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Short Communication

Reversed-phase high-performance liquid chromatography of functionalized dendritic macromolecules

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Abstract

Dendritic macromolecules substituted with various numbers of trimethylsilyl and dodecyl groups have been separated by reversed-phase HPLC. While size-exclusion chromatography only provides a rough picture of the composition of the mixture, reversed-phase chromatography allows the separation of individual components and estimation of the distribution of each component.

1. Introduction

Recently, macromolecules with architectures that differ significantly from those of classical linear and cross-linked polymers have emerged. Dendritic macromolecules are characterized by a tree-like structure with branches that converge to a single point. Each monomer unit is a branch point and, as a result, the molecule contains a large number of chain ends while adopting a globular shape due to steric considerations [1–5]. This is in contrast to the typical loose random coil of linear polymers in solution. The convergent method we use to prepare dendritic molecules allow the precise control of their chemistry as well as their sizes resulting in well defined architectures [3,4,6,7]. Other dendritic molecules such as carboxylated "Starburst" dendrimers appear to be excellent calibration standards for aqueous size-exclusion chromatography (SEC) [8].

Since the chain-ends have a great influence on ultimate properties of the dendritic macromolecules, we have developed precise methods that allow the replacement of one or more functionalities at the periphery of the globule [9–11]. More recently, a novel approach was developed to also substitute the internal sites of the dendrimers [12]. This involves metalation of the dendrimer by a superbase (SB) followed by reaction of the metalated dendrimer with an electrophile. The fourth generation dendrimer [G-4]-OH shown in Fig. 1 has a total of 46 activated sites (31 benzylic sites and 15 sites located on aromatic rings between two oxygen substituents) that can be metalated by the superbase. However, the simultaneous metalation of all of these reactive sites is not possible. For example, even the use of a two-fold excess of the SB with a molar ratio SB/[G-4]-OH = 92, only led to the incorporation of 34 deuterium atoms after reaction with ^2H_2O. Moreover, the reaction does not result in uniform products and a mixture of dendrimers with different numbers of substituents is formed. Therefore, a detailed analysis of the reaction products is essential for

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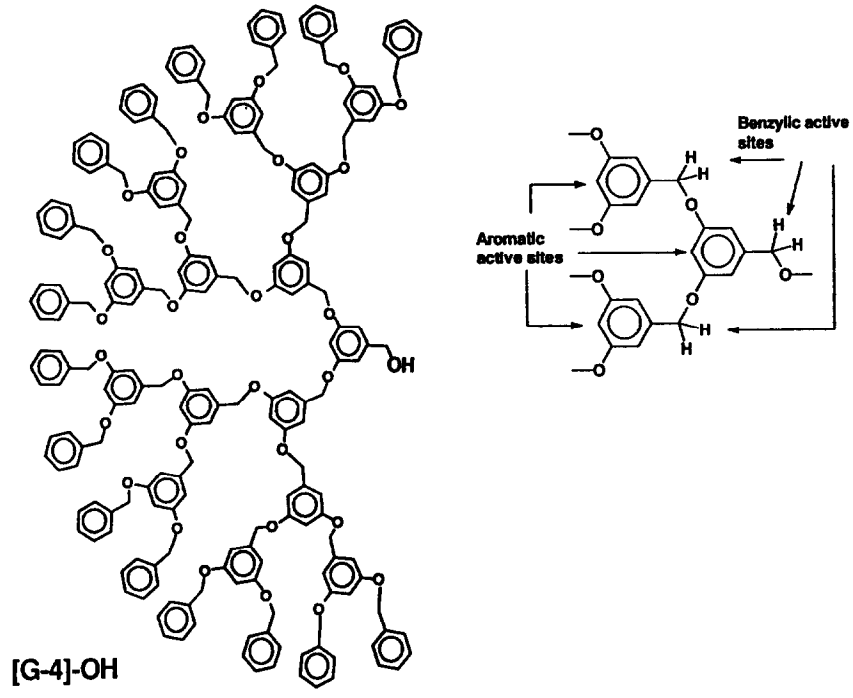

Fig. 1. Schematic representation of (G-4)-OH dendrimer.

optimization of this interesting functionalization reaction affecting "internal" sites of dendrimers.

This paper reports an application of reversed-phase HPLC to the characterization and separation of closely related functionalized dendritic macromolecules.

2. Experimental

2.1. Functionalized dendrimers

The functionalized dendrimers were prepared by metalation of dendrimer [G-4]-OH (Fig. 1), by SB prepared from butyllithium and potassium *tert.*-pentoxide [13,14], followed by reaction with electrophiles such as trimethylsilyl chloride and dodecyl chloride, according to the procedure described in detail elsewhere [12].

2.2. High-performance liquid chromatography

The SEC was performed with a Model 510 HPLC pump connected to a U6K injector (Millipore Waters Chromatography) to three 300 mm × 8 mm I.D. PL GEL columns (Mixed C, 100 Å and 500 Å, Polymer Labs.) thermostated at 30°C. The separation was monitored by a differential viscometer (Viscotek Corp.) and a differential refractometer Refractomonitor IV (Milton Roy).

The reversed-phase chromatographic separations were carried out in a commercial 150 mm × 3.9 mm I.D. stainless-steel column packed with Nova-Pak C_{18} 4 μm modified silica using an HPLC chromatograph comprised of two 501 HPLC pumps, an U6K injector and a 486 tunable absorbance UV detector. System control and data management was provided by a Millenium 2010 Chromatography Manager. All this equipment was from Millipore Waters Chromatography (Milford, MA, USA).

The isolated solid samples of functionalized dendrimers were dissolved in tetrahydrofuran, the solutions were then diluted with the highest amount of acetonitrile that did not induce precipitation prior to chromatography.

The mobile phase was a mixture of tetrafuran

(THF) and acetonitrile. The elutions were carried out at a flow-rate of 1 ml/min either in a gradient or in a combined isocratic and gradient mode at ambient temperature. The separations were monitored at 215 nm.

3. Results and discussion

Typically, the molecular mass distribution of a polymer is determined by SEC [15] while the interaction modes of HPLC are used less frequently for the separation of synthetic polymers, though the separation of oligomers and low molecular weight polymers can be achieved much faster. As the interactions of macromolecules depend on their composition, interactive HPLC is also an excellent tool for the determination of the chemical composition of copolymers in both reversed- and normal-phase mode [16,17].

The molecular mass of the starting [G-4]-OH is 3292. Substitution of a single activated site with a trimethylsilyl group increases the molecular mass by 72, for an increment of 2.25%, while modification with a dodecyl group (M_r 169) represents an increment of 5.13%. In theory, modification of half of all the potentially reactive sites should increase the average molecular mass of [G-4]-OH to 4944 upon functionalization with trimethylsilyl groups, and to 7129 upon modification with dodecyl groups. However, the reaction mixture may still contain the original unreacted [G-4]-OH, together with a variety of partly functionalized homologues and perhaps even a few fully substituted molecules with molecular masses as high as 6646 and 11 016, respectively. These molecular mass increases must be reflected in the hydrodynamic size of the dendrimers and produce a change of SEC trace. However, the increments are too small to allow precise monitoring of the individual components of the mixture. Therefore, the SEC analysis reveals only a peak covering all the species present in the sample. An example of such a SEC measurement is shown in Fig. 2.

The SEC data obviously fail to provide sufficient information about the composition of the

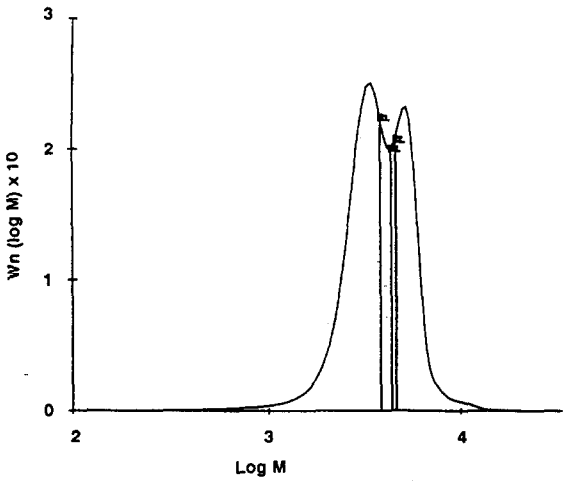

Fig. 2. Molecular mass distribution curve of dendrimer [G-4]-OH after metalation (SB/[G-4]-OH = 11) and reaction with trimethylsilyl chloride. Number-average molecular mass (M_n) = 3830; mass-average molecular mass (M_w) = 4370; *z*-average molecular mass (M_z) = 4610. M = Molecular mass; Wn = differential mass distribution function.

functionalized dendritic macromolecules. However, functionalization with trimethylsilyl and dodecyl groups changes not only the molecular size, but also the polarity of the dendrimers. Therefore, interactive modes of HPLC may be better suited to reflect these changes.

The size-exclusion chromatogram of a product prepared by metalation of [G-4]-OH with a two-fold excess (92 equivalents) of SB, followed by reaction with trimethylsilyl chloride, reveals a relatively narrow distribution of hydrodynamic sizes. Injection of the same sample into a reversed-phase column also results in observation of only one peak that despite its breadth confirms the relative homogeneity of the sample (Fig. 3a). Fig. 3b shows the separation of a product resulting from metalation of [G-4]-OH with only half of the theoretical amount (23 equivalents) of SB followed by reaction with the same electrophile. This chromatogram reveals that the sample contains some amount of less substituted dendrimers that are only partly separated at the front of the main peak. The reversed-phase separation of the reaction mixture obtained using an even lower molar ratio of superbase to dendrimer (SB/[G-4]-OH = 11, which is less than 1/4th of the theoretical

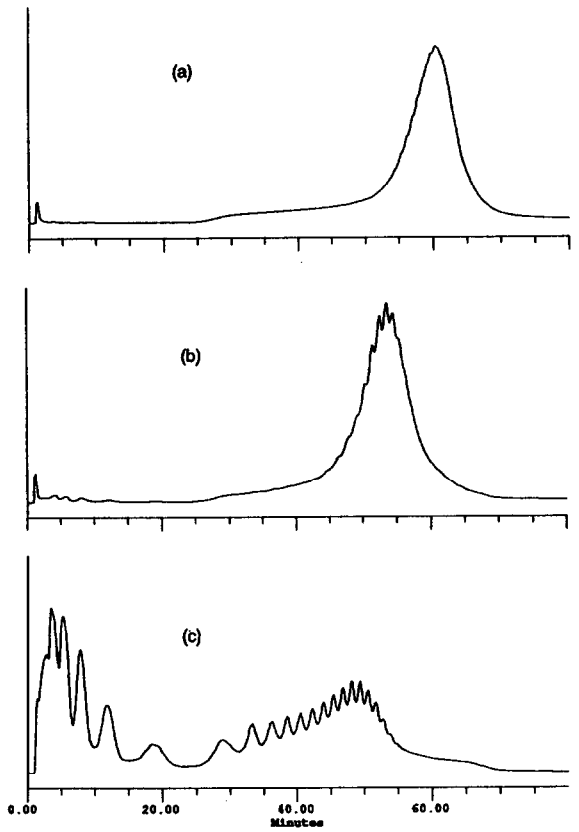

Fig. 3. Reversed-phase HPLC of dendrimer [G-4]-OH after metalation at a ratio SB/[G-4]-OH 92 (a), 23 (b) and 11 (c) and reaction with trimethylsilyl chloride. Conditions: Nova-Pak C_{18} column 150 mm × 3.9 mm I.D.; flow-rate 1 ml/min; isocratic elution with THF–acetonitrile (10:90) for 20 min followed by a linear gradient to THF–acetonitrile (50:50) within 40 min, detection at 215 nm.

amount) results in a chromatogram with 20 distinct peaks assigned to the original [G-4]-OH and 19 other species with different degrees of substitution (Fig. 3c). This chromatogram not only shows the composition of the sample, but it also serves as a reference for the assignment of peaks in chromatograms a and b. Because the last three peaks in Fig. 3c and the first three peaks in Fig. 3b overlap, the distinct peaks in Fig. 3b can be assigned to dendrimers substituted with 16 to 21 trimethylsilyl groups. Assuming that the peaks represent individual homologues with consecutively increasing number of substituents, a curve showing the retention time as a function of number of substituents can easily be drawn (Fig. 4). Extrapolation of the data allows the assignment of the maximum in Fig. 3a to a dendrimer with 29 substituents. This is in good agreement with the finding of 31 substituents calculated from ^{1}H NMR measurements [13,14]. The S-shape of the calibration curve a in Fig. 4 is due to the change in the elution mode from an isocratic elution to a THF–acetonitrile gradient after 20 min.

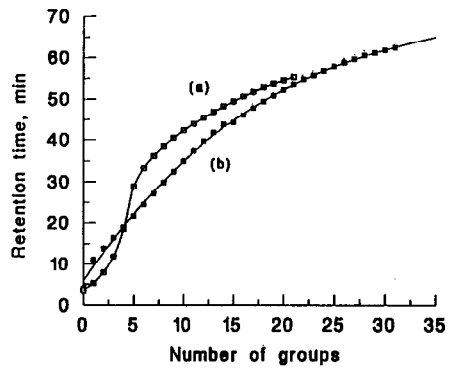

Fig. 4. Effect of degree of substitution of the [G-4]-OH dendrimer with trimethylsilyl (a) and dodecyl (b) groups on retention time of the homologues.

An even better separation was observed for dendrimers substituted with dodecyl instead of trimethylsilyl groups (Fig. 5). Fig. 5b and c show the separation of products containing 26 and 21 different compounds prepared in the reaction of [G-4]-OH with 23 and 11 equivalents of SB per [G-4]-OH, respectively, followed by quenching with dodecyl bromide. The separation shown in Fig. 5c is an excellent baseline separation of 22 different compounds. As the dodecyl group is more hydrophobic, the separation is better and 17 peaks are detected (Fig. 5a) in the product prepared from an intermediate resulting from metalation with equimolar amounts of SB and reactive sites (SB/[G-4]-OH = 46). Using again the calibration diagram (Fig. 4b) constructed from the data reported in Fig. 5b and c, peaks are assigned to dendrimers containing from 15 to 32 dodecyl chains per dendritic macromolecule. As no isocratic elution precedes the gradient in

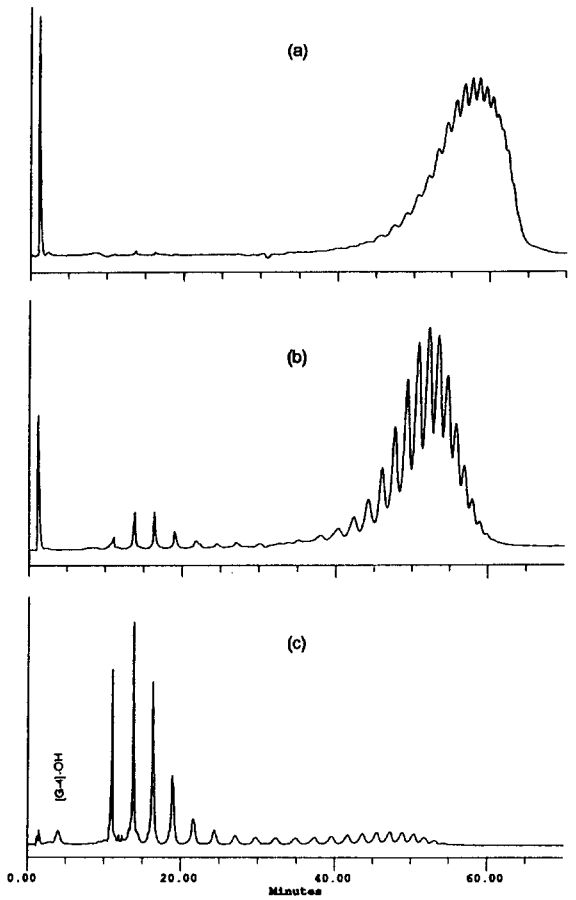

Fig. 5. Reversed-phase HPLC of dendrimer [G-4]-OH after metalation at a ratio SB/[G-4]-OH 46 (a), 23 (b) and 11 (c) and reaction with dodecyl chloride. Conditions: Nova-Pak C_{18} column 150 mm × 3.9 mm I.D.; flow-rate 1 ml/min; linear gradient of THF in acetonitrile from 50 to 80% within 60 min, detection at 215 nm.

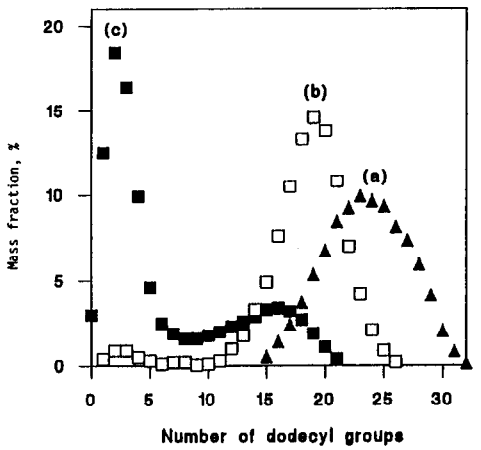

Fig. 6. Distribution diagram of dodecyl substituted homologues prepared from dendrimer metalated at a ratio SB/[G-4]-OH 46 (a), 23 (b) and 11 (c).

the chromatographic separation of the dodecyl derivatives, the calibration curve does not exhibit any inflection points.

The relative fraction of the homologues in the modified dendrimer mixtures can be easily calculated from the areas of peaks in the various chromatograms. For example, the relative fractions of dendrimers substituted with dodecyl groups are shown in Fig. 6. Unlike the substitution to a high degree of functionalization (curve a), the distribution curves of lesser substituted dendrimers (b and c) exhibit two clear maxima and suggest that the creation of some homologues is preferred. However, it must be emphasized that the distribution shown is not a continuous curve but only a schematic representation of the contents of discrete individual components in the sample.

With pure THF as solvent, the reversed-phase column used did not separate the homologues and no retention was observed. Therefore, a simple size exclusion mechanism is not operative in the reversed-phase separations shown in this paper. The solvophobic mechanism typical for very large molecules (polymers, proteins) which is based on contacts between the C_{18} hydrocarbon surface of the separation medium and the hydrophobic parts of the separated molecules [18,19] is also unlikely. The functionalization procedure used for the preparation of substituted dendrimers can occur on many sites. Each homologue is defined by its different number of substituents but these are attached at different points of the parent dendrimer molecule thus forming many possible isomers. For example, a homologue with 2 substituents has *theoretically* 820 isomers, while a homologue with 40 substituents has *theoretically* 41 isomers. Even if one takes into account the fact that many of the "theoretical" isomers are identical and that the

reactivity of all sites is not equal, the number of possible isomers remains high. Obviously, some isomers have the hydrophobic moiety more exposed on the outer surface of the globular dendritic macromolecule than others that have their hydrophobic component buried "inside" the globule. Therefore, if the solvophobic mechanism was operative, the strength of interaction of different isomers would depend on the accessibility of the hydrophobic patch rather than on the total number of hydrophobic groups and would result in an extensive overlap of the peaks. The chromatogram would be then an envelope covering all of the peaks and separation into distinct peaks would not be achieved.

Partition mechanisms are frequently encountered in the reversed-phase HPLC of small molecules [20]. The partition between the mobile phase and the organic modifier of the mobile phase which is adsorbed by the surface of the separation medium depends on the total hydrophobicity of the separated molecules and not on the extent of hydrophobic domains. Analysis of the chromatographic results achieved during this study supports the concept that the overall hydrophobicity of the individual moieties plays the most important role during the separation of dendrimers functionalized with hydrophobic substituents; the number of substituents rather than their location within the dendrimer is the key factor that determines the overall separation. This behavior is somewhat unexpected for molecules with molecular masses well over 3300. It implies that, owing to the dense globular character of the dendritic macromolecules, a partition mechanism is operative in the separation.

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5. References

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