Non-ionic Dendronized Multiamphiphilic Polymers as Nanocarriers for Biomedical Applications

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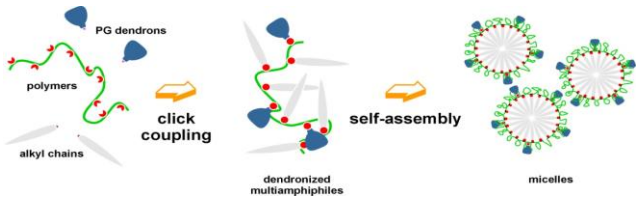
ABSTRACT

Several supramolecular architectures on the nanoscale have become important candidates for potential applications in nanomedicine, particularly for drug, dye, and gene delivery. Biocompatiblity and biodegradablity of these nanocarriers is especially important for the clinical application of therapeutic compounds. Out of various conceptual extensions for the development of such molecular objects, our approach uses polymers as multi-functional, polydisperse cores to which dendrons are connected via pendant functional groups at every repeating unit and are termed as 'dendronized polymers' or 'denpols'. Surface tension measurements and dynamic light scattering (DLS) studies revealed that all of the multiamphiphilic polymers synthesized spontaneously self-assemble in aqueous solution. Cryogenic transmission electron microscopy (cryo-TEM) further proved the formation of multiamphiphiles towards monodisperse spherical micelles of about 7-9 nm in diameter. The evidence from UV-vis and fluorescence spectroscopy suggest the effective solubilization of hydrophobic guests like pyrene and 1-anilinonapthalene-8-sulphonic acid within the hydrophobic core of the micelles. These results demonstrate the potential of these dendronized multiamphiphilic polymers for the development of prospective drug delivery systems for the solubilization of poorly water soluble drugs.

Key words: multiamphiphiles, dendronized polymers, bio-catalysis, micelles, encapsulation

1. Introduction

Recently, it became evident that the introduction of polyglycerol dendrons in monomolecular amphiphiles has a remarkable stabilizing effect, resulting in structurally very defined supramolecular architectures [1]. We assumed that polyglycerol dendrons would also improve the overall structural stability of polymer based supramolecular architectures if covalently linked to a linear polymer backbone. This would combine the biomedical benefits of PEG-type polymers, which have long clearance times, high stability and solubility, no immunogenicity, antigenicity, or toxicity [2, 3], better monodispersity than classical monomolecular amphiphiles [1] and enhanced stability and biocompatibility due to polyglycerol dendrons [4].



2. Results and Discussion

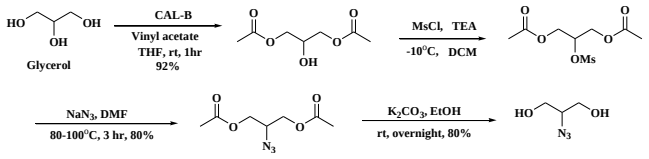
We have designed a biocatalytic approach for the synthesis of biodegradable $(AB)_n$-type diblock polymers from 2-azido-1, 3-propanediol (azido glycerol) and PEG diethylesters using Novozym-435 (*Candida antarctica* lipase) as biocatalyst which were dendronized further by making use of highly efficient click coupling reactions to attach different generation dendrons and alkyl chains. This study is focused on the supramolecular self-assembly of these dendronized multiamphiphilic polymers, in particular, the effect of dendron loading onto linear PEG-based polymers and the ability to solubilise hydrophobic guest molecules.

3. Synthesis

2-Azido-1, 3-propanediol (azido glycerol) was synthesized from glycerol in four steps (Scheme 1). Glycerol was first converted to 2-hydroxypropane-1, 3-diyl diacetate using vinyl acetate as the acylating reagent, by following the established procedure [5]. The secondary hydroxyl group of 2-hydroxypropane-1, 3-diyl diacetate was then mesylated and subsequently an azidation reaction was performed to obtain 2-azidopropane-1, 3-diyl diacetate. It was then deacetylated using K_2CO_3 in anhydrous ethanol to obtain the monomer 2-azido-1, 3-propanediol.

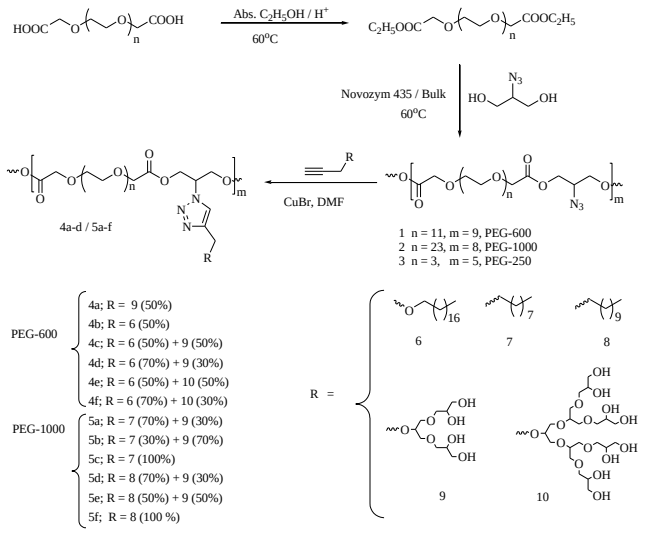
Earlier Novozym-435 catalyzed condensation of PEG-600 dimethylester and glycerol has been carried out by our group under solventless conditions [6]. Herein, polymers were synthesized from 2-azido-1, 3-propanediol and PEG (M_n 250/600/1000) diethylesters, following a well-established biocatalytic route in approx 80 % yield. PEG diesters were in turn synthesized from their corresponding diacids by a standard esterification protocol [6]. Though

PEG diacids (M_n 250/600) are commercially available but PEG diacid (M_n 1000) was synthesized from corresponding diol by $KMnO_4$ oxidation. Use of the diesters instead of the diacids not only enhances the degree of polymerization but also introduces an ethyl group that could be used for end-group analysis to determine the average molecular weight of the polymer by ^{1}H-NMR.



Scheme 1: Synthesis of azido glycerol

The base polymers were further functionalized with dendritic polyglycerols (G1 and G2) and alkyl chains in different functionalization levels via click chemistry to generate dendronized multiamphiphilic polymers. (Scheme 2).



Scheme 2: Synthesis of dendronised multiamphiphilic Polymers

4. Supramolecular Self Assembly in Aqueous Media

The supramolecular self-assembly of the synthesized non-ionic dendronized polymers (**4a-f**) in aqueous media was investigated using surface tension measurements, dynamic light scattering (DLS), and cryogenic transmission electron microscopy (cryo-TEM). Subsequently, the solubilisation capacities of the aggregates were evaluated using pyrene as a hydrophobic fluorescent guest and the interactions with the guest molecules were investigated by ANS binding experiments.

4.1 Critical Aggregation Concentration

Surface tension measurements were carried out in a pendant drop apparatus. The surface tension (γ) of the aqueous solutions was effectively reduced as the polymer concentration increased. The data were plotted as logarithmic functions of the surfactant concentrations. A clear break in the curve marks the critical aggregation concentration (CAC).

Polymer	Composition of R [%]			CAC	
	Alkyl Chain	PG [G1.0]	[G2.0]	(10^{-5} M)	(mgL^{-1})
4a	-	50	-	-	-
4b	50	-	-	2.00	153
4c	50	50	-	2.94	262
4d	70	30	-	2.50	224
4e	50	-	50	2.41	247
4f	70	-	30	2.08	203

Table 1. Critical Aggregation Concentration (CAC) of polymers **4a-f** in aqueous solution

Thus, the CAC of the dendronized multiamphiphiles was observed to decrease both by a larger proportion of hydrophobic side chains (**4d** < **4c** and **4f** <**4e**) as well as a higher generation of the dendrons (**4e** < **4c** and **4f** < **4d**). Similar correlations were found for monomeric non-ionic dendritic amphiphiles by Haag et al. [1] and for amphiphilic block copolymers composed of styrene and sodium acrylate by Eisenberg and co-workers [7].

Aggregation behaviour of the polymers was studied by cryo-TEM and DLS at a general concentration of 10.0 g L^{-1}, which is well above the CAC of the polymers. The cryo-TEM measurements confirmed that exclusively dendronized polymer (**4a**), completely dissolves in water without any sign of aggregation as was already anticipated from the absence of a CAC. All other multiamphiphilic polymers (4b-f) formed monodisperse spherical micelles.

Geometrical calculations of the micelles dimensions render overall diameters of 8.4 nm (**4b**), 8.8 and 9.2 nm (**4c** and **4e**) and 8.2 and 8.4 nm (**4d** and **4f**), and point to approximately 127 (**4c** and **4e**) or 60 (**4d** and **4f**) PG groups (with [G1.0] or [G2.0]) dendrons respectively, within their hydrophilic shells.

5. Encapsulation Studies

The polymeric multiamphiphiles were evaluated for their guest solubilization properties using pyrene as a hydrophobic guest molecule. To establish the polymer structure with guest solubilisation property, the quantity of encapsulated and, hence, solubilized pyrene in the different multiamphiphiles, was evaluated. Dendronized polymer (**4a**) which lacks any hydrophobic alkyl chains did not incorporate the hydrophobic guest pyrene, while polymers (**4b-f**) have considerable guest solubilisation properties. Thus, within this group of polymers the presence of an

internal hydrophobic core is the essential structural feature for the encapsulation of hydrophobic guest molecules.

Polymer	Solubilization Capacity [$n_{guest}/n_{polymer}$]	Solubilization efficiency [mg g^{-1}]
4a	-	-
4b	1.4	35.5
4c	1.0	20.9
4d	1.9	67.1
4e	2.1	42.4
4f	2.3	74.8

Table 2. Transport capacities and efficiencies of the polymers **4a-f** (conc. of 5 g/L) for solubilizing hydrophobic surrogate drug pyrene.

In the row of the three multiamphiphiles with the same percentage of hydrophobic content (50% alkyl), [G1.0] dendronized multiamphiphile (**4c**) has the lowest solubilisation capacity (1 mol pyrene per mol of polymer), followed by nondendronized multiamphiphile (**4b**) (1.4 mol pyrene per mol of polymer) and the [G2.0] dendronized multiamphiphile (**4e**) (2.1 mol pyrene per mol of polymer). In general, multiamphiphiles with [G2.0] dendrons (**4e**, **4f**) provide better guest solubilization capacities (2.1, 2.3 mol pyrene per mol of polymer, respectively) compared to those with [G1.0] dendrons (**4c**, **4d:** 1.0, 1.9) mol pyrene per mol of polymer.

Thus, the generation of the dendrons plays a significant role for the guest solubilization properties of these multiamphiphiles: [G2.0] dendrons stabilize the micelles, as indicated by the higher solubilization capacities, while [G1.0] dendrons seem to destabilize them, if compared to polymer (**4b**)**.** The guest solubilization efficiency (milligrams of guest per gram of amphiphile) increases, however, with an increased percentage of hydrophobic content. The dendronized multiamphiphiles (**4c** and **4e**) (50% alkyl grafted) encapsulate less pyrene (20.9 mg and 42.4 mg, respectively) than (**4d**) (67.1 mg) and (**4f**) (74.8 mg) (70% alkyl grafted), respectively. Altogether, our new dendronized multiamphiphilic polymers show solubilization efficiencies of up to 7.5 wt% (**4f**), which is considerably higher if compared with monomolecular non-ionic dendritic amphiphiles (up to 2 wt%), [1] and thus, highlights the versatility of the dendronized multiamphiphilic polymeric architectures for prospective drug delivery applications over corresponding monomolecular amphiphiles.

5.1 Binding Studies with 1-Anilino-naphthalene-8-sulfonic Acid

The encapsulation experiments with pyrene suggest that these new multi-amphiphiles can be employed for encapsulation of active agents/drugs and may find potential biomedical application as drug carriers. However, for drug delivery, it is essential to know the polymer's binding characteristics and association with guest molecules. Quantitative analysis of the polymer molecule binding capacity and affinity can be assessed by encapsulation of small fluorescent dyes [8]. Since all relevant multiamphiphiles (**4b–f**) show the similar micellization behaviours, the dendronized multiamphiphile (**4f**) with the most apparent guest solubilization properties was chosen for the ANS binding studies.

The fluorescence of 1-anilinonaphthalene-8-sulfonic acid (ANS) is sensitive to changes in the microenvironment [9], which allows the evaluation of host–guest binding interactions by spectrofluorometric methods. Due to dynamic quenching by water molecules, ANS shows only low fluorescence in the range between 400 and 600 nm with an emission maximum (λ max) at 525 nm in aqueous media. The fluorescence is greatly enhanced and is accompanied by a blue-shift of the band, as the solvents polarity decreases. Upon addition of polymer (**4f**)**,** the ANS fluorescence was significantly increased and gradually led to a blue shift of (λ max) to about 477 nm. Since the polymer solution did not show any bands in this range, these changes arose due to the binding of ANS and suggest that the dye was placed in a hydrophobic environment, well shielded from the surrounding water molecules. By further increasing the polymer concentration the fluorescence began to show signs of maximizing as more ANS was bound. Additional studies with ANS revealed unimolecular binding characteristics within the hydrophobic domain of the micelles that indicates that the guest molecules are predominantly located in the internal hydrophobic cores of the aggregates. All together, this new class of dendronized multiamphiphilic polymers demonstrate the formation of notable uniform micelles with higher guest solubilization capacities and efficiencies compared to monomolecular amphiphiles, added biocompatibility due to the use of PEG and PG dendrons, and lower exchange rates due to covalently linked multiamphiphilic architecture, which all ought to enhance the potential use as nanocarriers for biomedical applications. Further work is underway to study the aggregation behaviour, encapsulation of hydrophobic drugs by the non-ionic dendronised amphiphilic polymeric systems resulting from PEG (M_n 250/1000) and also the cellular uptake mechanism of these new multiamphiphiles, fine-tune the structure to achieve more stable architectures, and obtain controlled drug release profiles.

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