Role of Topology and Amphiphilicity for Guest Encapsulation in Functionalized Hyperbranched Poly(ethylenimine)s

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The promising potential of dendrimers in a variety of areas, such as catalysis, materials science and biomedicine is related to their globular shape, large number of modifiable surface functionalities and the presence of internal reservoirs.[1] Their use in liquid−liquid-phase transfer protocols, based on the encapsulation of guest molecules as drug delivery vehicles for pharmaceutical application, represents an important issue.[2] Unfortunately, dendrimer synthesis is time-consuming, which currently limits practical use to laboratory scale. For that reason, hyperbranched polymers prepared from AB_m-type monomers in one-step processes have gained increasing interest.[3] The development of the slow monomer addition technique has resulted in well-defined hyperbranched polymers ($1.3 < M_w/M_n < 1.5$) of narrow polydispersity with peculiar functional group distribution throughout the polymer scaffold, as demonstrated in detailed studies for hyperbranched polyglycerols.[4a] Partial modification of these hyperbranched polyethers with fatty acids or ketones leads to amphiphilic hyperbranched materials with compact core−shell structures. These structures exhibit dendrimer-like properties, such as the formation of unimolecular micelles.[4b,c] Hyperbranched poly(ethylenimine)s (PEI) have been used for a long time for various industrial purposes, e.g., as flocculating agents, thickeners, and dispersion stabilizers, and have recently been studied as a prototype for “weak” polyelectrolytes, as a component in hydrogenation processes, and for quantum dot nanocomposites.[5] Recently, partial functionalization of PEI via amidation with long alkyl chains afforded amphiphilic hyperbranched polymers with core−shell-type architectures, capable of stabilizing silver nanoparticles and transferring polar dyes into organic solvents.[6] In this paper, we describe a comparison of the amidated hyperbranched PEI with the linear analogue with respect to solution properties and phase transfer. Furthermore, the influence of the polarity difference of “core” and “shell” components in the specific function of a “molecular nanocapsule” prepared from hyperbranched polymers has not yet been detailed. Herein we report a comparative analysis of amidated hyperbranched PEI vs the linear analogue in solution and summarize the results of a study of the effect of core polarity on guest encapsulation, using quaternized, amidated-hyperbranched PEI.[7] The combination of selective external and internal functionalization of hyperbranched polymers to fine-tune their properties has hardly been addressed. In one previous paper a selective core-functionalization route for hyperbranched polyglycerol cores based on acetal formation has been presented; however, no detailed study of encapsulation properties was carried out.[4e]

Two commercially available hyperbranched PEI samples, namely **PEI10K** ($\bar{M}_n = 10^4$ g/mol, $\bar{M}_w/\bar{M}_n = 2.5$) and **PEI25K** ($\bar{M}_n = 2.5 \times 10^4$ g/mol, $\bar{M}_w/\bar{M}_n = 2.5$) have been partially amidated with palmitoyl chloride as previously reported,[6a] affording organo-soluble, hyperbranched **PEI10K—C16$_{0.6}$** and **PEI25K—C16$_{0.6}$**, respectively (Scheme 1). FT-IR spectra clearly show the typical band of the amide group ($\upsilon = 1635$ cm^{-1}). ^{1}H NMR analysis confirmed a degree of amidation of 60%. To render the interior of **PEI10K—C16$_{0.6}$** and **PEI25K—C16$_{0.6}$** more polar, further quaternization of the residual amine groups (40%) with methyl iodide afforded the fully modified and organo-soluble hyperbranched polymers, namely, **PEI10K—C16$_{0.6}$N$^+_{0.4}$** and **PEI25K—C16$_{0.6}$N$^+_{0.4}$** as indicated by the downfield shift of adjacent CH$_2$ protons of the amine groups in the ^{1}H NMR spectra (Supporting Information). The linear analogue with high molecular weight, **LPEI15K** ($\bar{M}_n = 1.5 \times 10^4$ g/mol), was obtained after hydrolysis of poly(2-ethyl-2-oxazoline) and subsequent partial amidation (60%) of the secondary amine groups, yielding **LPEI15K—C16$_{0.6}$** (Scheme 1).[8]

Covalently modified hyperbranched polymers with hydrophobic shell have been shown to sequester polar dyes from the aqueous phase into organic media.[6b] To confirm the micellar properties of the amidated PEIs with both neutral and cationic cores, their capacity for polar guest encapsulation have been evaluated and compared with that of the modified linear PEI. To this end, four water-soluble dye probes, namely Eosin Y (EY), Fluorescein Sodium (FS), Methyl Orange (MO) and Congo Red (CR) have been utilized (Figure 1). The encapsulation results are summarized in Table 1.

Whereas the linear polymer **LPEI15K—C16$_{0.6}$** only transports dyes to an insignificant extent, the hyperbranched amidated **PEI10K—C16$_{0.6}$** and **PEI25K—C16$_{0.6}$** encapsulate all dyes in large amounts more efficiently than the previously reported hyperbranched PEI containing an imine-bound shell.[6b] The higher encapsulation capacities of the amide shell in the hyperbranched PEI-based systems is ascribed to the additional contribution of the amide functional groups via hydrogen-bonding interactions. Such multiple secondary interactions originating from the presence of amide groups have been previously used to encapsulate and assemble end groups in a reversible way at the interior as well as at the periphery of dendrimers.[2e−f,9] Remarkably, the number of encapsulated dye molecules increased by a factor of about 3 after quaternization. For instance, the loading of encapsulated CR changed from 14.4 in **PEI10K—C16$_{0.6}$** to 41.2 dye molecules in the sample **PEI10K—C16$_{0.6}$N$^+_{0.4}$**.

The low loading of dyes by the linear amidated polymer was not unexpected, since a previous study on hyperbranched and linear polyglycerols had confirmed the peculiarity of the hyperbranched structure in the context of phase transfer.[7c] In the case of LPEI, we attribute the low, but not negligible fraction of dye

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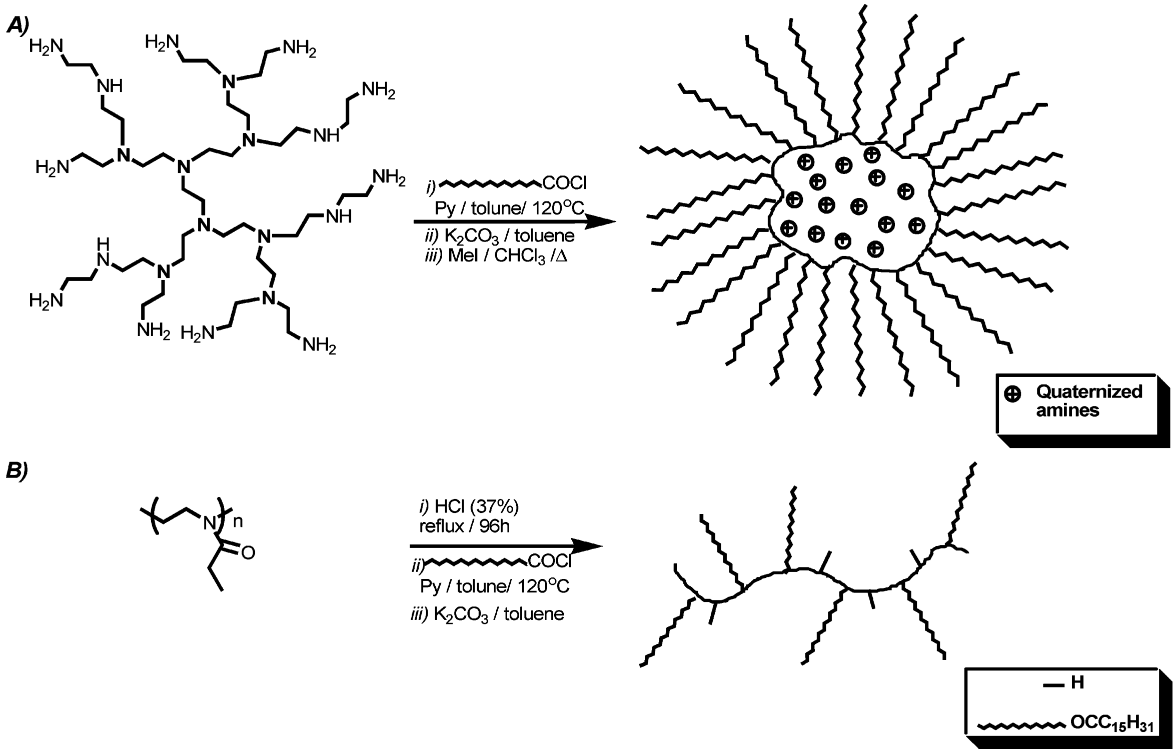
Scheme 1. Schematic Representation of the Synthesis of Amidated Hyperbranched Polyethylenimine, Displaying Core−Shell Structure (A) and the Linear Analogue (B)



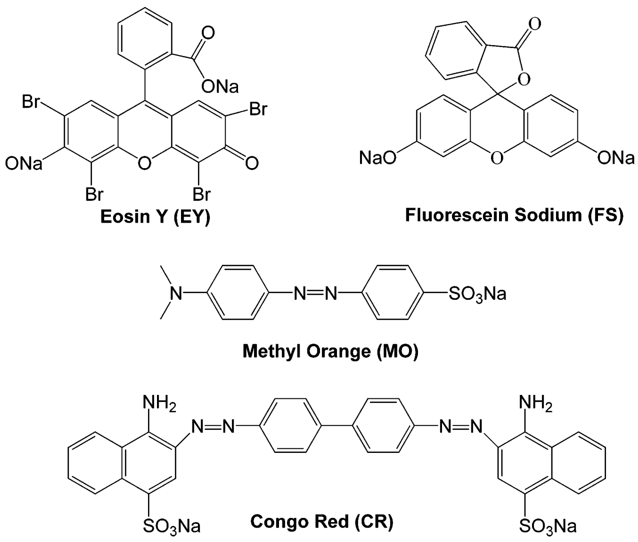


Figure 1. Water-soluble dye structures.

Table 1. Encapsulation Capacities of Amide-Modified Poly(ethylenimine) Samples

	dye loading capacity			
polymers[a]	*N*(EY)[b]	*N*(FS)	*N*(MO)	*N*(CR)
PEI10K—C16$_{0.6}$	14.4	7.0	25.6	16.9
PEI25K—C16$_{0.6}$	38.4	14.2	57.4	44.5
PEI10K—C16$_{0.6}$N$^+_{0.4}$	41.2	12.2	56.6	49.3
PEI25K—C16$_{0.6}$ N$^+_{0.4}$	71.4	41.7	153	90.1
LPEI15K—C16$_{0.6}$	4.2	0.9	5.3	0

[a] Nomenclature: PEIX-C16$_y$, PEI hyperbranched skeleton; X, M_n of PEI; C16, length of alkyl chain; y, degree of functionalization. In the case of quaternized polymers N^+_z was used where z indicates the degree of quaternized amines. [b] N(dye): transported dye number.

transported to the weak interaction of dyes with the remaining secondary amine groups. In contrast, the compact core−shell structure in the amidated hyperbranched PEI is relevant for encapsulating a higher amount of dye in the polar interior. The results show that the additional increase of the polarity of the core strongly enhances the guest encapsulation capacity of the "molecular nanocapsules". This is most probably due to the thermodynamically favored environment for the anionic dyes.[10] Further evidence for efficient dye encapsulation arises from fluorescence quenching of the encapsulated FS dyes, indicating their location within the Förster distance of the internal amine quenchers.

Experiments to assess the selectivity of dye encapsulation of the amidated hyperbranched PEIs have also been performed, using two different mixtures of FS: MO and EY: FS dyes, respectively, in 1:1 ratio. Both dyes were extracted, as observed from UV−vis spectra. However, a reasonable quantification of the ratio of transported dye fractions was difficult due to the featureless and overlapping maximum absorption bands in the corresponding UV−vis spectra of the encapsulated dyes. Detailed competitive experiments are underway to assess the encapsulation selectivity.

To obtain insight in the structure of the modified PEIs in solution, viscosimetry studies have been performed. The specific viscosity values measured for the linear material **LPEI15K—C16$_{0.6}$** are considerably higher than those observed for the hyperbranched polymers, as shown in Figure 2. The low intrinsic viscosity values [η], illustrating the compact nature of the polymers in solution,[11] were around 5.6 mL/g for both **PEI10K—C16$_{0.6}$** and **PEI25K—C16$_{0.6}$**, twice lower than for the linear analogue **LPEI15K—C16$_{0.6}$** ([η] = 12.5 mL/g). The quaternized polymers **PEI10K—C16$_{0.6}$N$^+_{0.4}$** and **PEI25K—C16$_{0.6}$N$^+_{0.4}$** show even lower intrinsic viscosity ([η] = 4.6 mL/g).

The remarkably low viscosity values for the amidated PEI species with cationic interior are attributed to the very compact structure adopted for the hyperbranched

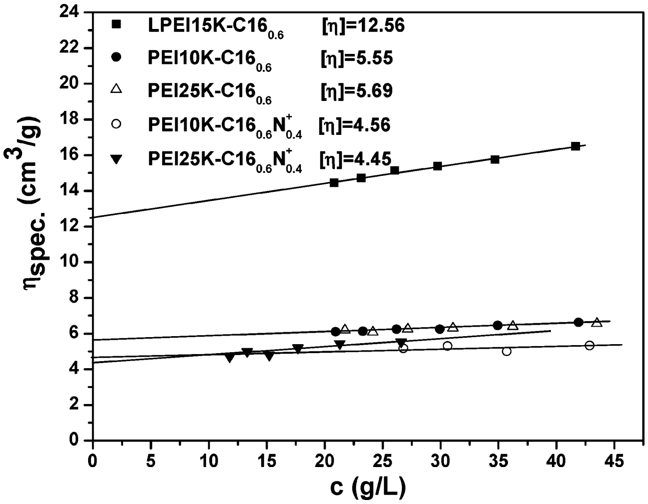


Figure 2. Representation of specific viscosity vs concentration for modified PEI systems.

PEIs in apolar media, caused by unfavorable interaction of the charged, polar interior with the apolar solvent. This obviously results in a "collapsed" topology. These properties associated with the modified hyperbranched PEIs have been studied at concentrations higher than those used for the phase transfer experiments.[12] Since dilute solutions have been used for the phase transfer protocols, the determination of the polymer structure at these conditions was necessary. Dynamic light scattering (DLS) experiments have been performed, confirming the absence of clustering between modified hyperbranched PEI and indicating single particle behavior of **PEI10K—C16$_{0.6}$** and **PEI25K—C16$_{0.6}$** with a hydrodynamic diameter of approximately 6 ± 0.05 and 9 ± 0.05 nm, respectively, while the quaternized species **PEI10K—C16$_{0.6}$N$^+_{0.4}$** and **PEI25K—C16$_{0.6}$N$^+_{0.4}$** display sizes of 5 ± 0.05 and 7 ± 0.05 nm, respectively, pointing to an inverted unimicellar behavior of the amidated-PEI species with both neutral and cationic interior.

In summary, partially amidated hyperbranched PEIs with cationic interior are attractive compounds to obtain unimolecular, hyperbranched nanocapsules for phase-transfer applications. Our data confirm the crucial role of both the hyperbranched structure and the polarity difference between core and shell in amphiphilic dendritic systems.

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Supporting Information Available: Experimental section consisting of text discussing the synthetic details, figures showing NMR and IR spectra, tables of calorimetric and viscosity data of the polymers, as well as a picture illustrating liquid−liquid extraction protocols of several dyes. This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) A comparative study with esterified, high molecular weight hyperbranched polyglycerols PG$_{269}$C16$_{0.6}$ was performed (Supporting Information) to study the importance of the polarity difference between core and shell components and H-bonds of amides for the observed higher transport capacities of functionalized PEIs. PG$_{269}$C16$_{0.6}$ was found to bind only 2.2, 0.2, 1.2, and 2.4 of EY, FS, MO, and CR, respectively.

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