Dendronized core-multishell nanocarriers for solubilization of guest molecules

Abstract
We have synthesized core-multishell (CMS) nanocarriers with different outer shells and the self-aggregation behavior and the transport capacity of these nanocarriers were studied with the hydrophobic guest molecules Nile red (NR) and methotrexate (MTX). The outer shell either consisted of methoxypoly(ethylene glycol) (mPEG) or polyglycerol (PG) dendrons of generation one or two. NR was solubilized by all CMS nanocarriers. The solubilization of MTX could only be achieved with mPEG-terminated CMS nanocarriers. Depending on the encapsulated guest, the CMS nanocarriers showed self-aggregation. The NR loaded CMS nanocarriers all formed bigger aggregates with a radius between 110 and 170 nm. In the case of the MTX loaded CMS nanocarriers, the MTX prevented the formation of CMS aggregates and therefore its efficient transport.

Keywords
copolyglycerol • dendron • Nile red • methotrexate • drug delivery • supramolecular aggregation

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1. Introduction
Poor bioavailability and systemic toxicity are the major limiting factors for establishing new drugs. A promising way to overcome these problems is the use of polymeric nanocarriers which can encapsulate and selectively transport drugs to the desired site of action. The most prominent examples for such nanocarriers are polymeric micelles[1-3] and liposomes [4,5]. However, a drawback of these systems is the disassembly of the carrier upon dilution [6]. Carrier platforms that consist of covalently bound amphiphilic structures, so-called unimolecular micelles or core-shell type architectures, are able to overcome this problem. These unimolecular core-shell architectures can be easily obtained from dendritic polymers. A recent example for an efficient core-shell architecture are PE-PG nanocarriers consisting of a dendritic hydrophobic poly(ethylene) core and a grafted, dendritic, hydrophilic polyglycerol (PG) shell [7]. The easy accessibility, low polydispersities, and possible variations in the degree of branching and molecular weight of dendritic polyglycerol (dPG) classify it as an excellent candidate for core-shell architectures [8,9]. Furthermore, its biocompatibility was shown to be comparable or even better than the widely used and FDA-approved poly(ethylene glycol)s (PEG) [10]. Therefore, dPGs have found many biomedical applications [11]. Our group has developed several core-shell architectures based on dPG as drug delivery systems [12-18]. Inspired by the polarity gradient of liposomes we designed core-multishell (CMS) architectures with either dendritic poly(ethylene imine) or dPG as core, a hydrophobic inner shell and a hydrophilic outer shell [19-21]. These CMS nanocarriers have a high potential as delivery platforms for therapeutic and diagnostic applications. They can solubilize hydrophilic as well as hydrophobic guest molecules and dissolve them in aqueous and many organic media [19]. CMS nanocarriers with an outer methoxypoly(ethylene glycol) (mPEG) shell (CMS-mPEG) have been shown to be non-toxic and non-irritating [20]. The transport of the guest molecules occurs in supramolecular aggregates of the CMS-mPEG

Abbreviations
CMS, core-multishell; NR, Nile red; MTX, methotrexate; mPEG, methoxypoly(ethylene glycol); PG, polyglycerol; dPG, dendritic polyglycerol; PEG, poly(ethylene glycol); CMS-mPEG, core-multishell nanocarrier with a mPEG outer shell; EPR, enhanced permeation and retention; PG[G1], polyglycerol dendron of generation 1; PG[G2], polyglycerol dendron of generation 2; CMS-G1, core-multishell nanocarrier with a PG[G1] outer shell; CMS-G2, core-multishell nanocarrier with a PG[G2] outer shell; DLS, dynamic light scattering; THF, tetrahydrofuran; DMF, N,N-dimethylformamide; CDI, carbonyldimidazole; RT, room temperature; DCM, dichloromethane; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

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can be considered as non-toxic. mPEG is FDA-approved and one of the most frequently used polymers in drug delivery. The 1,18-octadecanedioc acid is very similar to fatty acids present in the body and should therefore be non-toxic as well. dPG as mentioned above has been proven to be highly biocompatible [10]. The replacement of linear mPEG with PG dendrons is supposed to result in cavities in the CMS nanocarrier structure which should lead to an increase of the transport capacity which was investigated by loading these novel CMS nanocarriers with the hydrophobic guests NR (dye) and methotrexate (MTX, drug, see Figure 1). NR was chosen as a guest, because this dye has structural properties similar to some drug molecules, it has very low solubility in water, and its solubilization can be easily quantified by absorption spectroscopy. Furthermore, we have already gained detailed insight on the behavior of NR in combination with CMS nanoparticles [22]. MTX is an antimetabolite and an antifolate drug, which is used for the treatment of cancer. MTX is almost insoluble in water and therefore the solubilization of MTX with CMS nanoparticles could be a promising approach to enhance the bioavailability of the drug. The loaded CMS nanocarriers were investigated using UV/Vis spectroscopy and dynamic light scattering (DLS).

2. Methods

2.1. General.
Reactions requiring dry conditions were carried out in dried Schlenk glassware under argon. Analytical grade solvents and chemicals were purchased from Acros or Sigma Aldrich and used as received. Dry solvents were obtained from a MBraun SPS-
800 solvent purification system. The protected PG dendrons of generation 1 and 2 were synthesized with an amine group at the focal point (PG\[G1.0\]-NH₂ and PG\[G2.0\]-NH₂) according to literature procedures \[31,32\]. Dendritic CMS nanocarriers with mPEG350 as the outer shell were synthesized as described in the literature \[19,20\]. NMR spectra were recorded on a Jeol ECX 400, Jeol Eclipse 500 MHz, or Bruker Avance 700 spectrometer. The chemical shifts observed in proton and carbon NMR spectra are given relative to tetramethylsilane and were referenced to the indicated solvents \[34\]. NMR data was reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, m = multiplet), integration, and coupling constants in Hertz (Hz). Multiplets (m) were reported over the range (ppm) at which they appear at the indicated field strength. Mass spectrometry was performed on an Agilent 6210 ESI-TOF spectrometer. UV/Vis spectra were recorded on a Scinco S-3100 UV/Vis spectrometer. DLS experiments were performed by using a Malvern Zetasizer Nano instrument.

2.2. Sample Preparation.
Stock solutions of all three nanocarriers (CMS-mPEG, CMS-G1, and CMS-G2) with concentrations of 1 and 5 mg/mL in Millipore water were prepared. A 20 mM guest (NR or MTX) stock solution was stirred and heated to reflux. 1,1'-Carbonyldiimidazole (CDI, 2 eq.) was added to a 500 mL round-bottom flask containing 1,18-Octadecanedioic acid (10.0 g, 22.9 mmol, 3 eq.) were dissolved in 300 mL dry DCM. The solution was refluxed for another 30 min while being purged with dry argon.

2.3. Transport Capacity Determination.

100 µL of the aqueous solutions of the different nanocarriers with solubilized guest were freeze-dried and redissolved in 2 mL methanol. The concentrations of NR in methanol were estimated using the molar extinction coefficient (ε) of 45,000 M⁻¹ cm⁻¹ at 552 nm \[35\]. The concentrations of MTX in methanol were determined by a calibration curve.

2.4. Experimental Procedures

C18-PG\[G1\]: 1,18-Octadecanedioc acid (10.0 g, 31.8 mmol, 2 eq.) was added to a 500 mL round-bottom flask containing 300 mL dry THF and fitted with a reflux condenser. The solution was stirred and heated to reflux. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 4.4 g, 22.9 mmol, 10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, concentrated, and purified by flash column chromatography (silica, DCM with 0 to 4% MeOH). The product was obtained as a white wax-like solid (7.1 g, 73%).

\[M+Na\]^+; M(observd.) = 638.4244 [M+Na]^+.

1H-NMR (500 MHz, DCM-d2, 25 °C, ppm): δ = 6.16-6.08 (m, 1H, -NH-CO-), 4.25-4.19 (m, 2H, 2x (-CH2)2CHO- (PG\[G1\])), 4.18-4.11 (m, 1H, -NH-CH(CH2)2 (PG\[G1\])), 4.04-3.98 (m, 2H, 2x -OCHCHHO- (PG\[G1\])), 3.71-3.66 (m, 2H, 2x -OCHCHHO- (PG\[G1\])), 3.61-3.56 (m, 2H, -CH(CHH-O-) 2 (PG\[G1\])), 3.54-3.44 (m, 6H, -CH(CHH-O-) 2 and 2x -OCH-CHO- (PG\[G1\])), 2.30 (t, J = 7.5 Hz, 2H, -CH2CH2COOH), 2.15 (t, J = 7.6 Hz, 2H, -CH2CH2CONH-), 1.63-1.54 (m, 4H, -CH2CH2COOH and -CH2CH2CONH-), 1.38 (s, 6H, 2x (-O)2C(CH3)(CH3)), 1.32 (s, 6H, 2x (-O)2C(CH3)(CH3)), 1.31-1.24 (m, 24H, -CH2-(CH2)12-CH2-).

13C-NMR (125 MHz, DCM-d2, 25 °C, ppm): δ = 178.1, 173.8, 109.9, 109.9, 75.3, 75.3, 72.9, 72.9, 70.5, 70.5, 67.1, 67.0, 49.7, 37.2, 34.5, 30.2, 30.2, 30.2, 30.1, 30.1, 30.0, 29.94, 29.8, 29.8, 29.8, 29.7, 27.1, 26.3, 25.8, 25.7, 25.4.

HRMS (ESI-TOF): M(calcd.) for C_{51}H_{93}NO_{17} = 1014.6341 [M+Na]^+; (M(observed) = 1014.6341 [M+Na]^+).

C18-PG\[G2\]: 1,18-Octadecanedioc acid (4.16 g, 13.2 mmol, 2 eq.) was added to a 500 mL round-bottom flask containing 300 mL dry THF and fitted with a reflux condenser. The solution was stirred and heated to reflux. CDI (1.1 g, 6.6 mmol, 1 eq.) was added to the solution and stirred until the CO2 evolution had ceased. The solution was refluxed for another 30 min and it was purged with dry argon. Then PG\[G2.0\]-NH2 (4.6 g, 6.6 mmol, 1 eq.) was dissolved in 20 mL dry THF and added to the solution. The reaction was refluxed for another two hours and then allowed to reach RT. The mixture was concentrated under vacuum, and the residue was taken up in DCM and cooled in an ice bath. The formed precipitate (excess of 1,18-octadecanedioc acid) was filtered off. The DCM was evaporated and the remaining crude product purified by flash column chromatography (silica, CHCl3 with 0 to 4% MeOH). The product was isolated as colorless oil (4.4 g, 67%).

1H-NMR (400 MHz, CHCl3-d1, 25 °C, ppm): δ = 6.37-6.20 (m, 1H, -NH-CO-), 4.28-4.20 (m, 4H, 4x (-CH2)2CHO- (PG\[G2\])), 4.18-4.10 (m, 1H, -NH-CH(CH2)2 (PG\[G2\])), 4.06-4.00 (m, 4H, 4x -OCHCHHO- (PG\[G2\])), 3.80-3.40 (m, 26H, 2x -OCHCHHO- (PG\[G2\])), 2.31 (t, J = 7.5 Hz, 2H, -CH2CH2COOH), 2.14 (t, J = 7.6 Hz, 2H, -CH2CH2CONH-), 1.67-1.55 (m, 4H, -CH2CH2COOH and -CH2CH2CONH-), 1.40 (s, 12H, 4x (-O)2C(CH3)(CH3)), 1.34 (s, 12H, 4x (-O)2C(CH3)(CH3)), 1.31-1.20 (m, 24H, -CH2-(CH2)12-CH2-).

13C-NMR (175 MHz, MeOH-d4, 25 °C, ppm): δ = 176.3, 174.7, 131.7, 128.5, 109.1, 78.5, 78.4, 74.9, 74.7, 72.6, 72.6, 72.1, 72.0, 71.1, 70.8, 69.8, 68.6, 66.2, 63.1, 49.5, 49.2, 35.8, 33.6, 29.4, 29.4, 29.3, 29.3, 29.2, 29.1, 28.9, 28.9, 25.8, 25.7, 24.7, 24.3.

HRMS (ESI-TOF): M(calcd.) for C_{18}H_{35}NO_{2} = 638.4244 [M+Na]^+; (M(observed) = 638.4232 [M+Na]^+).

NHS-C18-PG\[G1\]: In a 500 mL Schlenk-flask C18-PG\[G1\] (4.7 g, 7.6 mmol, 1 eq.) and N-hydroxy succinimid (NHS, 2.6 g, 22.9 mmol, 3 eq.) were dissolved in 300 mL dry DCM. The reaction was cooled to 0 °C in a water-ice bath. Then 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC, 4.4 g, 22.9 mmol, 1 eq.) was added to the solution. The reaction was refluxed for another 30 min while being purged with dry argon. Then PG\[G1.0\]-NH2 (5.0 g, 15.9 mmol, 1 eq.) was added to the solution. The reaction was refluxed for another two hours and then cooled to room temperature (RT). The mixture was concentrated under vacuum, and the residue was taken up in dichloromethane (DCM), filtered, and washed with water (3 x 10 mL). The organic phase was dried over anhydrous sodium sulfate, filtered, concentrated, and purified by flash column chromatography (silica, DCM with 0 to 4% MeOH). The product was obtained as a white wax-like solid (7.1 g, 73%).
After 30 min the cooling bath was removed and the reaction was stirred for 16 hours at RT. The solution was concentrated under vacuum and washed with water (3 x 10 mL). The organic phase was washed once with brine (15 mL) and then dried over sodium sulfate. The pure product was obtained after flash column chromatography (silica, DCM with 0 to 4% MeOH). The product was obtained as a white wax (4.4 g, 79%).

1H-NMR (500 MHz, DCM-d2, 25 °C, ppm): δ = 6.61-6.08 (m, 1H, -NH-CO-), 4.24-4.18 (m, 2H, 2x (-CH2CH2CHO-), 4.16-4.10 (m, 1H, -NH-CH(=CH)2-NH-), 4.02-3.98 (m, 2H, 2x -OCH3CH2-), 3.71-3.65 (m, 2H, 2x -OCH2CH2-), 3.60-3.55 (m, 2H, -CH(=CH-OH)-), 3.54-3.44 (m, 6H, -CH(OH)-), and 3.45-3.35 (m, broad, 4H, -CH2-CHO-), 2.79 (s, broad, 4H, -OCOCH2CH2-), 2.58 (t, J = 7.5 Hz, 2H, -CH2CH2CONH-), 1.71 (dt, J = 15.2, 7.5 Hz, 2H, -CH2CH2CONH-), 1.62-1.52 (m, 2H, -CH2CH2CONH-), 1.37 (s, 6H, 2x (-O)2C(CH3)(CH3)), 1.32 (s, 6H, -O(CH2)2(OH)2), 1.31-1.22 (m, 24H, -CH2-(CH2)12-CH2-).


CMS-G1 protected: NHS-C18-PG[G2] (0.2 g, 0.2 mmol, 1.1 eq, per amine group on dPG) was dissolved in 1.5 mL of DMSO and added dropwise to 1 mL of a stock solution of hyperbranched polyglycerol amine (M = 10 kDa, degree of amination = 70%, dPG10kDa(-NH2)0.7) in MeOH. The reaction mixture was stirred for 24 hours. Then the reaction mixture was transferred into a dialysis tubing (MWCO 2 kDa) and was dialyzed against pure methanol. Finally the solvent was removed under vacuum yielding 0.5 g (74%) of a slightly yellow solid.

1H-NMR (500 MHz, MeOH-d4, 25 °C, ppm): δ = 4.24-4.13 (m, 4H, 4x (-CH2)2CHO-), 4.19-4.12 (m, 1H, -NH-CH(=CH)2-NH-), 4.09-4.02 (m, 4H, 4x -OCH3CH2-), 3.90-3.83 (m, broad, dPG backbone and PG[G2]), 2.82-2.14 (m, 4H, 2x -CH2CH2CONH-), 1.69-1.54 (m, 4H, 2x -OCH2CH2CONH-), 1.39 (s, 12H, 4x (-O)2C(CH3)(CH3)), 1.37-1.24 (m, 36H, 4x (-O)2(CH2)2(OH)2), 1.29-1.12 (m, 24H, -CH2-(CH2)12-CH2-).

13C-NMR (175 MHz, MeOH-d4, 25 °C, ppm): δ = 174.7, 165.1, 155.3, 113.8, 110.0, 78.5, 78.5, 78.4, 78.3, 72.6, 72.6, 71.6, 71.0, 70.9, 70.8, 70.8, 69.8, 69.6, 68.7, 68.5, 68.4, 66.8, 66.6, 49.0, 49.0, 48.6, 36.6, 33.8, 30.9, 29.6, 29.5, 29.4, 29.4, 29.3, 29.0, 28.7, 26.8, 25.7, 25.6, 25.4, 24.9, 24.5.

IR (cm⁻¹): ν = 3346, 2971, 2914, 1671, 1522, 1637, 1374, 1211, 1091, 972, 900, 822.

CMS-G1: The acetal protected CMS-G1 (0.49 g) was dissolved in 10 mL methanol and one drop of concentrated hydrochloric acid was added. The reaction was stirred at RT for 18 hours. The solution was concentrated and diluted with water. After freeze-drying 0.37 g (97%) of a slightly yellow foam was obtained.

1H-NMR (500 MHz, MeOH-d4, 25 °C, ppm): δ = 4.23-4.13 (m, 1H, -NH-CH(=CH)2-NH-), 4.00-2.50 (m, broad, dPG backbone and PG[G1]), 3.82-3.72 (m, 2H, -OCH2CH2CONH-), 3.65-3.40 (m, 12H, -CH2CH2CONH-), 2.15-2.28 (m, 4H, 2x -CH2CH2CONH-), 1.69-1.53 (m, 4H, 2x -CH2CH2CONH-), 1.5-1.1 (m, 24H, -CH2-(CH2)12-CH2-).
NHS activation was chosen since it provides an activated and stable product which could be easily stored for prolonged time. The protected dendronized CMS nanocarriers were obtained by stirring the NHS-ester with dPG amine (MW$_\text{dPG} = 10,000$ g/mol, degree of amination 70%) in methanol. Purification was performed via dialysis (MWCO 2 kDa) in methanol. The deprotection of the outer shell was performed in methanol with a drop of concentrated hydrochloric acid. The pure product was obtained after freeze-drying. Since all other byproducts were volatile under the freeze-drying conditions (0.08 mbar) no further purification was necessary.

3.2. Transport Capacities.

In order to investigate their suitability as delivery agents the different CMS nanocarriers were loaded with the dye NR or the drug MTX. The transport capacities were determined via UV/Vis spectroscopy. To avoid any undesired effects that could occur due to the aqueous environment the samples were freeze-dried and redissolved in organic media. In a previous report we were able to show self-aggregation of NR in aqueous mPEG-terminated CMS nanocarrier solutions [22]. The obtained transport capacities are given in Table 1. All carriers were able to enhance the solubility of NR. The NR transport capacity was doubled by changing the outer mPEG shell to PG[G1]. Here, the cavities formed by the dendronized outer shell may have been supportive for the transport capacity and thus could be loaded with additional NR. The transport capacity of the CMS-G2 nanocarriers for both carrier concentrations was comparable to the mPEG-terminated CMS nanocarriers. In this case, the higher generation dendrons seem to form cavities which are not supportive for the transport capacity. For MTX, on the other hand, only mPEG-terminated CMS nanocarriers achieved good transport capacities, which

![Figure 2](image_url)

**Figure 2.** Synthesis of CMS nanocarriers with PG dendrons as outer shell. The synthesis is shown exemplary for CMS-G1. The synthesis of CMS-G2 was performed using the same synthetic pathway. For simplicity only one arm of the functionalized dPG is shown. a) CDI, dry THF, reflux, 1 h, PG[G1] amine or PG[G2] amine, reflux, 2 h; b) NHS, EDC, dry DCM, 0 °C to RT, 16 h; c) dPG amine, MeOH, RT, 24 h; d) HCl, MeOH, 18 h.
were comparable to the transport capacity of NR for the CMS-mPEG nanocarrier. Both dendronized CMS nanocarriers did not enhance the solubility of MTX. The fact that mPEG-terminated CMS nanocarriers were able to transport MTX as efficiently as NR, but the dendronized CMS nanoparticles were not, reveals that the composition of the outer shell can directly influence the transport capacities for different guests. Overall, it seems that the type of guest plays an important role for the solubilization with the different CMS nanocarriers. However, the CMS-mPEG nanocarriers seem to be more universal than PG-terminated nanocarriers.

### 3.3. Size and Aggregation Behavior of Unloaded CMS Nanocarriers.

In order to better understand the influence of the outer shell on the transport and aggregation behavior of the different CMS nanoparticles, we investigated loaded and unloaded CMS nanocarriers with the help of DLS. First of all, we studied the unloaded CMS nanocarriers in methanol and in water. In methanol the CMS nanocarriers had hydrodynamic radii of around 3 nm, 3-4 nm, and 5-6 nm for mPEG-terminated CMS, CMS-G1, and CMS-G2, respectively (see Table 2). These differences in size go along with the changes one would expect. The used mPEG and PG[G1] have approximately the same molecular weight and therefore it makes sense that they have a similar steric demand since mPEG is most likely in a collapsed state. PG[G2] is significantly bigger than PG[G1] and therefore a bigger particle size is observed for CMS-G2 nanocarriers. In the case of CMS-G1 and CMS-G2 nanocarriers with a concentration of 5 mg/mL, bigger aggregates were observed in the intensity distribution of the DLS measurements. The aggregates were between 40-50 nm in radius for the CMS-G1 nanocarriers and around 66 nm for the CMS-G2 nanocarriers. In water the mPEG-terminated CMS nanocarriers had a hydrodynamic radius of 6-8 nm, the CMS-G1 nanoparticles a radius of 3-6 nm, and the CMS-G2 nanoparticles of 6-7 nm. The obtained values for the radii of single particles in water again go along with what one would expect taking into account that mPEG stretches out in water. Therefore, the CMS-mPEG nanocarriers are as big as the ones with PG[G2] and the CMS-G1 are the smallest particles. All nanocarriers showed bigger aggregates in the intensity distributions with radii of 80-110 nm. An aggregate peak of around 70 nm was only observable in the volume distribution for 5 mg/mL mPEG-terminated CMS nanocarriers. This shows that CMS-mPEG nanocarriers in water at higher concentrations have a slightly increased tendency to form aggregates on their own. For the dendronized nanocarriers (CMS-G1 and CMS-G2) the aggregation behavior is less pronounced than for mPEG-terminated CMS nanocarriers in the case of aqueous solutions. This is in line with earlier results when grafted, hyperbranched PG shells were used [21]. However, the 100% branched, dendronized shells cannot fully prevent aggregation.

### 3.4. Size and Aggregation Behavior of Loaded CMS Nanocarriers.

In order to study the influence of the guest molecules on the aggregation behavior and to see whether the transport occurs

<table>
<thead>
<tr>
<th>Nanocarrier</th>
<th>Carrier Conc. [mg/mL]</th>
<th>Transport Capacity [mgguest/gcarrier]</th>
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<tbody>
<tr>
<td>NR MTX</td>
<td></td>
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</tr>
<tr>
<td>CMS-mPEG</td>
<td>1</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>3.4</td>
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<tr>
<td>CMS-G1</td>
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<td>15.3</td>
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<td></td>
<td>5</td>
<td>7.1</td>
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<tr>
<td>CMS-G2</td>
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<td>6.0</td>
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<tr>
<td></td>
<td>5</td>
<td>3.9</td>
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</table>

Table 1. Transport capacities of CMS-mPEG, CMS-G1, and CMS-G2 nanocarriers for the guest molecules Nile red (NR) and methotrexate (MTX) in water at different carrier concentrations determined via UV/Vis measurements. Transport capacities are corrected in respect to the remaining water solubility of the guest molecules.

<table>
<thead>
<tr>
<th>Nanocarrier</th>
<th>Carrier Conc. [mg/mL]</th>
<th>Size in MeOH [nm]</th>
<th>Size in H2O [nm]</th>
</tr>
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<tbody>
<tr>
<td>CMS-mPEG</td>
<td>1</td>
<td>3.3 ± 0.3</td>
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<td>2.5 ± 0.1</td>
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<td>72.3 ± 2.9 (56%)</td>
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<td>6.3 ± 0.1 (44%)</td>
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<td>5.2 ± 0.2 (99%)</td>
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<td></td>
<td></td>
<td>58.1 ± 0.2 (&lt;1%)</td>
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<tr>
<td>CMS-G1</td>
<td>1</td>
<td>44.7 ± 4.0 (56%)</td>
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<td>3.7 ± 0.2 (44%)</td>
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<td>2.7 ± 0.1</td>
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<td>6.5 ± 0.1 (58%)</td>
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<td>101.5 ± 4.2 (42%)</td>
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<td>4.8 ± 0.1</td>
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<tr>
<td>CMS-G2</td>
<td>1</td>
<td>66.6 ± 1.7 (61%)</td>
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<td>6.1 ± 0.2 (39%)</td>
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<td>4.5 ± 0.1</td>
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<td>103.1 ± 1.2 (82%)</td>
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<td>6.4 ± 0.7 (18%)</td>
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<td>4.7 ± 0.5</td>
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* $r_{Hint}$ and $r_{Hvol}$ are the average hydrodynamic radii calculated by intensity and volume given with the mean standard deviation. In the case of two visible peaks in the size distribution the abundance is given in brackets.

Table 2. Hydrodynamic radii of CMS-mPEG, CMS-G1, and CMS-G2 nanocarriers in water and methanol at different nanocarrier concentrations.
via the formation of aggregates, the NR and MTX-loaded CMS nanocarriers were analyzed by DLS (see Table 3). The NR-loaded CMS nanocarriers all formed aggregates. For mPEG-terminated CMS nanocarriers aggregates with a hydrodynamic radius of 120-130 nm were observed, while the CMS-G1 nanocarriers had a radius of about 170 nm at a concentration of 1 mg/mL, and a radius of around 100 nm at a concentration of 5 mg/mL, the CMS-G2 nanocarriers had a radius of 110-120 nm. The volume based size distributions further confirmed this, as all CMS nanocarriers showed only one peak. This clearly shows that all CMS nanocarriers form aggregates if they are loaded with NR. The CMS-G1 nanocarriers formed noticeably larger aggregates, which seems to correlate with the higher transport capacity of this nanocarrier.

In the intensity based size distributions the MTX loaded CMS nanoparticles all showed peaks corresponding to aggregates (Table 4). Based on the volume distributions, however, the single nanocarriers are the predominant species for all carriers.

The influence of the outer shell of different CMS nanocarriers on the aggregation behavior seems to be negligible. Instead the guest molecule seems to have a bigger influence on the aggregation behavior. When NR was used as the guest all CMS nanocarriers formed aggregates, while the presence of MTX inhibits aggregation and therefore only unimolecular nanocarriers were observed.

4. Conclusion

We synthesized a new type of dendronized CMS nanocarrier with PG dendrons of the 1st or 2nd generation as the outer shell and compared them to CMS nanocarriers with a mPEG outer shell. By DLS analysis we investigated the influence of the outer shell on the aggregation behavior of the CMS nanocarriers which were either unloaded or loaded with Nile Red (NR) or methotrexate (MTX). While the exchange of mPEG to PG dendrons of the 1st generation led to an increase in the transport capacity of NR. The 2nd generation only reached transport capacities comparable to the values of the mPEG-terminated CMS nanocarriers. In case of the drug MTX, only the mPEG-terminated CMS nanocarriers were able to solubilize the guest. In case of NR-loaded CMS nanocarriers, it was found that all carriers exclusively form aggregates. In contrast to NR loaded CMS nanocarriers, the MTX loaded carriers showed sizes corresponding to single CMS nanocarriers. Overall, the experiments show that one cannot in general prevent the formation of CMS nanocarrier aggregates by replacing the mPEG outer shell even with fully branched PG dendrons. Furthermore, we conclude that the aggregation behavior is strongly influenced by the guest molecule. The transport capacities, on the other hand, are influenced by the material used as outer shell of the CMS nanocarriers. These results indicate that the careful design of nanocarriers allows the fabrication of tunable drug delivery systems.

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References


