihydrochloride] was used as initiator. V50/4VP = 0.02.

the microgel solution into a seamless semipermeable cellulose tube; then, the tube was further immersed in a large amount of deionized water for over 2 weeks at room temperature. Water was changed every day.

The quarternizing agents, iodomethane and bromoacetic acid, were purchased from Tokyo Chemical Industries Co. They were used as received. Water was purified by ion-exchange resin. Methanol was used without further purification.

Modification of Chromato-gel by Microgels

The chromato-gel (1 g) was added into the microgel aqueous solution (100 mL) of 2 wt %. The chromato-gel would float on the surface of solution because the interfacial tension between chromato-gel and water was large. Here, methanol (10 mL) was added into the solution to allow the chromato-gel dispersed into the solution by decreasing the interfacial tension between chromato-gel and solution. Then, the solution was heated at 60°C for the chemical fixation of the microgel on the chromato-gel. The reaction was continued under gentle stirring for 48 h. Finally, the chromato-gel was separated from the residual microgel solution by a G3 glass filter and was washed by methanol until the methanol became transparent. The chromato-gel obtained was further dispersed in methanol and shaken by ultrasonic irradiation wave (50 W, 25 kHz) to remove completely the microgels absorbed physically on the chromato-gel. At last, the chromato-gel was collected by filtration and was washed by methanol as before.

Quarternization of Microgels on Chromato-gel

To investigate the effects of the chemical characters of microgels on the chromatographic character, the chromato-gel modified by P4VP microgels was further quarternized by iodomethane or bromoacetic acid. The chemical reactions are shown schematically in Figure 2. The process was as follows: First, the chromato-gel was immersed into the iodomethane/methanol or bromoacetic acid/methanol solution (2 wt %). Then, the solution was heated at 60°C for 48 h. After the reaction was finished, the chromato-gel was collected and washed as before. Because the quarternization reaction of the pyridine ring with alkyliodite proceeds easily even at room temperature,[12] it can be considered that the fixation of microgels on the chromato-gel and the quarternization of microgels on the surface of the chromato-gel were carried out steadily. After quarternization, the microgel quarternized by iodomethane would show cationic character in water due to the dissociation of the iodine ion, whereas that quarternized by bromoacetic acid would show amphoteric character due to the dissociation of both bromine and hydrogen ions, as shown in Figure 2. Furthermore, because the ionization would enhance the hydrophilic character of microgels, the swelling degrees of both quarternized samples in water would become larger than that of the nonquarternized sample. Also, due to the dissociation of the hydrogen ion of bromoacetic acid beside the bromine ion, the hydrophilicity of microgels quarternized by bromoacetic acid would be enhanced further and the swelling degree would become larger than that quarternized by iodomethane. Thus, because the swelling degree and chemical character can be varied by changing the quarternizing agents, the different character of the chromato-gel can be expected.

The above modification and quarternization condition are shown in Table III. To investigate the effects of microgels sizes, the MGV-7Br chromato-gel was modified by microgels of 70 or 300 nm diameter, and MGV-8Br chromato-gel was modified by the microgels of 300 nm diameter and then that of 70 nm diameter stepwisely or 700 nm diameter. For the MGV-8Br chromato-gel, the microgels of 70 nm diameter were fixed in order to decrease the pore size among larger microgels of 300 nm diameter. Furthermore, to investigate the effects of chemical character of microgels, the MGV-8Br samples after being fixed by microgels were further modified by iodomethane or bromoacetic acid.

Measurement of Character of Chromato-gel

The character of the chromato-gel before and after modification was investigated by measuring the retention volume of dextran and pullulan standards by the gel permeation chromatography (GPC) method at room temperature after they were packed into a stainless-steel column. The diameter and length of the column were 4.6 and 150 mm, respectively. Deionized water was used as a eluent, and the flow rate of water was 1.0 mL/min.

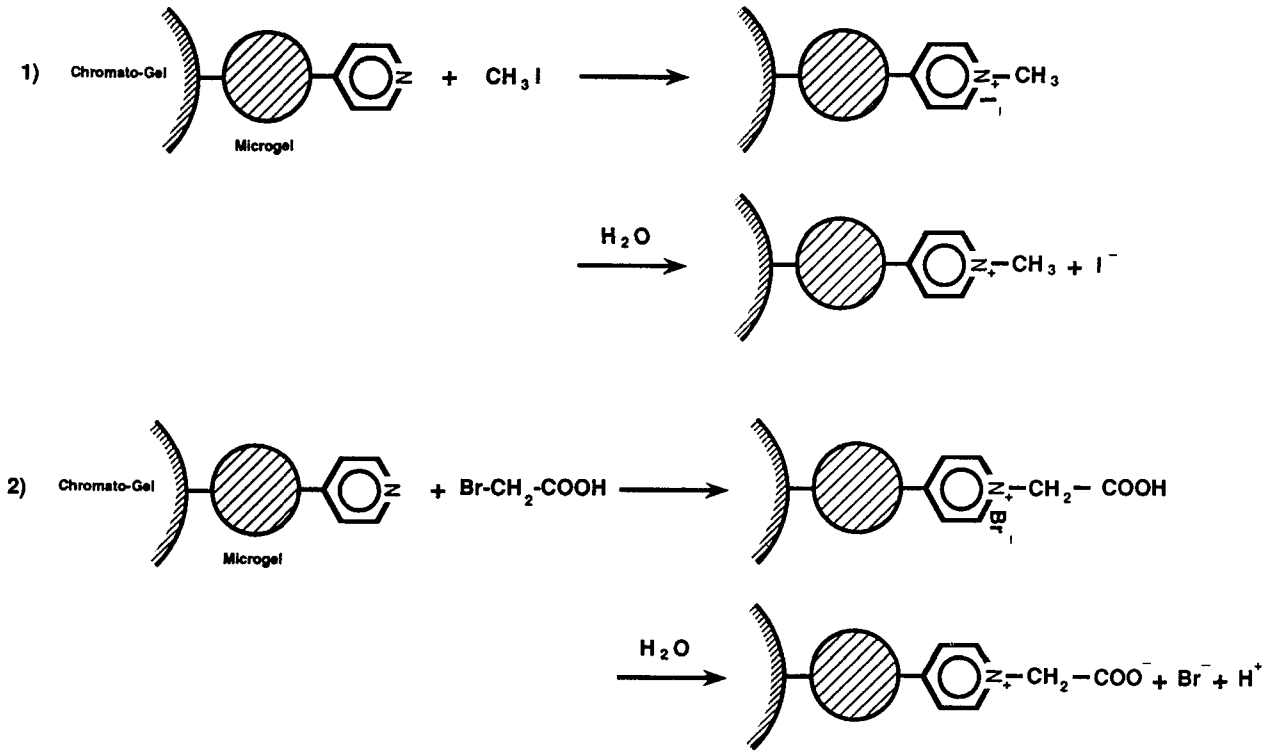

Figure 2 The reaction scheme for the quarternization of the microgel and the dissociation of the products in water.

Electron Microscopic Observation

The surfaces and cross sections of films before and after fixation were observed by a JSM-5200 scanning electron microscope (SEM) or H-700H transmission electron microscope (TEM). The specimens for SEM observations were prepared by sputtering and coating a thin Pd—Pt film (about 60 Å in thickness) on the sample under reduced pressure of below 0.05 Torr with a Hitachi E 102 ion sputter. Because the sample was porous, the sputtering and coating process was carried out from various angles to allow the surface of the chromato-gel to be covered by Pt—Pd thoroughly. The TEM specimens for the observations of cross sections were prepared by cutting down the ultrathin films (ca. 100 nm) from the chromato-gels with a microtome and setting them on the copper mesh.

Table III The Conditions of Modification of Chromatogels

Chromatogels	Diameter of Microgel (nm)	Quarternizing Agent
MGV-7Br	300	—[a]
	70	—
MGV-8Br	700	—
	700	CH_3I
	700	$BrCH_2COOH$
	300 + 70[b]	—
	300 + 70	CH_3I
	300 + 70	$BrCH_2COOH$

[a] —: Microgels were not modified.
[b] 300 + 70 nm: The microgels of 300 and 70 nm were fixed stepwisely. Refer to text.

RESULTS AND DISCUSSION

Effect of Size of Microgels

On the MGV-7Br chromato-gel with small hole size (18 nm), two kinds of microgels (70 and 300 nm in diameter) were fixed, respectively (Table III). Examples of SEM and TEM micrographs for the sample modified by the microgels of 300 nm diameter are shown in Figure 3. Figure 3(a) and (b) are the SEM micrographs of the surface, and Figure 3(c) is the TEM micrograph of the cross section. From Figure 3, we found that numbers of microgels have been fixed compactly on the surface of the chromato-gel and a lot of places showed closed hexagonal packing, although the covering percentage was dif-

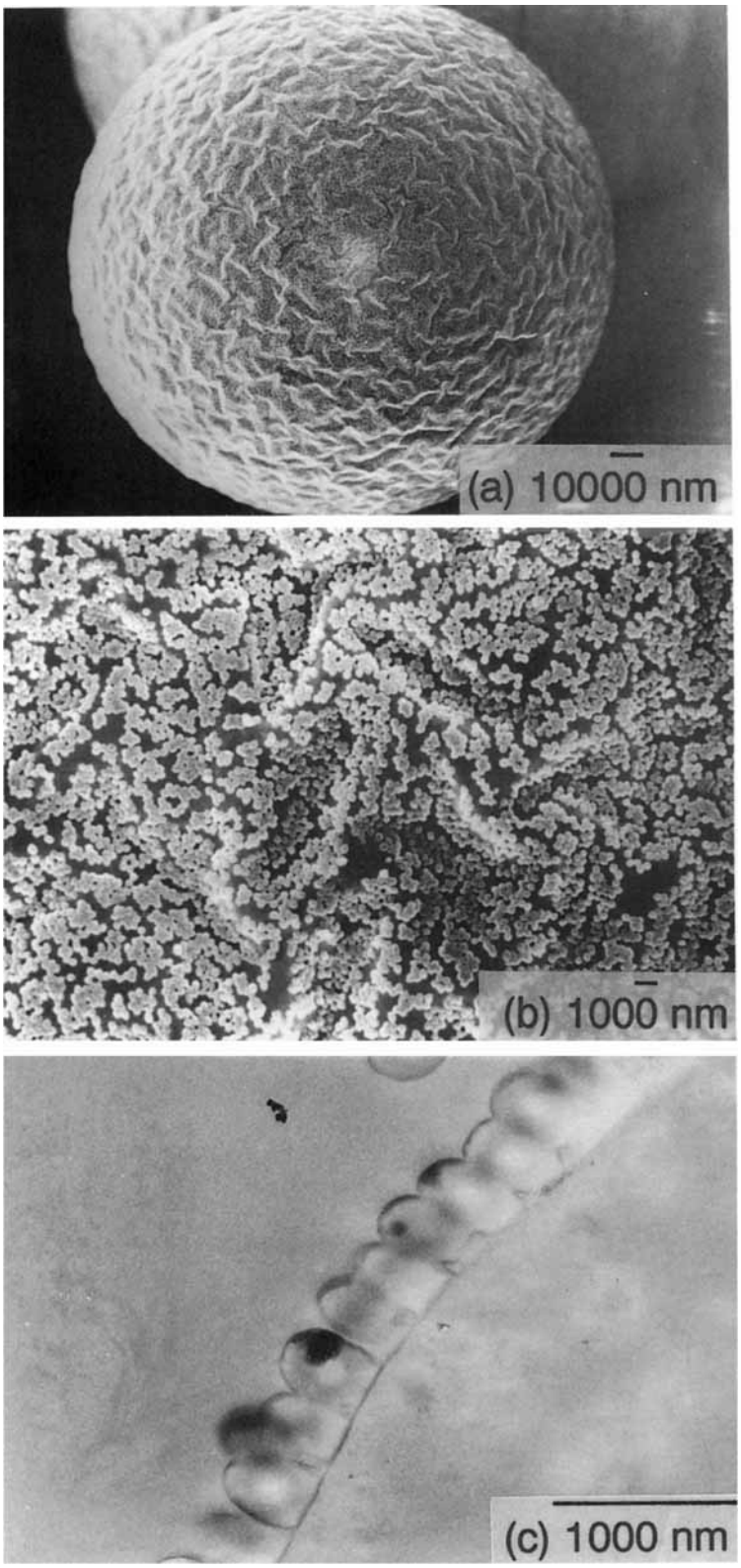

Figure 3 The electron micrographs of porous chromoto-gel MGV–7Br modified by P4VP microgels: (a,b) SEM micrographs of the surface; (c) TEM micrograph of the cross section. Diameter of microgel: 300 nm.

ficult to know exactly because the surface of the chromato-gel was very complicated. But we can see from the SEM and TEM micrographs that the chromato-gel has been covered compactly by a single layer of microgels. The phenomenon that the microgels showed closed hexagonal packing was similar to that in the preparation process of microgels film,[11] so the fixation process of microgels may be followed the arrangement process of microgels on the chromato-gel. The detailed reason must be investigated further.

The results of the retention volume of the original and modified MGV-7Br chromato-gel measured by the gel permeation chromatography (GPC) method are shown in Figure 4. The relationship between the molecular weight (MW) of dextran, of pullulan standards, and their relative retention volumes, *Ve/Vt* (*Ve* is the retention volume of dextran or pullulan and *Vt* is that of air dissolved in the solution) was represented. Reviewing the relationship between *Ve/Vt* and MW, we found that the *Ve/Vt* changed abruptly, i.e., the $d(Ve)/d(\mathrm{MW})$ showed a maximum value (the slope of curve was close to 0) at a certain molecular weight. This molecular weight was defined as the limit exclusion molecular weight (LEMW). The molecules above and below this LEMW can be fractionated. It can be seen that the character of the chromato-gel was changed largely

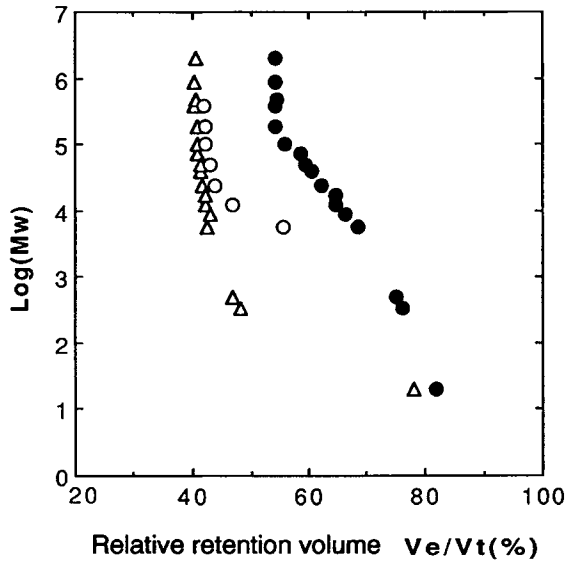

Figure 4 The relationship between molecular weight (MW) and relative retention volume (*Ve/Vt*) of dextran and pullulan standards for chromato-gel MGV-7Br; (○) before fixation of microgels; (●) after fixation of microgels of 300 nm diameter; (△) after fixation of microgels of 70 nm diameter.

after the microgels were fixed, and it depended remarkably on the sizes of microgels. For the sample on which the microgels of 70 nm diameter were fixed, the value of *Ve/Vt* did not change apparently in the range of molecular weight higher than 10^4 g/mol or in the very low region (2×10 g/mol), whereas this value changed drastically in the range of molecular weight between 10^4 and 10^2 g/mol. The LEMW decreased largely as expected. It changed to the order of 10–10^2 g/mol from 10^4 g/mol. This result can be simply ascribed to the very small pore sizes formed by the microgels, i.e., only 10 nm (15% of diameter of microgels) in diameter if the hexagonal packing of the microgels was assumed. The *Ve/Vt* values of the large molecules (MW > 10^4 g/mol) were not affected by the fixation of smaller microgels because they had been excluded even before the fixation of the microgels. Of course, the *Ve/Vt* values of dextran and pullulan with very low molecular weight (<10^2 g/mol) would not also be affected by the fixation of this small microgel, apparently so because the pore sizes among microgels were large enough to let them pass and the path of pores formed by microgels was much shorter compared with the holes of the original chromato-gel. The above phenomena resulted in that the LEMW became smaller.

Contrary to above result, for the sample on which the microgels of 300 nm diameter were fixed, the reverse result was observed. The *Ve/Vt* values for all of dextran and pullulan (2×10–2×10^6 g/mol) increased compared with the original chromato-gel, and the LEMW increased to 10^5 from 10^4 g/mol. In addition, the slope of the curve at the LEMW region was farther from 0, i.e., the $d(Ve)/d(\mathrm{MW})$ became smaller. This resulted from the fact that the size of pores formed by the microgels of 300 nm diameter (45 nm in diameter) was much larger than the holes of the chromato-gel. Therefore, just because larger pores than those of the chromato-gel were formed on the surface of the chromato-gel, the dextran and pullulan with higher molecular weight (10^4–10^6 g/mol) would also enter these pores and their retention volumes would be elongated to a certain extent and the LEMW would become larger. However, because the path of these larger pores formed by the microgels was shorter than that of the holes of the original chromato-gel, the retention volume of the larger molecules that entered these pores would be still much smaller than the other relatively smaller molecules that entered further the chromato-gel. As a result, the slope of the curve at LEMW region was farther from 0.

The explanation about the chromatographic character of the chromato-gel modified by microgels

of small size (70 nm diameter) or larger size (300 nm diameter) is shown schematically in Figure 5, where the hexagonal closed packing of microgels was assumed for convenience.

Furthermore, comparing the samples before and after the fixation of the microgels, it was found that the point of inflection at the beginning of the LEMW region became clearer for the samples modified by the microgels, especially by that of 300 nm diameter. This can be ascribed to the narrow size distribution of pores formed by the microgels of homogeneous size.

To summarize the above results, when the small-size microgels (70 nm diameter), which formed smaller size pores than the holes of the chromato-gel itself, were fixed on the surface, the microgels took a screen role, and dependent on which molecules with relatively lower molecular weight were also excluded, their retention volume became smaller. As a result, the LEMW became smaller, and the slope of curve at the LEMW region was not affected by the microgels so apparently. On the other hand, when the large size microgels (300 nm diameter), which formed larger pores than the holes of chromato-gel, were fixed, the pores formed by the microgels also took a large role in the increment of the retention volume of larger molecules. So, the LEMW was raised. But because the path of these pores was very short, the slope of the curve at the LEMW region was far from 0 and the chromatographic character, thus, became worse. This disadvantage was expected to be solved by fixing several layers of microgels on it or by preparing a new chromato-gel with microgels of homogeneous size.

From above results, we can know that the microgel size, i.e., the pore size of the microgels, played a large role in the chromatographic character of the chromato-gel, although the path of the pores formed by the microgels was much shorter than that of the original chromato-gel. The retention volumes of molecules that entered the chromato-gel were determined by the sizes and quantities of original chromato-gel, but the possibility or impossibility of entering the chromato-gel were determined by the pores of the microgels. As a result, the LEMW was determined by these two factors.

To confirm the above results, the same experiment was carried out for the MGV–8Br sample that had a larger pore size (100 nm). It was modified by the microgels of 300 nm diameter and then those of 70 nm diameter stepwisely, or those of 700 nm diameter. The examples of SEM micrographs for the samples modified by the microgels of 300 nm and 70 nm diameter stepwisely are shown in Figure 6.

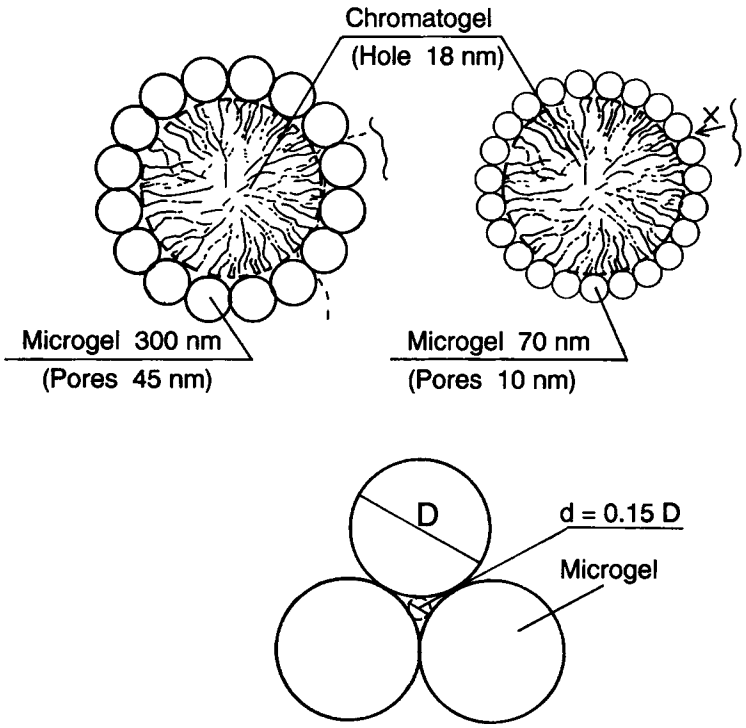

Figure 5 The scheme for the explanation of chromatographic character of chromato-gels modified by microgels.

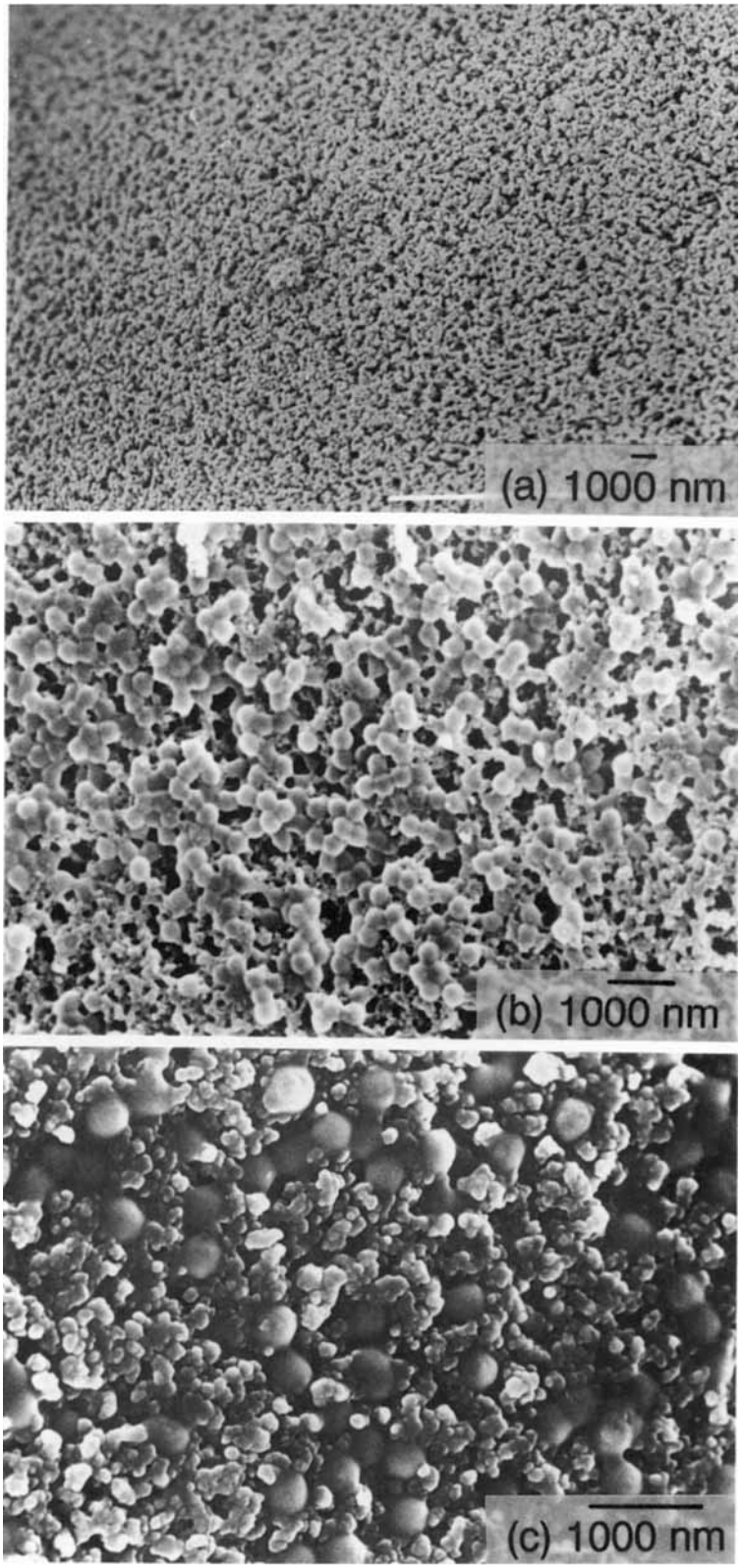

Figure 6 The SEM micrographs of porous chromato-gel MGV–8Br modified by P4VP microgels: (a) before fixation of microgels; (b) after fixation of microgels of 300 nm diameter; (c) after fixation of microgels of 300 and 70 nm diameter.

According to above results, a single layer of microgels has been fixed.

The chromatographic characters of them are shown in Figure 7. Similar results were observed for this sample. The LEMW decreased to 3×10^4 g/mol from the original 1.5×10^5 g/mol after the fixation of the microgels of diameters of 300 and 70 nm. The pores formed by them were clearly smaller than the holes of the original chromato-gel. On the other hand, the LEMW did not change apparently after the fixation of microgels of 700 nm diameter because the pore size (ca. 105 nm for the closed hexagonal packing) was almost the same as that of the original chromato-gel. In addition, the point of inflection at the beginning of the LEMW region was also found to become clearer after the fixation of the microgels.

Effect of the Chemical Character of Microgels

The MGV-8Br samples modified by the microgels were quarternized further by bromoacetic acid and iodomethane. Their chromatographic characters are shown in Figure 8 and 9, respectively.

For the samples modified by the microgels of 300 and 70 nm diameter, as shown in Figure 8, we found that the *Ve/Vt* value and the LEMW further decreased largely after the quarternization by bromoacetic acid or iodomethane. Comparing the samples quarternized by bromoacetic acid and iodomethane, the *Ve/Vt* value of the former in the region of above the LEMW was smaller than that of the

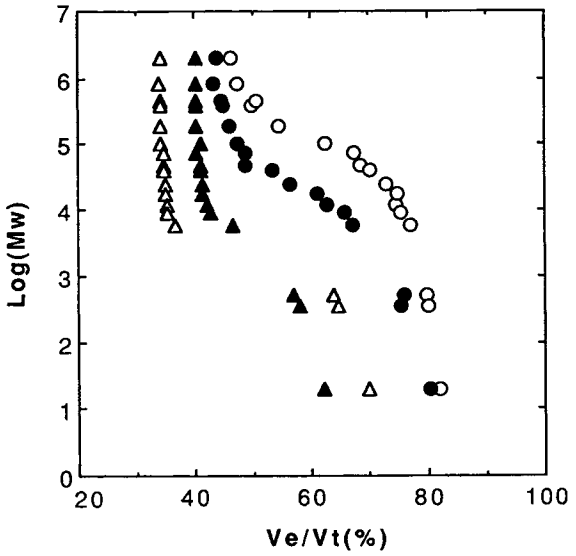

Figure 8 The relationship between molecular weight (MW) and relative retention volume (*Ve/Vt*) of dextran and pullulan standards for chromato-gel MGV-8Br: (○) before fixation of microgels; (●) after fixation of microgels of 300 and 70 nm diameter; (△) after quarternization of microgels by bromoacetic acid; (▲) after quarternization of microgels by iodomethane.

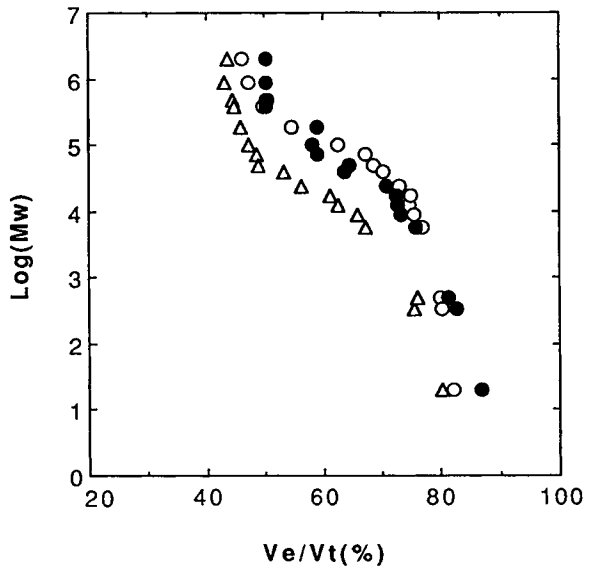

Figure 7 The relationship between molecular weight (MW) and relative retention volume (*Ve/Vt*) of dextran and pullulan standards for chromato-gel MGV-8Br: (○) before fixation of microgels; (●) after fixation of microgels of 700 nm diameter; (△) after fixation of microgels of 300 and 70 nm diameter.

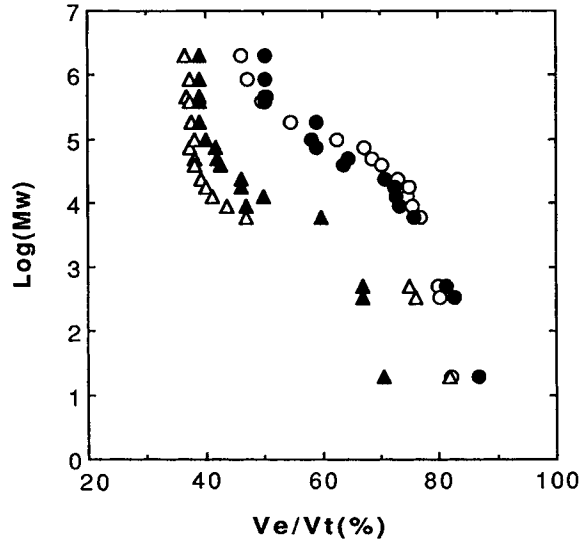

Figure 9 The relationship between molecular weight (MW) and relative retention volume (*Ve/Vt*) of standard dextran and pullulan for chromato-gel MGV-8Br: (○) before fixation of microgels; (●) after fixation of microgels of 700 nm diameter; (△) after quarternization of microgels by bromoacetic acid; (▲) after quarternization of microgels by iodomethane.

latter, whereas in the region of below the LEMW, the Ve/Vt of the former became larger than that of the latter. Then, the slope of the curve at the LEMW region was closer to 0 for the former than for the latter or other samples before quarternization. As a result, the chromatographic character became better after the quarternization of the microgels by bromoacetic acid. The behavior of the molecules above the LEMW may be ascribed mainly to the different swelling degrees of the microgels in water. The pores among microgels would become smaller if the swelling degree of the microgels in water is larger. Figure 10 shows the SEM micrographs of chromato-gels dried from water solvent after the quarternization by bromoacetic acid and iodomethane; the microgels fixed on the surface of chromato-gel in the former case apparently became softer than that in the latter case because the swelling degree of the former microgels in water was larger than that of the latter microgels as described before (Fig. 2). But the behavior of smaller molecules below the LEMW cannot be explained by only the swelling degrees of microgels. A possible explanation for this result is the different chemical characters of the microgels fixed. The microgels quarternized by bromoacetic acid showed amphoteric character (because the dissociation of the bromine ion and hydrogen ion), whereas those by iodomethane showed cationic character (because the dissociation of the iodine ion) in water (Fig. 2). The interaction between microgels and dextran and pullulan standards is supposed to be different from each other. The details are not pos-

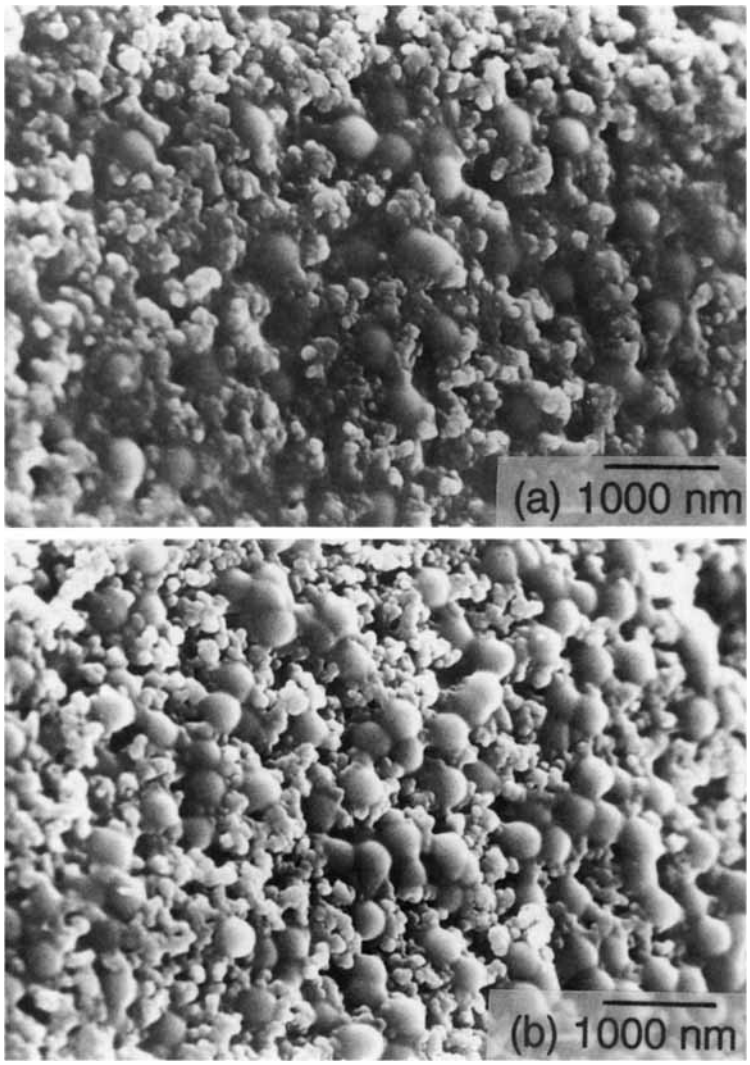

Figure 10 SEM micrographs of chromato-gel MGV-8Br after quarternization of microgels: (a) quarternized by bromoacetic acid; (b) quarternized by iodomethane. Diameter of microgel: 300 and 70 nm.

sible to discuss in this study. It remains to be investigated by changing the standard substance.

For the samples modified by the microgels of 700 nm diameter, similar results as shown in Figure 9 were found, i.e., the Ve/Vt value and LEMW decreased remarkably after the quarternization by bromoacetic acid or iodomethane. Also, the Ve/Vt value of the sample quarternized by bromoacetic acid was smaller than that of the sample modified by iodomethane in the higher molecular weight region, whereas in the lower molecular weight region, the Ve/Vt value of the former was larger than that of the latter. The slope at the LEMW region of the former was closer to 0 than that of the latter or of other samples before quarternization.

As a conclusion, due to the effects of both the sizes and chemical characters of microgels, the chromatographic characters were largely changed and the samples after modification by bromoacetic acid showed excellent chromatographic character.

Certainly, the quarternized ratio may also affect the behavior of the chromato-gel more or less when we studied the effects of the chemical characters of microgels. The quarternization reaction of pyridine proceeded easily even at lower temperature,[12] so it is difficult to control the quarternized ratio of microgels in the experimental condition of this study. Therefore, the results in this study were that the microgels on the surface chromato-gel had been quarternized thoroughly. In the near future, we expect to be able to discuss the effect of the quarternized ratio of P4VP microgels.

From the above results, we can say that this study provided an easy method to change the chromatographic characters of the chromato-gel. By this method, a series of chromato-gels with various characters can be obtained easily from one kind of original chromato-gel. Especially, the difficulties of the preparation of chromato-gel of smaller homogeneous pore size and the adjustment of pore sizes can be overcome easily. Furthermore, the result that the chromato-gel showed excellent chromatographic character if the P4VP microgels were quarternized further by bromoacetic acid was very attractive and very useful.

CONCLUSION

A single layer of poly(4-vinylpyridine) microgels can be fixed compactly on the surface of porous chromato-gel for liquid chromatography where bromo groups have been introduced. The chromatographic characters of chromato-gel chromatography can be varied easily by fixing the microgels with different sizes and characters on their surface. The retention volume and limit exclusion molecular weight (LEMW) of dextran and pullulan standards can be decreased when the pores formed by the microgels are smaller than the holes of the chromato-gel, whereas they can be elongated when the pores among microgels are larger than the holes of chromato-gel. The former result provided a good and easy way to lower the limit exclusion molecular weight, which cannot be achieved in the process of the production of the chromato-gel. The retention volume decreased further after the fixed microgels were quarternized by iodomethane or bromoacetic acid, and the chromato-gel quarternized by bromoacetic acid showed excellent chromatographic character.

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