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Immobilization of Amphiphilic Dendron on Silica Particles Toward the Application to Ultrahigh Pressure Liquid Chromatography

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Amphiphilic poly(amido amine) (PAMAM) dendrons were immobilized on the surface of silica particles, following the solid phase strategy. PAMAM dendrons were constructed by the repetitive feeding of methyl acrylate and ethylenediamine as the monomers. The peripheral functionalization of amine to long alkyl ends generated the amphiphilic PAMAM dendron on the silica surface. These amphiphilic dendron-modified silica particles were applicable as new packing materials for ultrahigh pressure liquid chromatography.

Keywords: Amphiphilic Dendron, Immobilization, Silica Particle, Chromatography.

Recently developed ultrahigh pressure liquid chromatography (UHPLC) provides very short separation time to enhance the performance of on-line liquid chromatography/mass spectrometry (LC/MS).[1–3] Considering fast but efficient separation, small silica particles with a dimension of 1.5–2 μm are usually utilized as spherical packing materials due to reduce eddy diffusion and lower mass transfer resistance of mobile phase.[4,5] Especially non-porous silica particles are easily prepared in the 1–2 μm size range and they are also mechanically strong enough to withstand very high pressure. However, the low surface area of non-porous silica suppresses the surface modification by organic molecules, such as long alkyl chains, for providing effective partition of analyte between the mobile and stationary phases. Therefore, beyond the strategy by using small non-porous silica particles for UHPLC, alternatively our attention was paid on the developing methods to create surface-modified non-porous silica particles with higher organic content. In the present study, amphiphilic poly(amido amine) (PAMAM) dendrons were immobilized on the non-porous silica particles, in order to generate higher carbon contents, based on the multivalent functionality of dendrons during surface modification, and large number of reaction sites for the effective partition of analytes based on the nanocavities inside the dendritic structure.

Silica particles (named "1100" silica by Shiseido Co. Ltd.) were prepared by heating porous silica particles (particle size: 5 μm, pore size: 8.5 nm, and specific surface area: 460 m^2/g) in an oven at 1100 °C for 24 h under air flow. Aminopropyltriethoxysilane (APTES), methyl acrylate, ethylenediamine, octanoyl chloride, nonadecanoyl chloride, and pyrene carboxylic acid were purchased as the reagent grade, and used without further purification. All the organic solvents were also obtained as the reagent grade for following reactions.

Silica particles were pretreated overnight at room temperature under vacuum. 1 g of the pretreated particles were reacted overnight with 10 cm^3 APTES/toluene (1% v/v) solution at room temperature, and then the wet particles filtered were transferred to a vacuum oven. Curing was performed for 24 h under vacuum at 150 °C to yield aminopropyl-immobilized silica particles (APS). Bare silica particles and thus-prepared APS were immersed into an ethanol solution of pyrene carboxylic acid for 10 min. The pyrene-labeled silica samples were then filtrated off, stripped by a nitrogen flow, and photographically recorded by optical and fluorescence microscopy.

Propagation of PAMAM dendrons on APS was followed by a conventional divergent procedure (Fig. 1).[6] For the construction of methyl ester terminated PAMAM dendron-modified silica particles, large excess of methyl acrylate was slowly added into a methanol solution of APS or amino-terminated PAMAM dendron-modified silica particles, and then the mixture was reacted at 40 °C with mild

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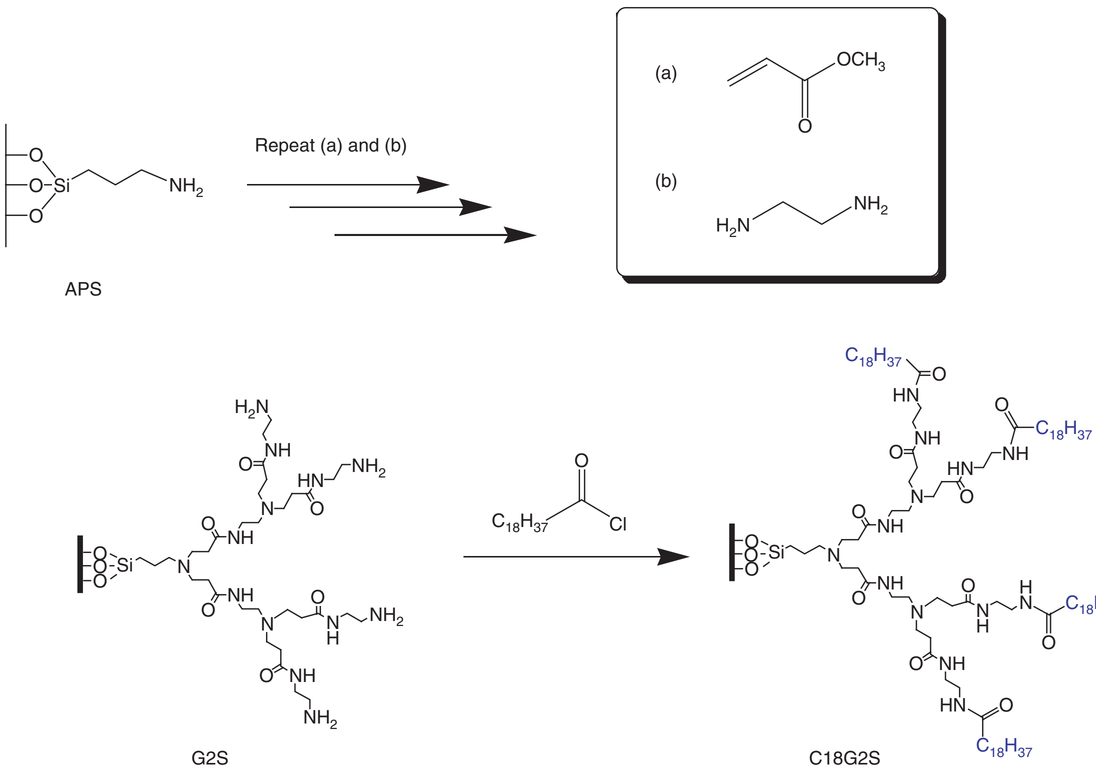

Fig. 1. A solid phase strategy based on divergent pathway for the synthesis of amphiphilic PAMAM dendrons on silica particles.

stirring under N_2 inert atmosphere. After 72 h, the solvent was filtrated, and the remaining silica particles were repeatedly rinsed by methanol. The products were dried under vacuum to yield methyl ester terminated PAMAM dendron-immobilized silica particles as white fine powder. PAMAM dendron-immobilized silica particles (GnS) was synthesized from methyl ester terminated PAMAM dendron-immobilized silica particles by the same procedure, except that only large excess of ethylenediamine was introduced as a monomer instead of methyl acrylate.

A dimethylformamide (DMF) solution of a large excess of either octanoyl chloride (C7) or nonadecanoyl chloride (C18) was added into a DMF solution of GnS, and then the mixture was reacted at 70 °C with mild stirring under N_2 inert atmosphere (Fig. 1). After 24 h, the solvent containing excess alkanoyl chloride was filtrated, and the remaining silica particles were thoroughly rinsed by chloroform and methanol. The products with long alkyl-terminals were dried under vacuum to yield amphiphilic silica particles (CmGnS) as white fine powder.

Calcinated porous silica particles were selected as the starting materials for the surface modification by amphiphilic PAMAM dendrons. The surface area of regular porous silica particles determined by nitrogen adsorption/desorption method is 400∼500 m^2/g. However, the present calcinated porous silica particles show a much lower surface area of 9 m^2/g, due to serious shrink of the mesopores on silica surface. Then these particles belong to the category of "non-porous" silica. Therefore, the reaction efficiency of organic modification on this silica particle is not comparable to the case of porous silica particles bearing higher surface area.

In association with this surface character, the procedure for the preparation of APS, based on the flip mechanism, was carried out by immersing calcinated particles into an APTES/toluene solution, ensuring the quantitative adsorption of APTES molecules on silica surface through electrostatic interaction.[7] Then, the curing step leads to not only the amine flipping over the surface but also the polycondensation of those alkoxysilane and silanol groups on the surface. It is noticed that the pretreatment for removal of the surface moisture on silica particles was carried out at room temperature, because the total surface loading of APTES molecule, decreasing with increasing the pretreatment temperature, depends on the degree of surface hydration.[8]

By using this protocol, nitrogen contents of the surface-modified silica particles determined by elemental analysis came very close to the saturated values (0.2∼0.3%), which was calculated for ideal occupation by APTES molecules on a surface (9 m^2/g for calcinated silica), using molecular area calculated from CPK (Corey-Pauling-Koltun) model. Table I lists the carbon and nitrogen contents of APS by determined with elemental analysis. Moreover, a simple ninhydin spraying test shows a deep purple color of APS, indicating the existence of primary amines on the silica surface.

Table I. Carbon and nitrogen contents (wt%) of organically modified silica particles.

	Carbon	Nitrogen
1-1		
APS	0.90	0.27
G1S	2.85	0.76
C7G1S	3.03	0.78
1-2		
APS	1.26	0.40
G2S	2.30	0.57
C18G2S	2.95	0.57
1-3		
APPS	6.90	2.1
G1PS	13.9	4.1
C18G1PS	25.8	4.1

In addition, as shown in Figure 2, silica particles without APTES treatment reveal only weaker fluorescent spherical images due to the physical adsorption of dye molecules (pyrene carboxylic acid) on silica surface; in contrast, pyrene carboxylic acid-labeled fluorescence microscopic image of APS clearly demonstrates the enhanced core–shell structure through the much stronger acid–base interaction. This result suggests the successful surface modification of silica particles by APTES molecules.

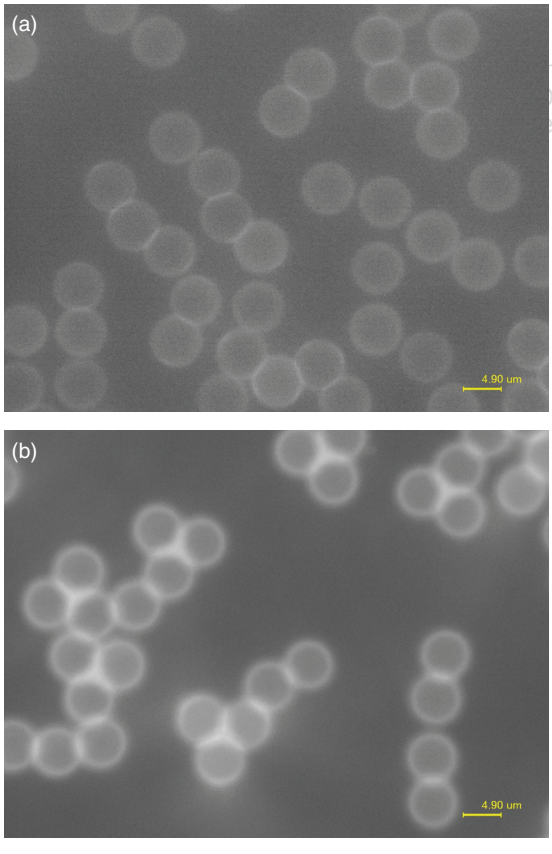

Fig. 2. Fluorescence microscopic images of pyrene carboxylic acid-labeled non-porous silica particles before (a) and after (b) APTES modification. The enhanced core–shell structure on aminopropyl-modified silica particles (b) indicates strong adsorption of pyrene carboxylic acid to amines on particle surface via electrostatic interaction.

PAMAM dendrons were synthesized on thus-prepared APS via the solid phase strategy. Table I lists the increasing carbon and nitrogen contents after either first or second generation (G1 or G2) dendron modification, indicating a successful propagation of PAMAM dendron on particles, because of the increasing carbon and nitrogen contents. It is mentioned that the elemental analysis for APS often results in different numbers of carbon and nitrogen contents during each repetitive measurement; nevertheless these values are quite reproducible for measuring the dendron-modified samples. The results suggest that APTES molecules cannot homogeneously cover each silica particle to form a uniform organic layer because of too low surface area of silica particles. If PAMAM dendrons were gradually react to immobilize on the silica surface, the flexible dendritic structure is able to well-cover the surface, making a better dispersion of these silica particles in the reaction media for dendron construction. Thus, this effect brings about a preferable surface modification and a constant organic readout in elemental analysis.

Generation of either heptyl (C7) or octadecyl (C18) peripheries for producing amphiphilc dendron-modified silica particles was carried out by a facile amidation between amine terminals on PAMAM dendron and excess alkanoyl chloride under basic condition. Table I shows the increasing carbon contents without increase in nitrogen contents, indicating a successful end group transformation.

The amphiphilic dendron-immobilized "porous" silica particles (C18G1PS), bearing abundant carbon content (25.8%, Table 1-3), was prepared in the same procedure from porous silica particles without heating for the sake of comparison of the regular reverse-phase HPLC with UHPLC. The chromatogram shown in Figure 3 was performed on a stainless column (2.0 mm Int.$\varphi \times 150$ mm) packed with C18G1PS, and seven analytes were sequentially eluted out at the duration of 25 min with meaningful separation by methanol/H_2O eluent. In contrast, the C7G1S and C18G2S were packed into a "mini" stainless columns (2.0 mm Int.$\varphi \times 20$ mm) for UHPLC test. Mixtures of 4 analytes were rapidly (at 2 min) eluted out by acetonitrile/H_2O eluent. Figure 4 depicts the chromatograms of the four analytes. In the case of a C7G1S column, a very poor separation of the mixtures was observed, indicating that the holding capacity for these standards by C7G1S is quite low; however, a C18G2S column demonstrates a much better performance to separate four standards in a short period.

It is noticed that the carbon content of C18G2S is much lower than the values of C18G1PS for providing effective separation in conventional reverse-phase HPLC (see Table 1-1). Since the modification for porous silica particles is regarded as a nearly homogeneous reaction due to the porous structure, the construction of amphiphilic dendrons is easily achieved to give desired carbon content

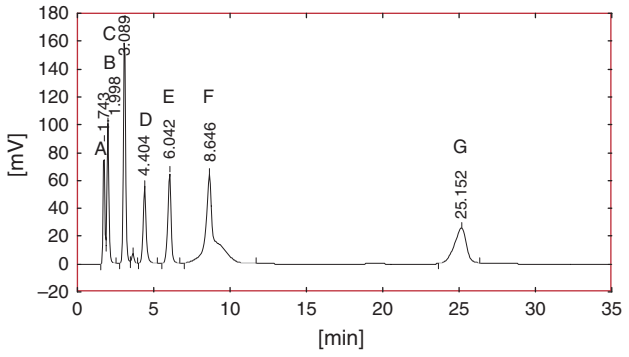

Fig. 3. Reverse-phase HPLC chromatogram of uracil (A), caffeine (B), phenol (C), methyl benzoate (D), benzene (E), toluene (F), and naphthalene (G) under C18G1PS column. Mobile phase: methanol/H_2O (1:1 v/v%); flow rate: 200 mm^3/min; injection volume: 0.5 mm^3.

for chromatography purpose. However, the surface modification carried out in the case of non-porous silica particles is less effective, because heterogeneous reaction takes place between a solid phase (non-porous silica) and solution phase (reagents). Therefore, the carbon content of both C7G1S and C18G2S is not necessarily comparable to that of amphiphilic dendron surface-modified porous silica particles.[6]

Moreover, the organic contents for C7G1S and C18G2S, starting from two different batches of APS, are quite close, because the successive propagation of dendron was usually suppressed by extremely low surface area of silica particles and by the less effective heterogeneous reaction, compared to the reaction carried out in the case of porous silica particles. Then, the desirable carbon content is difficult to be predicted and manipulated for each preparation. However, C18G2S column is still assured of remarkable separation ability for the UHPLC purpose. Therefore, it is supposed that the uniformly covered surface by C18G2 dendrons contributes to the meaningful separation in UHPLC, being independent to the carbon content, because the organic content on non-porous silica particles is always far away from that on porous case.

In summary, PAMAM dendrons were propagated on the aminopropyl-immobilized non-porous silica particles, although the efficiency of each preparation was incomparably lower than the case of the porous silica particles. Because of too low surface area of the non-porous silica and less effective heterogeneous reaction, the dendron modification must be non-stoichiometrically carried out. However, from the UHPLC result, it is found that discriminable separation efficiency is attributed to the well-covered silica surface by the flexible C18G2 dendrons, but not to the carbon contents of the organically modified silica particles. It is expected that amphiphilic dendron-immobilized non-porous silica, once efficient higher generation of dendron was propagated, it could be a new packing material for UHPLC, providing a rapid and efficient separation.

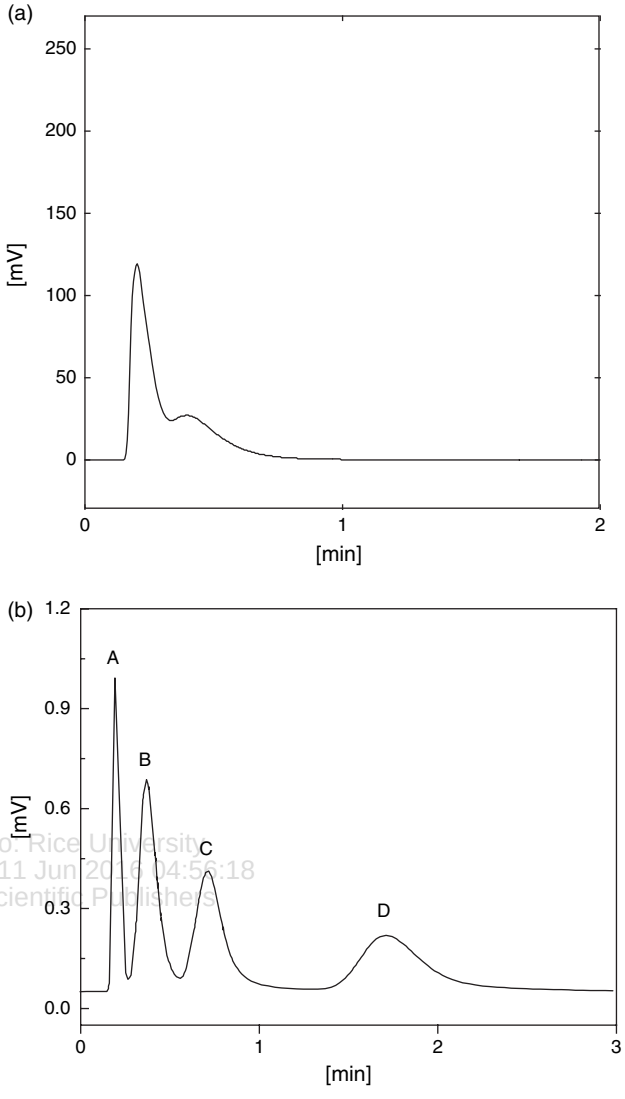

Fig. 4. UHPLC chromatograms of uracil (A, 0.05 mg/cm^3), methyl benzoate (B, 1.1 mg/cm^3), toluene (C, 4.4 mg/cm^3), and naphthalene (D, 0.5 mg/cm^3) under (a) C7G1S and (b) C18G2S columns. Mobile phase: acetonitrile/H_2O (1:4 v/v%); flow rate: 200 mm^3/min; injection volume: 0.5 mm^3.

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