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

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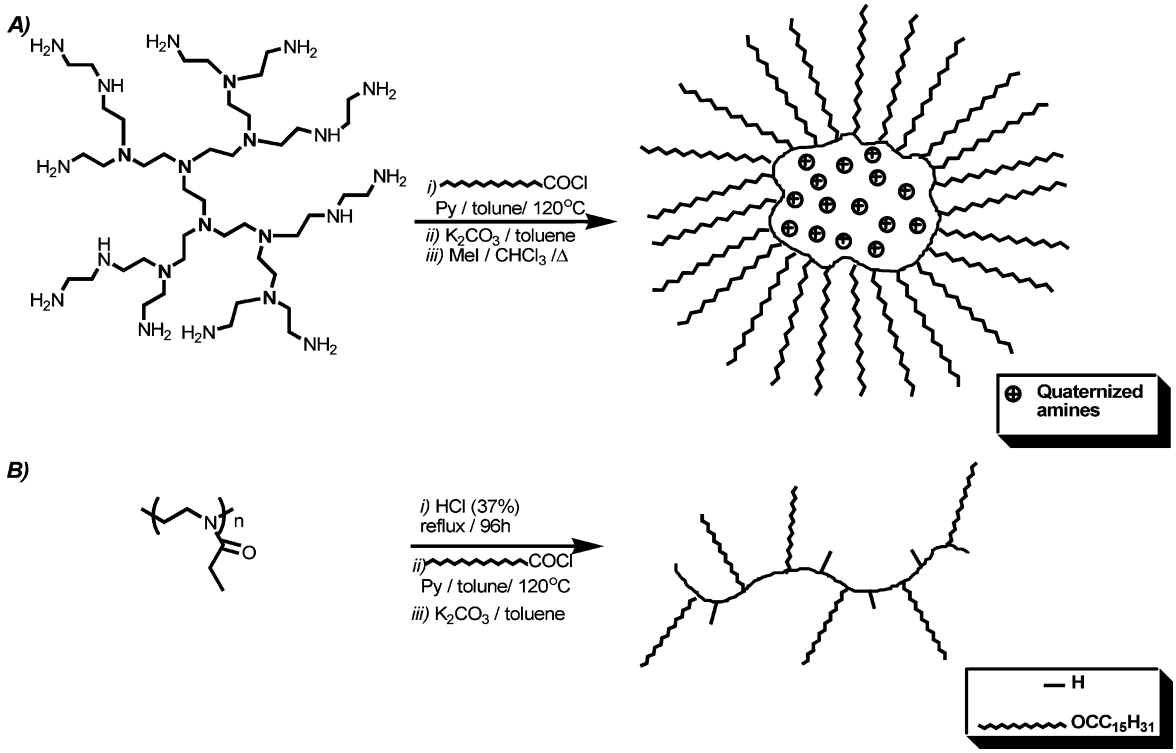
Scheme 1. Schematic Representation of the Synthesis of Amidated Hyperbranched Polyethylenimine, Displaying Core−Shell Structure (A) and the Linear Analogue (B)



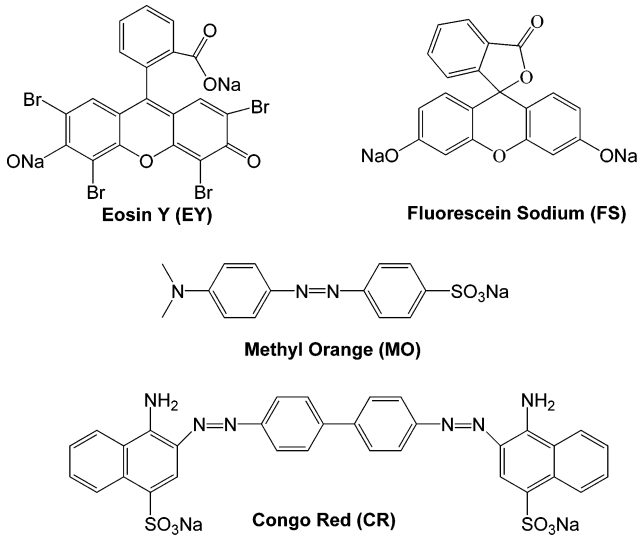


Figure 1. Water-soluble dye structures.

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

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.

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

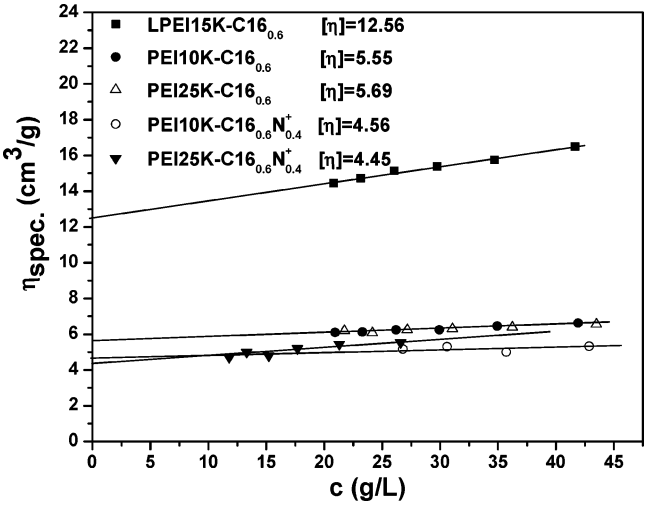


Figure 2. Representation of specific viscosity vs concentration for modified PEI systems.

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.

Acknowledgment. The Ministerio de Educación y Ciencia (Spain) is acknowledged for support to (S.-E.S.) in the context of "Ramón y Cajal" program. The assistance of Mrs X. Yuan with dynamic light scattering (DLS) measurements is greatly appreciated.

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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(8) The linear analogue with high molecular weight **LPEI15K** was prepared by hydrolysis of poly(2-ethyl-2-oxazoline). ^{1}H, ^{13}C NMR and FT-IR analysis support the incorporation of the hydrophobic alkyl chains via amidation (Supporting Information).

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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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(12) The concentrations used in viscosity measurements for **LPEI15K—C16$_{0.6}$** (41.6 g/L), **PEI10K—C16$_{0.6}$** (41.9 g/L), **PEI25K—C16$_{0.6}$** (43.5 g/L) **PEI10K—C16$_{0.6}$N$^+_{0.4}$** (42.8 g/L), and **PEI25K—C16$_{0.6}$N$^+_{0.4}$** (26.7 g/L) were much higher than those handled for DLS analysis and phase transport studies. The concentrations of **PEI10K—C16$_{0.6}$**, **PEI25K—C16$_{0.6}$**, **PEI10K—C16$_{0.6}$N$^+_{0.4}$**, and **PEI25K—C16$_{0.6}$N$^+_{0.4}$** used for the dye transport experiments were 2.36×10^{-6}, 9.45×10^{-7}, 9.65×10^{-7}, and 2.85×10^{-7} M, respectively. Concentrations used for DLS were 5×10^{-5} (2.13 g/L), 2×10^{-5} (2.13 g/L), 9.1×10^{-5} (5 g/L), and 3.3×10^{-5} M (5 g/L), respectively.

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Supplementary Materials

Macromolecules

Experimental Setup

1. Materials

Hyperbranched polyethylenimine PEI10K (M_n=1x10^4 g/mol, M_w/M_n=2.5) was purchased from Aldrich. PEI25K (M_n=2.5x10^4 g/mol, M_w/M_n=2.5) was provided from Hyperpolymers GmbH (Freiburg, Germany. *http://www.hyperpolymers.com*). Poly(2-ethyl-2-oxazoline) (M_w=5x10^4 g/mol) was ordered from Aldrich. The polydispersity was found to be 1.44 by SEC using THF as eluent, indicating that M_n of this polymer is 3.5 x10^{4}g/mol. Polyglycerol PG_{269} (M_n=2x10^4 g/mol, M_w/M_n= 2.0) was prepared as reported previously,[1] using trimethanolpropane (TMP) as initiator. Esterified polyglycerol was prepared as described elsewhere.[2] Methyl iodide was purchased from Acros. Palmitoyl chloride (99%) was used as received from Fluka. Benzoylated cellulose tubing (MWCO 1,000) was purchased from SIGMA.

2. Characterization Methods

^{1}H NMR spectra were recorded on a Bruker ARX 300 spectrometer, operated at 300 MHz. Molecular weight distributions were determined on PSS GPC at 30˚C, using chloroform or THF as eluent and linear polystyrene standards for calibration. UV-Vis spectra were obtained from Perkin-Elmer Lambda 2 UV/Vis Spectrophotometer. Fluorescence spectra were obtained from Spex Fluorolog 2 1681 spectrometer. IR spectra were recorded on a Nicolet 5DXC FTIR spectrometer. Viscosities were measured by Lauda Processor-Viscosity-System 2.52a using $CHCl_3$ as solvent at 20°C. Hydrodynamic radii (R_h) of modified PEI have been determined by dynamic light scattering (DLS) using an experimental setup consisting of a He-Ne laser (JDS Uniphase 1145p-3083, JDS Uniphase, 632.8 nm, 25 mW output power), goniometer SP-86(ALV), and an ALV-3000 digital correlator/structurator. All light scattering

measurements have been carried out with filtered samples (Millipore dimex-13 filter, pore size 0.2 μm). The experimental error of DLS measurement is around 5%.

3. Amidation of hyperbranched polyethylenimine

The preparative procedure for partial amidation of PEI10K (T=33.3%, D=26.7%, L=40%), and PEI25K (T=33.3%, D=26.9%, L=39.8%) affording PEI10K$C16_{0.6}$, PEI25K$C16_{0.6}$, respectively was carried out as reported in literature.[3] ^{1}H NMR data are given because of their significance to support the incorporation of palmitoyl chain in the polyethylenimine.

Polymer PEI10K-$C16_{0.6}$:

^{1}H NMR ($CDCl_3$): δ = 3.36 (br, -$CH_2C\underline{H}_2$NHCO-, -$CH_2C\underline{H}_2$NCO-); 2.55 (m, -$CH_2C\underline{H}_2$NH-, -$CH_2C\underline{H}_2$N-); 2.40-2.00 (br, -NCOC$\underline{H}_2$-); 1.56 (br, -NCOC$H_2C\underline{H}_2$-); 1.22 (br, -NCOC$H_2CH_2(C\underline{H}_2)_{12}CH_3$); 0.85 (t, -NCOC$H_2CH_2(CH_2)_{12}C\underline{H}_3$). IR: ν=1635cm^{-1} (amide group)

Polymer PEI25K-$C16_{0.6}$:

^{1}H NMR ($CDCl_3$): δ = 3.38 (br, -$CH_2C\underline{H}_2$NHCO-, -$CH_2C\underline{H}_2$NCO-); 2.58 (m, -$CH_2C\underline{H}_2$NH-, -$CH_2C\underline{H}_2$N-); 2.45-2.00 (br, -NCOC$\underline{H}_2$-); 1.58 (br, -NCOC$H_2C\underline{H}_2$-); 1.24 (br, -NCOC$H_2CH_2(C\underline{H}_2)_{12}CH_3$); 0.85 (t, -NCOC$H_2CH_2(CH_2)_{12}C\underline{H}_3$). IR: ν=1635cm^{-1} (amide group)

4. The quaternization of PEI10K-$C16_{0.6}$ by methyl iodide

PEI10K-$C16_{0.6}$ (1.97g) was dissolved in 25ml of chloroform, and then 0.72g of K_2CO_3 and 2.93ml of methyl iodide (10 eq. relative to the remaining amine groups of PEI10K$C16_{0.6}$) was added. The mixture was refluxed for 36h. After filtration and removal of the volatile, partially quaternized polymer PEI10K-$C16_{0.6}N^+_{0.4}$ was obtained. ^{1}H NMR ($CDCl_3$): δ = 4.39-3.03 (br, -$CH_2C\underline{H}_2$NHCO-, -$CH_2C\underline{H}_2$NCO-, -$CH_2C\underline{H}_2N^+$-, $C\underline{H}_3N^+$-); 2.50-2.00 (br, -NCOC$\underline{H}_2$-); 1.56 (br, -NCOC$H_2C\underline{H}_2$-); 1.22 (br, -

$NCOCH_2CH_2(\underline{C}H_2)_{12}CH_3$); 0.85 (t, -$NCOCH_2CH_2(CH_2)_{12}C\underline{H}_3$). IR: ν=1635cm^{-1} (amide group).

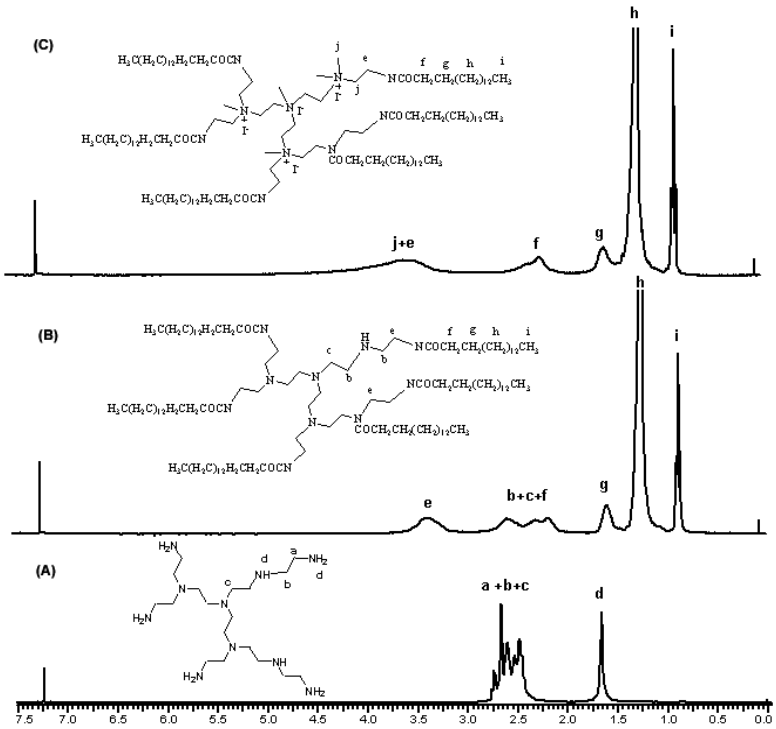


Figure 1. ^{1}H NMR spectra of (**A**) hyperbranched **PEI10K**, (**B**) **PEI10K-C16$_{0.6}$** and (**C**) **PEI10K-C16$_{0.6}$N$^+_{0.4}$**

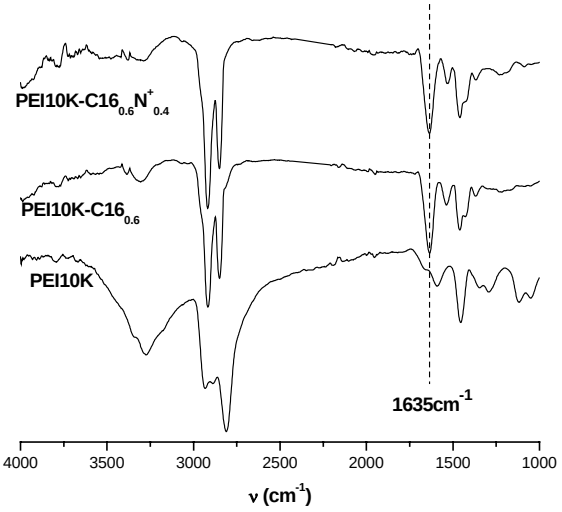
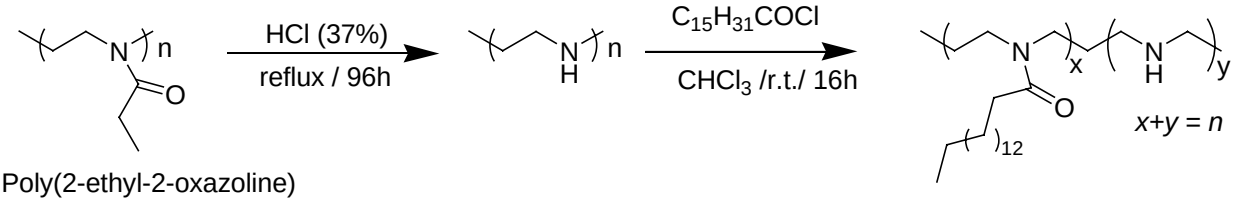


Figure 2. Comparison of the FT-IR spectra of hyperbranched **PEI10K**, **PEI10K-C16$_{0.6}$** and **PEI10K-C16$_{0.6}$N$^+_{0.4}$**

5. Preparation of the linear polyethylenimine LPEI15K



Poly(2-ethyl-2-oxazoline)

Linear PEI was prepared from Poly(2-ethyl-2-oxazoline) according to Kem's methods[4]: A solution of 25g Poly(2-ethyl-2-oxazoline) in 65ml of deionized water was introduced into a 250ml three necked flask equipped with funnel, magnetic stirrer and condenser. Then 40ml of 37% HCl was added. The mixture was refluxed for 96 hours. All the volatile was removed under reduced pressure and the resulting product was redissolved in deinoized water. The solution was neutralized with NaOH solution and then evaporated to dryness. The opaque residue is taken up in isopropanol and the precipitated sodium chloride was filtered off. The cycle was repeated twice to remove all NaCl completely. The pure linear PEI (LPEI15K, average functionality of amine is 348, M_n=1.5x10^{4}g/mol) was purified by dialysis against chloroform. ^{1}H NMR ($CDCl_3$): δ = 2.67 (-C<u>H</u>$_2$C<u>H</u>$_2$NH-); 1.76 (-CH$_2$CH$_2$N<u>H</u>-). IR (NaCl): ν=1633 cm^{-1} (amide group)

6. Amidation of the linear polyethylenimine LPEI15K

The amidation of the linear polyethylenimine was performed as reported for the hyperbranched isomers.[3]

Polymer LPEI15K-C16$_{0.6}$:

^{1}H NMR ($CDCl_3$): δ = 3.40 (br, -CH$_2$C<u>H</u>$_2$NCO-); 2.67 (br, –C<u>H</u>$_2$NH-); 2.39-2.06 (m, -CH$_2$C<u>H</u>$_2$NH-, -NCOC<u>H</u>$_2$-); 1.56 (br, -NCOCH$_2$C<u>H</u>$_2$-); 1.22 (br, -NCOCH$_2$CH$_2$(C<u>H</u>$_2$)$_{12}$CH$_3$); 0.85 (t, -NCOCH$_2$CH$_2$(CH$_2$)$_{12}$C<u>H</u>$_3$). IR: ν=1633cm^{-1} (amide group)

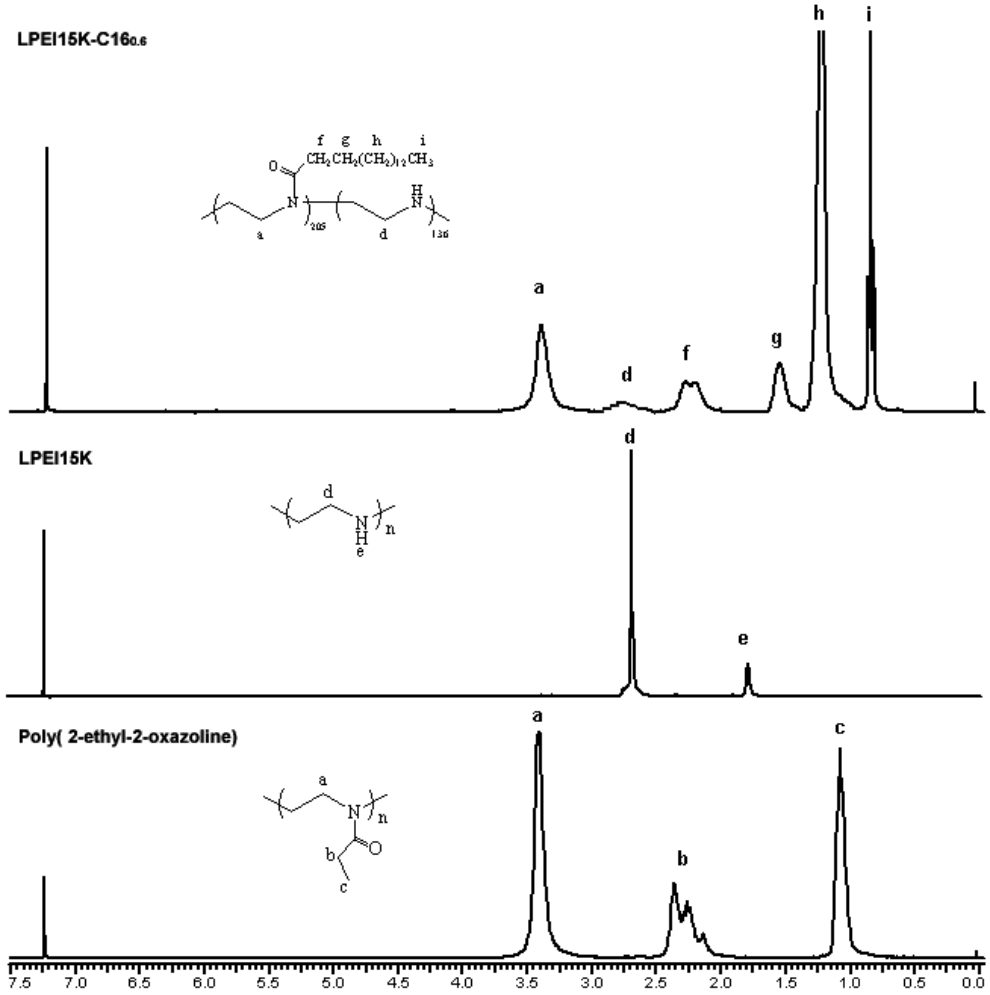


Figure 3. Comparison of the ^{1}H NMR spectra of poly(2-ethyl 2-oxazoline), pure linear **LPEI15K and LPEI15K-C16$_{0.6}$**

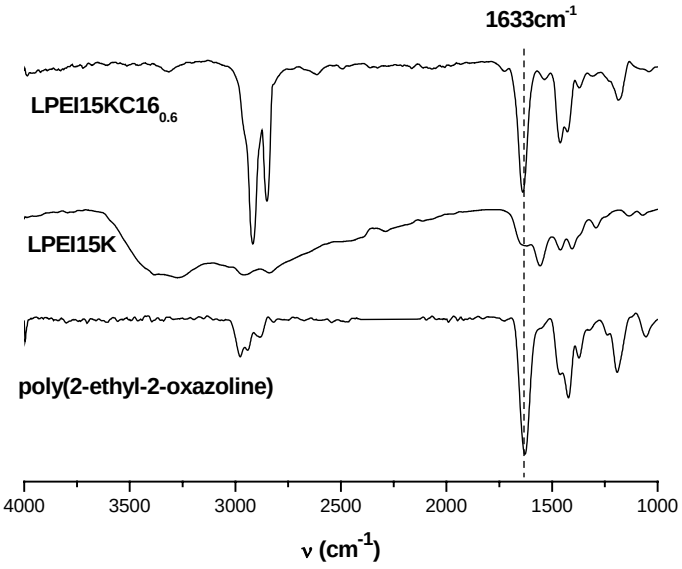


Figure 4. Comparison of the FT-IR spectra of poly(2-ethyl 2-oxazoline), pure linear **LPEI15K** and **LPEI15K-C16$_{0.6}$**

7. Esterified Hyperbranched Polyglycerol P($G_{269}C16_{0.6}$)

Hyperbranched polyglycerol (DP_n= 269; 16 g; 215.9 mmol of OH groups) was dissolved in 300 mL of freshly distilled pyridine at 80°C in the presence of *N*-methyimidazole (2 mL) under argon. Palmitoyl chloride (39.26 mL, 129.5 mmol, 0.6 equivalents) was dissolved in toluene (200 mL) and added dropwise to the pyridine solution of polyglycerol. The reaction mixture was kept at 120°C overnight. After removal of most of the solvent, a stochiometric amount of K_2CO_3 was added and pyridine residues were removed by azeotropic distillation with toluene (300 mL). The product was purified by dialysis (dialysis tubing, benzoylated cellulose MWCO 1,000) in $CHCl_3$ over one day to afford P($G_{269}C16_{0.6}$) as a waxy white solid. Yield: 90%. ^{1}H NMR ($CDCl_3$): 0.81 (t, CH_3), 1.18 (s, 24H, CH_2), 1.53 (m, 2H, C<u>H</u>$_2$CH_2CO), 2.22-2.27 (m, 2H, C<u>H</u>$_2$CO), 3.47-4.02 (br, 5H, glycerol), 4.59 (br, OH). ^{13}C NMR ($CDCl_3$): 14.49, 23.07, 25.30, 29.55, 29.65, 32.30, 34.23, 34.53, 34.72, 62.14, 63.96, 65.50, 68.91, 69.95, 71.53, 173.45. IR ($CHCl_3$) ν= 1737.72 (C═O), 3400 cm^{-1} (O─H). α (Degree of alkyl substitution per hydroxyl group) = 60%. M_w/M_n= 1.17. M_n = 58,770 g/mol.

8. The measurement of dye transport using PEI nanocapsules

In a typical UV-Vis experiment, 4 mL of an aqueous Congo red solution (λ_{max}=498nm, ε_{max}=2.50x10^4 L mol^{-1} cm^{-1}) with a concentration in the range of 7.1x10^{-6}- 7.8x10^{-5}M was shaken for 10 minutes with 4 mL of a chloroform solution of PEI25K-$C16_{0.6}$ (c=9.07x10^{-7}M). After standing and phase separation, a sample of the clear red chloroform solution was transferred into a UV-Vis cuvette and its absorption spectrum was measured.

9. Viscosity data

Table 1. Viscosity data of amidified PEI and esterified PG

Samples	M_n (x10^{-4})	M_w/M_n	$\eta_{spec.}$ (mL/g)[a]	$\eta_{dynamic}$ (mPa·s)[b]
PEI10K-C16$_{0.6}$	4.32	2.4	6.31	5.46
PEI10K-C16$_{0.6}$N$^+_{0.4}$	5.68	2.5	5.15	4.45
PEI25K-C16$_{0.6}$	11.7	2.3	6.34	5.48
PEI25K-C16$_{0.6}$N$^+_{0.4}$	15.0	2.4	5.78	5.00
LPEI15K-C16$_{0.6}$	6.48	1.7	15.4	13.3
PG$_{269}$C16$_{0.6}$	5.87	1.3	6.68	5.78

[a] The specific viscosities correspond to toluene solutions of the related polymer at 30 g/L; [b] dynamic viscosities were determined at 30 g/L and determined from the following equation: $\eta_{dynamic} = \upsilon \cdot \rho$, where υ (mm^2/s) and ρ = 0.865 g/cm^3 are the absolute (kinetic) viscosity and the density of toluene at 20°C respectively; [c] n.d. = not determined.

10. UV-vis and fluorescence measurements:

Table 2. UV-vis data of hydrophilic dyes in water and in chloroform solution containing amidated PEI

Solution	EY		FS		MO		CR	
	λ_{max} (nm)	ε_{max} x 10^{-4}	λ_{max} (nm)	ε_{max} x 10^{-4}	λ_{max} (nm)	ε_{max} x 10^{-4}	λ_{max} (nm)	ε_{max} x 10^{-4}
H_2O	516	8.65	488	6.06	464	2.16	498	2.50
PEI25K-C16$_{0.6}$[a]	533	4.86	506	1.58	428	2.32	500	1.72
PEI10K-C16$_{0.6}$N$^+_{0.4}$[a]	533	5.32	506	3.02	428	2.60	500	2.15
PG$_{269}$C16$_{0.6}$[a]	533	4.13	502	1.73	423	2.66	510	2.93

[a] The encapsulated dyes in PEI nanocapsules have been measured in chloroform solution.

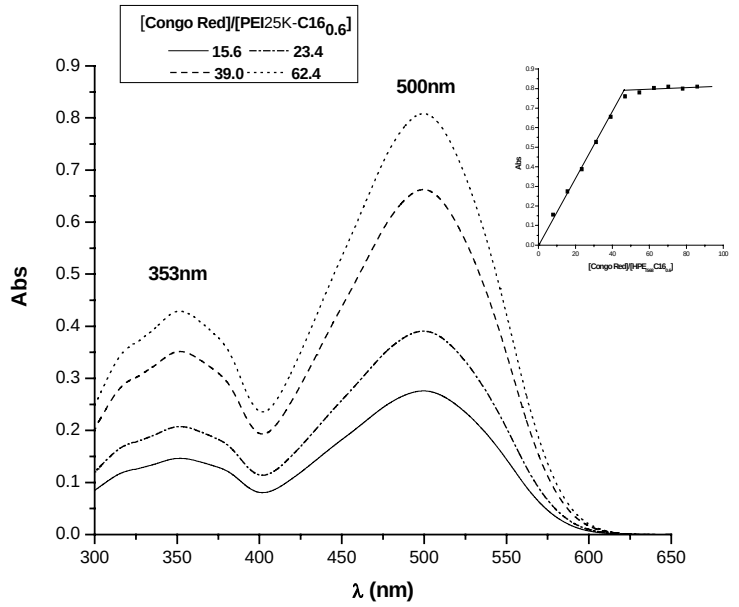


Figure 5. UV-Vis spectra and titration curve (inset) for **PEI25K-C16$_{0.6}$** encapsulating Congo red (CR) in chloroform phase

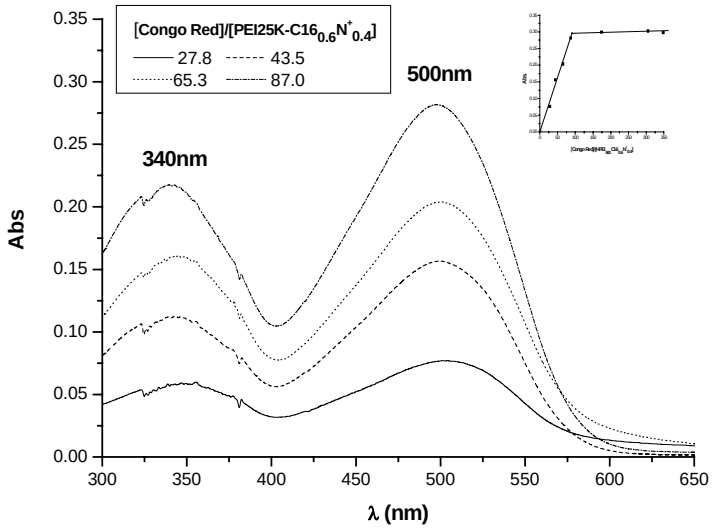


Figure 6. UV-Vis spectra and titration curve (inset) for **PEI25K-C16$_{0.6}$N$^{+}_{0.4}$** encapsulating Congo red (CR) in chloroform phase

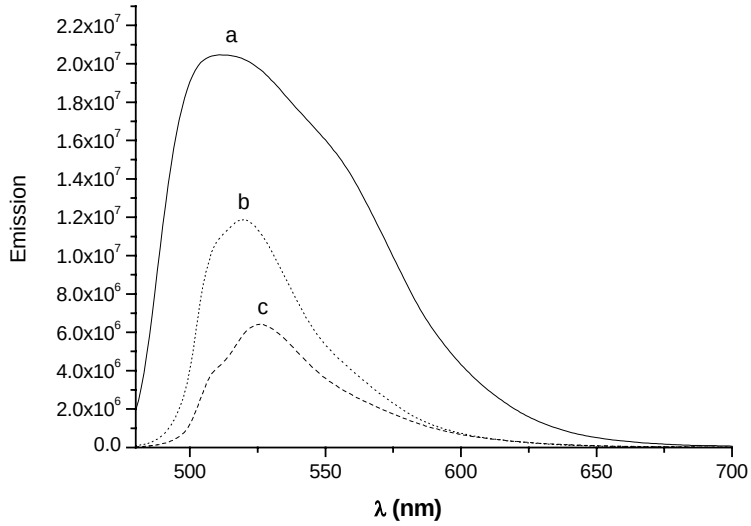


Figure 7. The fluorescence spectra of (a) Fluorescein sodium in water (Abs=0.047, λ_{ex}= 488 nm), (b) Fluorescein sodium encapsulated by **PG$_{269}$C16$_{0.6}$** in chloroform (Abs= 0.048, λ_{ex} = 506 nm) and (c) Fluorescein sodium encapsulated by **PEI25K-C16$_{0.6}$** in chloroform (Abs=0.048, λ_{ex}= 506 nm)

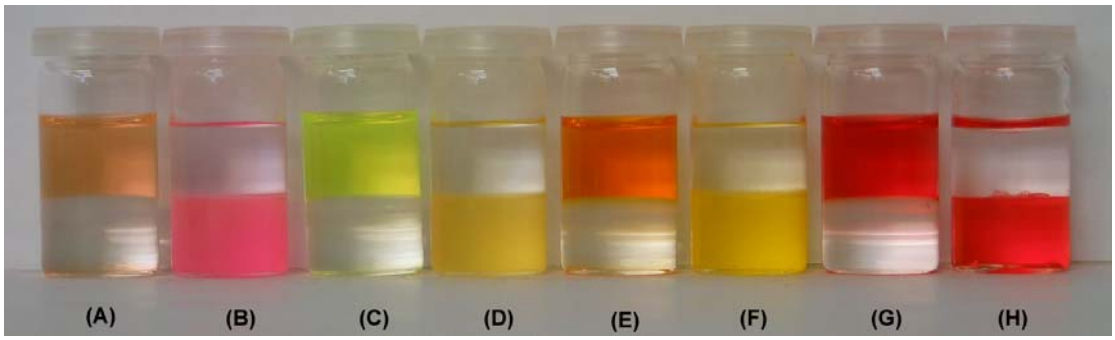


Figure 8. Demonstration of the solubilization effect (bottom layer: chloroform, upper layer: water). (A) Eosin Y; no polymer in organic layer; (B) Eosin Y; **PEI10K-C16$_{0.6}$N$^{+}_{0.4}$** in organic layer; (C) Fluorescein Sodium; no polymer in organic layer; (D) Fluorescein Sodium; **PEI10K-C16$_{0.6}$N$^{+}_{0.4}$** in organic layer; (E) Methyl Orange; no polymer in organic layer; (F) Methyl Orange; **PEI10K-C16$_{0.6}$N$^{+}_{0.4}$** in organic layer; (G) Congo Red; no polymer in organic layer; (H) Congo Red; **PEI10K-C16$_{0.6}$N$^{+}_{0.4}$** in organic layer

11. References

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2. (a) Slagt, M. Q.; Stiriba, S.-E.; Klein Gebbink, R. J. M.; Kautz, H.; Frey, H.; van Koten, G. *Macromolecules* **2002**, *35*, 5734-5737. (b) Stiriba, S.-E.; Kautz, H.; Frey, H. *J. Am. Chem. Soc.* **2002**, *124*, 9698-9699.
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