

Designable Size Exclusion Chromatography Columns Based on Dendritic Polymer-Modified Porous Silica Particles

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Received March 18, 2003. Revised Manuscript Received July 30, 2003

Dendritic polymer-modified porous silica particles prepared by a poly(amidoamine) (PAMAM) dendrimer synthesis reaction were characterized by elementary analysis (EA) and size exclusion chromatography (SEC). EA demonstrated the successful alternate growth of dendritic layers on the particles. The phased growth of polymer moieties by cascade reactions filled mesopores on the silica particles and decreased the exclusion limit molecular weight (M_{lim}) step by step. The density of the dendritic polymer moieties was affected by the reaction conditions, such as reaction time, temperature, and generation. The mesopore size on the particles, indicated as M_{lim} , was controlled by the grafting ratio, which could be adjusted by the reaction conditions. Furthermore, the elution behaviors of SEC standard samples with a M_w lower than the M_{lim} were governed by the local microstructure of the dendritic polymer moieties on the silica particles, most notably by polymer density. It was found that the local microstructure of the dendritic polymer moieties could also be controlled by the reaction conditions.

Introduction

The synthesis and application of hyper-branched polymers such as dendrimers was reported for the first time by Tomalia and co-workers in 1985 and has been widely researched since in the development of new classes of polymer materials.¹ Dendrimers and related materials continue to attract much attention as key materials in nanoscale science due to their interesting characteristics, such as their regularly branched structure, spherical shape, monodispersion, controllable size, and easy modification.^{2–5} The structural component of dendrimers can be classified into a “core”, a “framework”, and a “terminal group”. As cores of dendrimer molecules, not only simple amine molecules but also functional molecules, such as porphyrins and fullerene, have been applied.^{6,7} In addition, polymers with dendritic side groups have been synthesized and investigated as cylindrical dendrimers.^{8–10} A variety of den-

dimer frameworks have been proposed and synthesized. The most widely used divergent-type and convergent-type dendrimers are the polyamidoamine-type and Fréchet-type dendrimers, respectively. One of the representative modifications of terminal groups is the sugar-terminated dendrimers, “sugar balls”, as investigated by Aoi and co-workers¹¹

The cascade reactions utilized for dendrimer synthesis can also be applied to create surface modifications. The immobilization of dendrimer molecules on electrode surfaces has been reported by Crooks and co-workers.^{12,13} In 1998, Tsubokawa and co-workers reported the synthesis of dendritic polymer-modified particles from a silica surface using a PAMAM synthesis reaction.¹⁴ PAMAM-type dendritic polymer-modified particles have also been synthesized from polymer particle cores with primary amino units, such as chitosan powder¹⁵ and zirconia–urea–formaldehyde (ZrO_2 –UF) resin.¹⁶ Dendron based on amino acids^{17–19} and

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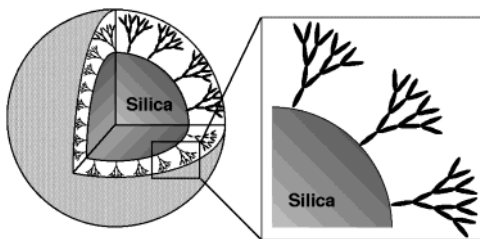


Figure 1. Schematic illustration of a dendritic polymer-modified particle prepared by a dendrimer synthesis reaction initiated on a silica surface.

Fréchet-type dendron²⁰ have also been applied in the modification of silica particle surfaces.

Catalytic applications utilizing dendritic polymer-modified particles have been researched by several groups. Alper and co-workers studied the hydroformylation reaction, Heck reaction, and carbonylation reactions on dendritic polymer-modified silica with a rhodium complex.^{21–23} Chung and Rhee estimated the ability of the chiral auxiliary for enantioselective addition of diethylzinc to benzaldehyde using dendritic polymer-modified silica with a terminal chiral group.^{24,25}

Modified particles with a hyper-branched structure are also attractive for high-performance liquid chromatography (HPLC) applications. Chromatographic experiments have been attempted on L-glutamic acid-based dendritic polymer-modified particles as a stationary phase using chiral analysis under reverse and normal phase conditions, although chiral resolution has not yet been achieved.¹⁸ In our previous paper, we reported the chromatographic application of dendritic polymer-modified silica particles under size exclusion conditions.²⁶ We indicated that the density of the moieties of dendritic polymer-modified particles can be controlled by the cascade reaction involving PAMAM dendrimer synthesis and the regulation of generation and reaction time. In addition, Lei and co-workers reported affinity chromatography of RNA-terminated PAMAM-type dendritic polymer-modified ZrO₂-UF resin for separation of AMP, ADP, and ATP.¹⁶

In this article, we report the synthesis and characterization of dendritic polymer-modified porous silica particles (Figure 1). We will focus on size exclusion chromatography (SEC) using dendritic polymer modified silica, as an approach to the design of surface modifications of HPLC gels using cascade reactions.

Experimental Section

1. Synthesis of an Aminated Silica Particle. Scheme 1 shows the reactions for synthesis of dendritic polymer-modified

silica. Spherical Silica Gel 60 (Kanto Chemical Co., Inc., Japan) was used as the base silica particle. The particle size and pore size were 40–100 μm and 55–75 \AA , respectively. The silica particles were dried over 24 h at 150 $^{\circ}\text{C}$ and aminated silica particles were synthesized by reaction with 3-aminopropyltriethoxysilane (APTES, Nacalai Tesque) in toluene flowing nitrogen for 12 h at 95 $^{\circ}\text{C}$. After the reaction, the particles were filtered out and washed with methanol (Nacalai Tesque). The surface concentrations of initial primary amino groups were measured by the Ninhydrin method. To evaluate the influence of the surface concentration of starting amino units, aminated silicas (I) and (II) (AS-I and AS-II) were prepared and used for subsequent dendritic polymer modifications. Table 1 shows the aminated reaction conditions and surface concentrations of initial primary amino units of AS-I and AS-II.

2. Dendritic Polymer Modification on Silica Particles. DPS particles were prepared by the PAMAM synthesis reaction, which involved alternate reactions with methylacrylate (MA: Wako Pure Chemical Industries) at the $(n+0.5)$ th generation and ethylenediamine (EDA: Wako Pure Chemical Industries) at the n th generation. Both reactions were repeated for a certain number of cycles. Various modified silicas were synthesized by the control of reaction conditions, not only by control of generation but also by control of reaction time and temperature. Typical synthetic procedures were as follows.

(a) *Reaction with MA for the $(n+0.5)$ th Generation.* Aminated silica particles (AS) or n th generation particles (ca. 60 g) were stirred with MA (54 mL, concentration 2.0 M) and hydroquinone (Wako Pure Chemical Industries) as an inhibitor in 300 mL of methanol in a flask at 25 $^{\circ}\text{C}$ for 48 h. After filtration through a glass filter, the product was thoroughly washed with 500 mL of methanol.

(b) *Reaction with EDA for the n th Generation.* Ester-terminated particles [$(n+0.5)$ th generation particles] were reacted with excess EDA (150 mL, concentration 7.5 M) in methanol. The $(n+0.5)$ th particles were stirred with EDA and 300 mL of methanol in a flask at 25 $^{\circ}\text{C}$ for 48 h. After filtration, the product was washed and purified with 500 mL of methanol.

3. Characterization of Modified Particles. The modified particles were characterized by elementary analysis (EA) and the Ninhydrin test. The grafting ratios as defined in eqs 1 and 2 were calculated using the EA data and corresponded roughly to the ratio of modified polymer moieties on the silica over the number of silica particles core moieties.

$$\text{sum\%} = \text{H\%} + \text{C\%} + \text{N\%} \quad (1)$$

$$\text{grafting ratio} = \text{sum\%}/(100 - \text{sum\%}) \quad (2)$$

The grafting ratio as determined by thermal gravimetry (TGA, SEIKO TG/DTA 300) was also calculated on the basis of weight percent decrease due to thermal decomposition. As an example, the grafting ratio for the 10th generation dendritic polymer-modified silica particles synthesized with AS-II (10th generation DPS-II) prepared by the reactions (25 $^{\circ}\text{C}$, 48 h) and analyzed by EA and TGA were 0.90 and 0.97, respectively.

The Ninhydrin test was conducted using a commercially available kit (Ninhydrin Test Solution Set, Wako Pure Chemical Industries) for measurements of the surface density of primary amino groups.

4. Size Exclusion Chromatography for Dendritic Polymer-Modified Silica. Degassed aqueous slurries of the particles were packed into a stainless steel column (150 \times 4.6 mm i.d.). The SEC experiments were conducted using an ordinary HPLC pump (Jasco 880PU), RI detector (Jasco RID-6A), and recorder (Shimadzu C-R6A). The mobile phase was degassed ultrapure water. As standard samples, homologous pullulans ($M_w = 5800, 12\,200, 23\,700, 48\,000, 100\,000, 186\,000, 380\,000, 853\,000$, Showa Denko), maltoheptaose (Nacalai Tesque), maltohexaose (Nacalai Tesque), maltotriose (SIGUMA Chemicals Co.), maltose (Katayama Chemical, Inc.), glucose (Nacalai Tesque), and D₂O (Nacalai Tesque) were used.

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Scheme 1. Reaction Cycle for Growth of PAMAM Dendritic Polymer on a Silica Particle

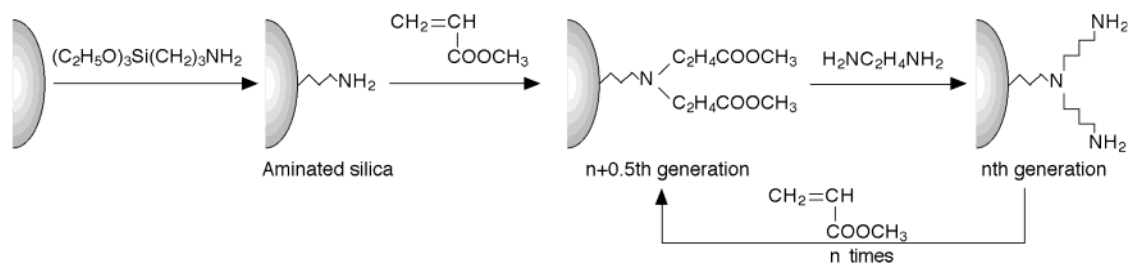


Table 1. Reaction Conditions and Primary Amino Group Content (PAC) of Aminated Silica Particles Used as Initial Gels^a

aminated silica particles	reaction conditions			PAC (mmol/g)
	silica (g)	APTES (g)	toluene (mL)	
AS-I	200	120	780	1.0
AS-II	100	6	390	0.01

^a APTES, 3-aminopropyltriethoxysilane; AS, aminated silica. Reaction temperature, 95 °C; reaction time, 12 h.

Results and Discussion

1. Preparation and Characterization of DPS.

Figure 1 and Scheme 1 show a schematic representation of dendritic polymer-modified silica particles (DPS) and the cascade reaction scheme for the growth of dendritic polymers on silica particles. The core particles were modified by the dendrimer synthesis reaction initiated from primary amino units on porous silica surfaces. The primary amino groups were introduced onto silica surfaces by reaction with 3-aminopropyltriethoxysilane (APTES).^{14,26} As shown in Scheme 1, the 0, ($n+0.5$)th, and n th generation indicate aminated, ester-terminated, and amine-terminated silica particles after n th times of the repeated dendrimer synthesis reaction, respectively. The cycle number, n , corresponds to the number of cycles of the reaction with EDA.

Aminated silica particles (AS-I and AS-II) with different surface concentrations of primary amino groups were prepared by control of the concentration of APTES, as shown in Table 1. The introduction of APTES was confirmed by EA and the surface concentrations of initial primary amino groups contents (PACs) were determined by the Ninhydrin test. The PACs of AS-I were 100 times greater than those of AS-II. Both series of dendritic polymer-modified silica particles, DPS-I and DPS-II, were synthesized from AS-I and AS-II, respectively. As mentioned later, the initial surface concentration of primary amino groups was very crucial for the structure and characteristics of growing dendritic polymers.

Successive growth of dendritic polymers on silica particles was confirmed by the EA and the Ninhydrin test to determine the PACs. Figure 2A shows the C/N ratios (C%/N%) of the modified silica against the generation. The zigzag lines of the C/N ratio in Figure 2A represent the alternate introduction of MA and EDA even at the 10th generation. The introduction of MA resulted in the increased C content and C/N ratio at the ($n+0.5$)th generation. On the other hand, the reactions with EDA gave rise to an increased N content, and thus a decrease in the C/N ratio was observed at the n th generation. Until the third generation, large changes

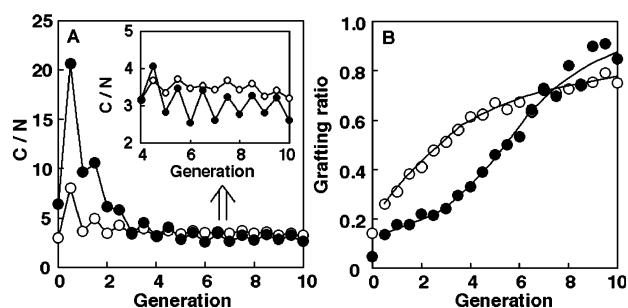


Figure 2. Changes in the C/N ratios (A) and grafting ratios (B) plotted against the n th generation for modified particles used DPS-I (open circles) and DPS-II (solid circles). Both reactions were conducted at 25 °C for 48 h.

in the C/N ratio between the n th generation and the ($n+0.5$)th generation were observed. The DPS-II series, which started with a smaller surface density of amino groups, gave notably higher C/N ratios than the DPS-I series. After the fourth generation, the differences in the C/N ratio between n th and ($n+0.5$)th generation became almost constant and the C/N ratios for both series (DPS-I and DPS-II) were almost equal. The large C/N ratio, especially for DPS-II, indicates more free space remaining for the growth of dendritic polymers in the early generations. After the fourth generation, the surfaces of both gel series would be totally covered by densely packed dendritic polymers, reducing any differences in the C/N ratios.

Figure 2B shows the typical changes in the grafting ratio of DPS based on the number of reaction cycles. The grafting ratio increased with successive repeats of the reaction for both series. In the case of DPS-I, the initial increases in the grafting ratio until the fourth generation showed a remarkable increase but the increases in the grafting ratio gradually decreased with successive generations. In contrast, the initial increases in the grafting ratio of DPS-II were small but the increases in the grafting ratio showed exponential increases until the sixth generation. Subsequently, the degree of increment of grafting ratios decreased at each generation. Eventually, the grafting ratios for both series (DPS-I and DPS-II) exceeded 0.7 at the 10th generation and the growth of polymers continued beyond the 10th generation.

The differences in the growth behaviors between the DPS-I and DPS-II series were clearly revealed in plots of the grafting ratios against each generation. Ideally, each cycle of the branching reaction should bear 2 times the number of amino groups and a plot of the grafting ratio against generation should yield an exponential curve. Certainly, an exponential increase in the grafting ratio was observed for DPS-II, which started with a smaller surface density of amino groups, up to the sixth

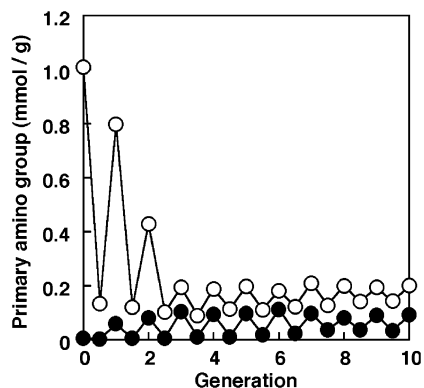


Figure 3. Changes in primary amino group content (PAC) against the generation number for DPS-I (open circles) and DPS-II (solid circles). The synthesis reactions on both particles were conducted at 25 °C for 48 h.

generation. However, the incremental changes in the grafting ratio for DPS-II decreased at each generation after the sixth generation. In the case of DPS-I, the increases in the grafting ratio diminished with each generation from the early generations onward. This observation might be attributed to steric hindrance of the hyper-branched structure, as well as the premature termination of the reactions in the pores of the porous silica. The lateral density of dendritic polymers increases with growth of the polymers, preventing an ideal branching reaction. The dendritic moieties on the silica obviously included some unreacted residues, which remained as primary or secondary amino groups. Tsubokawa pointed out that the modified particles were more likely to have a highly branched polymer rather than a dendrimer, especially at higher generations.¹⁴

To investigate the growth of dendritic polymers on particles, the primary amino group, which was reproduced by the reaction with EDA, was measured by the Ninhydrin test at each generation. Figure 3 shows the changes in the PACs of DPS-I and DPS-II synthesized at 25 °C for 48 h. As observed for C/N ratios in Figure 2, DPS showed zigzag lines in plots of the PACs against each generation. At the $(n+0.5)$ th and the n th generation, PACs repeatedly decreased and then increased with the introduction of MA and EDA, respectively.

The PACs of DPS-I decreased with an increasing number of generations until the third generation and the PACs remained constant after the third generation. Nevertheless, the EA results obviously proved that the dendritic polymer grew continuously. The decrease in the PACs for the DPS-I might be due to decreases in the surface area with the filling up of the mesopores on the silica surfaces. On the other hand, the PACs of DPS-II at the n th generation increased with increasing number of generations until the third generation. The increase in the PAC of DPS-II indicates that there was free space between dendritic polymers, which is necessary for relatively ideal growth. After the third generation, the PACs of DPS-II remained constant at values similar to those for DPS-I. The generation where the constant PAC for DPS-II was reached corresponded to that where the exponential growth of the grafting ratio changed to nearly saturated growth (Figure 2B).

At the $(n+0.5)$ th generation, the PACs for DPS-II were almost zero. This indicates that the primary amino

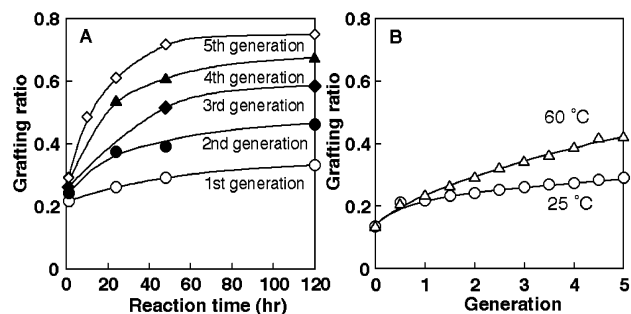


Figure 4. Influence of reaction time (A) and temperature (B) on the grafting ratios of DPS-I. In part A, open circles, solid circles, solid rhombuses, solid triangles, and open rhombuses indicate the particles at the first to fifth generations, respectively. In part B, open triangles and open circles indicated the particles synthesized at 60 and 25 °C, respectively. The reaction temperature (A) and the reaction time (B) were 25 °C and 1 h, respectively. The concentrations of MA and EDA were 2.0 and 7.5 M, respectively.

groups at the $(n+0.5)$ th generation were almost entirely consumed by the reaction with MA at the n th generation. The PACs at the $(n+0.5)$ th generation for DPS-I also indicated that primary amino groups remained in the dendritic polymers, probably due to steric hindrance. The primary amino groups located deep inside the dendritic polymers could not react with Ninhydrin molecules due to the densely packed structure at the surface of the dendritic polymers because the size exclusion limit molecular weight (<180) for DPS-I at the fourth generation was smaller than expected, as mentioned later in the chromatographic results.

2. Influence of Reaction Conditions. The DMS-I and DMS-II series discussed here were prepared by reaction of particles with 2.0 M MA at the $(n+0.5)$ th generation and reaction with 7.5 M EDA at the n th generation. Preliminary results revealed that high concentrations of MA and EDA were crucial for successive growth of dendrimer polymers. In cases of relatively lower concentrations of EDA, the incremental increase in the grafting ratio was too small to achieve successive modification, even if the reaction was conducted over a long time. This may be attributable to cross-linking between neighboring branches by EDA, which prevents reaction in subsequent generations.

Figure 4A shows the dependence of the grafting ratio of DMS-I on reaction time and generation. The grafting ratio for every generation obviously increased with increasing reaction time and saturation of grafting ratios was reached at almost 48 h. The grafting ratios for the fifth generation of DMS-I reacted for 1, 10, 24, 48, or 120 h were 0.29, 0.48, 0.61, 0.72, and 0.74, respectively.

Figure 4B shows a comparison of the grafting ratios after 1 h of reaction at 25 or 60 °C in each generation. The modified silica prepared at 60 °C revealed higher grafting than particles prepared at 25 °C. The grafting ratios at the fifth generation reached 0.29 and 0.42 for particles synthesized at 25 and 60 °C, respectively. When the reaction was conducted for more than 1 h at 60 °C, the grafting ratio was increased and reached a constant (0.75 at the fifth generation), which was almost the same as that for silica prepared by the 48 h reaction. This observation indicates that both conditions gave the same maximum introduction of dendritic moieties.

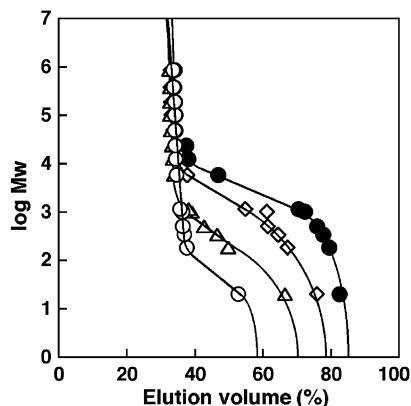


Figure 5. Calibration curves of aminated silica (AS-I) and dendritic polymer-modified particles (DPS, fifth generation) synthesized under different conditions. AS-I, DPS-I (25 °C, 48 h), DPS-I (60 °C, 1 h), and DPS-II (25 °C, 48 h) are represented by solid circles, open circles, open triangles, and open rhombuses, respectively. Measurement conditions: column, 150 × 4.6 mm; mobile phase, water; temperature, 25 °C; flow rate, 0.5 mL/min; standard samples were pullulanes, oligosaccharides, and D₂O.

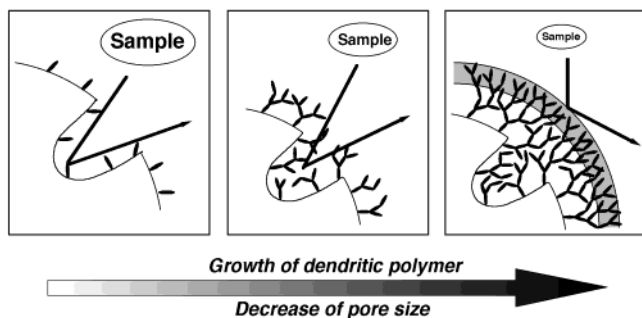


Figure 6. Schematic illustration of mesopores on the silica particles, which were gradually filled up by growing dendritic polymer.

3. Chromatographic Characterization of Dendritic Polymer-Modified Silica Particles. SEC techniques have frequently been used for the purification and size evaluation of dendrimers. In our experiments, the SEC technique was applied to evaluate the properties of dendritic polymer-modified porous silica particles as a stationary phase for HPLC.^{27,28} The porosity of the stationary phase directly affects the properties and separation characteristics in HPLC. Figure 5 shows typical aqueous SEC calibration curves using standard saccharides and D₂O for AS and for DPS (fifth generation) synthesized under different reaction conditions. The curves obtained with AS and DPS reveal typical size exclusion behavior without any specific adsorption or partition of pullulanes, oligosaccharides, and D₂O in the aqueous system. However, the size exclusion behaviors were markedly changed after dendritic polymer modification. The graph clearly shows that the modification of dendritic polymers on porous silica leads to a decrease in the elution volume, which indicates a decrease in pore size, as mentioned in a previous paper.²⁶ This change is due to the growth of dendritic polymers in mesopores on the silica particles. It also

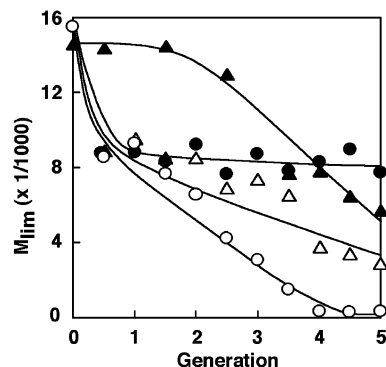


Figure 7. Generation dependence of M_{lim} 's using dendritic polymer-modified particles synthesized under different conditions. DPS-I (25 °C, 48 h), DPS-I (25 °C, 1 h), DPS-I (60 °C, 1 h), and DPS-II (25 °C, 48 h) series are indicated as open circles, solid circles, open triangles, and solid triangles, respectively. Measurement conditions: column size, 150 × 4.6 mm; mobile phase, water; temperature, 25 °C; flow rate, 0.5 mL/min; standard samples were pullulanes, oligosaccharides, and D₂O.

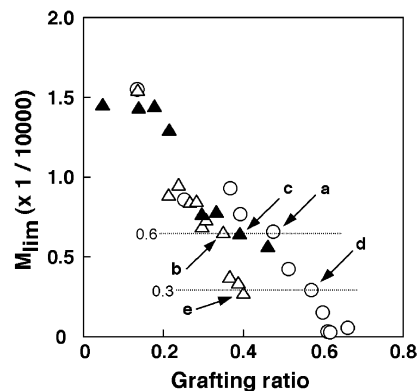


Figure 8. Correlation between grafting ratio and M_{lim} 's for the DPS-I (25 °C, 48 h; open circles), DPS-I (60 °C, 1 h; open triangles), and DPS-II (25 °C, 48 h; solid triangles) series. Measurement conditions: column size, 150 × 4.6 mm; mobile phase, water; temperature, 25 °C; flow rate, 0.5 mL/min; standard samples were pullulanes, oligosaccharides, and D₂O. The arrows indicate five selected particles (a–e) as shown in Table 2 and Figure 9.

indicates that even on a mesoporous silica surface, dendritic reactions can be conducted as long as there is space for penetration of the molecules. Figure 6 represents a schematic illustration of the growth and filling up of dendritic polymers into mesopores on the particle surface.

No remarkable morphology change by the dendritic polymer modification was observed by scanning electron microscopy at all because the resolution of SEM did not allow us to visualize mesopores on silica. Therefore, the pore-size distribution of DPS was estimated by the exclusion limit molecular weight (M_{lim}). The M_{lim} 's for porous silica, AS-I, and AS-II after reaction with APTES was 12 000, 16 000, and 14 000, respectively. The modification by APTES leads to roughening of the silica surface, causing a slight increase in the M_{lim} . Figure 7 shows the changes in M_{lim} for DPS with each generation. The introduction and growth of dendritic polymer on the surface leads to a continuous decrease in the M_{lim} 's with an increasing number of generations. When the reaction was conducted at 25 °C for 48 h with AS-I, the M_{lim} decreased with increasing generation number, until the M_{lim} 's finally reached a value of <180. Surprisingly, the

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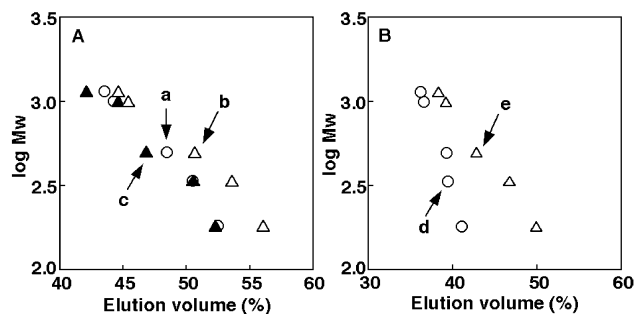


Figure 9. A comparison of calibration curves below M_{lim} 's for DPSs (A, M_{lim} ca. 6500; **a**, open circles; **b**, open triangles; **c**, solid triangles) and (B, M_{lim} ca. 3000; **d**, open circles, and **e**, open triangles). The synthesis conditions for the particles **a–e** are indicated in Table 2. Measurement conditions: column size, 150 × 4.6 mm; mobile phase, water; temperature, 25 °C; flow rate, 0.5 mL/min; standard sample, oligosaccharides.

particles at the fifth generation, and which were reacted at 25 °C for 48 h, excluded saccharides, including even the smallest sugars such as glucose, although D₂O was not excluded. The lowest M_{lim} of the particles reacted for 48 h ($20 < M_{lim} < 180$) indicates that the mesopores on the silica surface were completely filled and that the modified dendritic layers were densely packed.

On the other hand, when the reaction conditions were altered at 25 °C for 1 h, the M_{lim} dropped to approximately 9000 at the first generation, but few changes in the M_{lim} were observed after the second generation, although the grafting ratio increased with an increasing number of generations. Furthermore, when the reaction temperature and time were 60 °C and 1 h, a decrease in the M_{lim} with increasing number of generations was observed, as observed in particles prepared over a long reaction time. Moreover, when the AS-II, which possessed amino surface concentrations that were 100 times smaller than those of AS-I, was modified by the reaction at 25 °C and 48 h, the M_{lim} did not decrease markedly until the second generation, after which the decrease in M_{lim} was drastic. This observation indicates that the surface coverage of primary amino groups at early generations of AS-II was too low to fill up the mesopores effectively.

The pore distribution was notably affected by the grafting ratio; the pore size decreased with increased grafting ratio of the dendritic moieties and was moderated not only by the generation but also by the reaction conditions. Figure 8 shows the correlation between grafting ratios and M_{lim} 's for three series of DPS, which were synthesized under different reaction conditions.

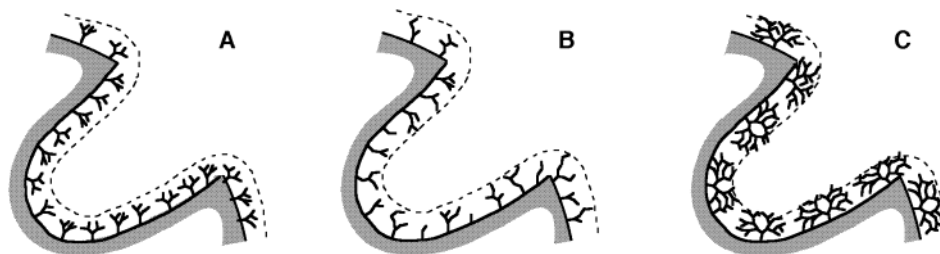


Figure 10. Schematic illustrations of the structural models for dendritic polymer moieties in silica mesopores prepared under different reaction conditions. A, B, and C illustrate the particles prepared by three different typical conditions: high initial amino content and long reaction time at low temperature, high initial amino content and short reaction time at high temperature, and low initial amino content and long reaction time at low temperature, respectively. The dotted lines show modified silica mesopores, which give the same M_{lim} .

Table 2. Preparation Conditions for DPS, Which Had Almost the Same Exclusion Limit Molecular Weights, $M_{lim} = 6500$ (a, b, and c) or $M_{lim} = 3000$ (d and e)

dendritic particle	aminated silica	reaction conditions			grafting ratio	M_{lim}
		generation	time (h)	temp (°C)		
a	I	2	48	25	0.47	6600
b	I	3.5	1	60	0.35	6600
c	II	4.5	48	25	0.39	6500
d	I	3	48	25	0.57	3000
e	I	5	1	60	0.40	2800

The growth of dendritic polymers in the pores gradually fills up the pores with progressive increases in the generation number or grafting ratio. Interestingly, the M_{lim} 's for the three series of modified silica particles showed roughly the same inverse proportional relationship-to-grafting ratios. This indicates that the pore size was predominantly regulated by the grafting ratio rather than by the generation.

However, the data for the modified particles reacted at 60 °C for 1 h were obviously shifted compared to that for the particle series reacted at 25 °C for 48 h. The former series gave smaller M_{lim} 's than the latter. This observation should be related to differences in the inner structures of dendritic polymers prepared under different reaction conditions. Therefore, the size exclusion behaviors of oligosaccharides with molecular weights less than M_{lim} were investigated using DPS particles which had almost the same M_{lim} (6500 and 3000), but which were prepared by different conditions, as shown in Table 2. The corresponding points of five selected particles (**a–e**) are indicated by the arrows in Figure 8. Among the particles with $M_{lim} = 6500$ (Figure 9A), particle **b** showed a larger elution volume than particles **a** or **c**. A higher elution volume would indicate lower density or greater dead volume in the dendritic polymer moieties, affecting pullulane samples penetration. These results were supported by the observation that the grafting ratio of particle **b** was smaller than that of particles **a** and **c**. On the other hand, particles **a** and **c**, which were prepared under the same reaction conditions but starting with different particles with a different initial amino content, showed similar elution behaviors as shown in Figure 9A. This suggested that both particles **a** and **c** would possess a similar packing density of dendritic moieties. In the case of the particles with $M_{lim} = 3000$ (Figure 9B), particle **e** with a smaller grafting ratio also showed a larger elution volume than that for particle **d**. These results indicate that the reaction at 60 °C for 1 h gave a lower polymer density

of dendritic moieties than reaction at 25 °C for 48 h. From these results, the reaction conditions, specifically reaction temperature and time, seemed to be crucial in determining the density of dendritic moieties on particles rather than the initial amino content and generation. Figure 10 shows schematic illustrations of the density of dendritic polymer moieties prepared under different reaction conditions. These results indicate that it is possible to control the local microstructure of dendritic polymer moieties on silica particles by manipulation of reaction conditions.

Conclusions

In this article, we reported details regarding the synthesis and SEC characterization of dendritic polymer-modified particles (DPS). Mesopore size on modified silica particles, as indicated by M_{lim} , was controlled by the grafting ratio which was adjusted by altering the dendritic polymer synthesis conditions, such as initial amino content, reaction time and temperature, and generation. When sample eluents with a lower M_w than

M_{lim} were applied in SEC conditions, the elution behaviors were governed by the local microstructure of the dendritic polymer moieties on the silica particles, such as polymer density. Moreover, it was found that the local microstructure of the dendritic polymer moieties could be controlled by manipulating the reaction conditions. The combination of stepwise modifications based on cascade reactions and the introduction of ligand moieties will lead to novel designable gel packings for multi-separation applications, which involve a combination of SEC and other separation modes such as partition, adsorption, ion exchange, and affinity chromatography. Information about the density of hyper-branched structures of dendritic polymer modifications will be important not only for chromatographic gel packing but also for other nanoscience applications.

Acknowledgment. This work was supported in part by CREST-JST.

CM0302936