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Topologically Controlled Inter-Polyelectrolyte Complexes between Molecular Bottlebrushes and Dendrimers

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ABSTRACT: The complexation of a cylindrical polyelectrolyte−brush-surfactant complex (PMMPSS−C_{12}) and an oppositely charged generation-5 PAMAM dendrimer (G5-PAMAM) in methanol (MeOH) leads to kinetically controlled formation of interpolyelectrolyte complexes. In contrast, the complexation of the same starting materials in solvents with higher dielectric constant such as dimethylformamide (DMF) or *N*-methylformamide (NMF) results in the formation of topologically controlled complexes of cylindrical morphology. The composition of the complexes is analyzed by static and dynamic light scattering (SLS, DLS) in solution and atomic force microscopy (AFM) after spin-casting the complexes onto mica. The analyzed complexes seem to consist of one PMMPSS−C_{12} molecule and, with increasing G5-PAMAM content, of an increasing number of G5-PAMAM dendrimers.

Introduction

Interpolyelectrolyte complexes form spontaneously upon mixing solutions of oppositely charged polyelectrolytes. The main driving forces are the gain of entropy due to the release of counterions as well as the strong ionic interactions.[1,2] Usually this process is kinetically controlled which leads to compact globular structures consisting of several molecules of each component. The process is significantly dependent on the composition and on the overall polymer concentration.[3,4]

Complexes between DNA and positively charged polyelectrolytes provide promising applications in gene therapy[5,6] where an effective DNA encapsulation is required. For these applications, it is advantageous if the complexation takes place in a rather controlled way leading to complexes with a defined number of molecules per complex, or even enabling topological control.

So far the influence of the topology of the starting materials has rarely been investigated.[7,8] Duschner et al. obtained cylindrical interpolyelectrolyte complexes between a poly(styrenesulfonate)−surfactant complex brush (PMMPSS−C_{12}) and a slightly charged PMMPEI−PEO whereas the complexation with highly charged PMMPEI−PEO yielded globular nonequilibrium structures. Even with DNA as a semiflexible polyelectrolyte interpolyelectrolyte complexes with well-defined structure could hardly been observed.[9] Goessel et al.[10] published one example about the complex formation of DNA with a positively charged cylindrical polymer densely grafted with second to fourth generation dendrons. Depending on the sequence of addition and within a small range of composition they found structures with the DNA wrapped around the cylindrical core.

The presented work focuses on the investigation of the experimental conditions leading to the formation of interpolyelectrolyte complexes which are either kinetically controlled and therefore irreversibly frozen nonequilibrium structures or thermodynamically controlled representing reversible complex formation.

The influence of the topology of the starting components is investigated by using a negatively charged polyelectrolyte brush (poly(styrenesulfonate) polymacromonomers with a polystyrene main chain (PMMPSS)) with well-defined shape, typically obtained by free radical polymerization of polymacromonomers.[11] The complexation with a polyamidoamin dendrimer of generation 5 as cationic component (G5-PAMAM, Figure 1) is investigated to improve the understanding about the mechanism and the experimental conditions where a controlled complex formation is possible.

The complexation in water employing PMMPSS leads to the typical kinetically controlled nonequilibrium structures. For the complexation in organic solvents such as methanol, DMF, and NMF, PMMPSS is first complexed with the surfactant

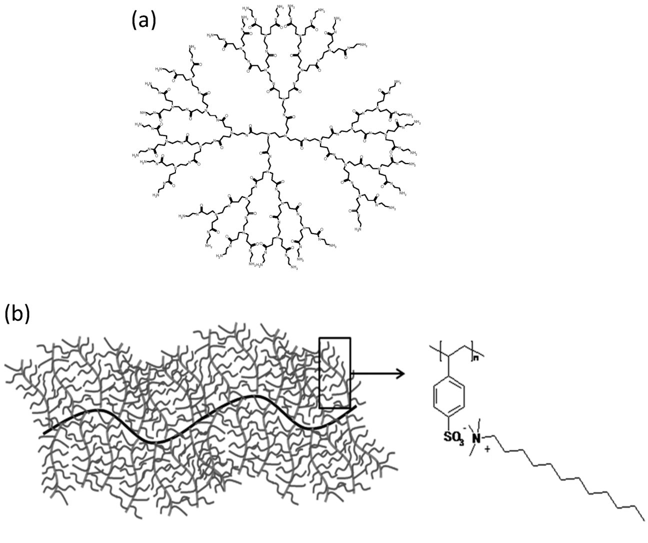

Figure 1. Schematic pictures of the employed cationic and anionic polyelectrolytes. (a) PAMAM-dendrimer (generation 3 exemplarily shown) (b) PMMPSS−C_{12} (n = 35).

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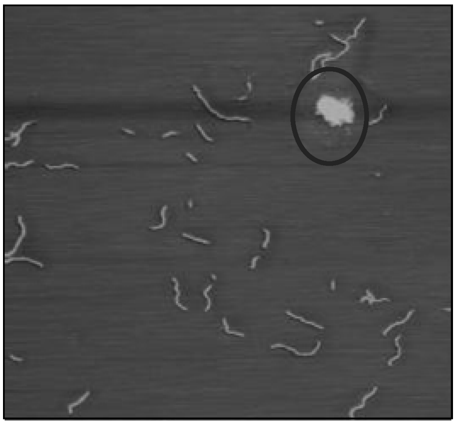

Figure 2. AFM picture (height, 1.8 μm × 1.8 μm) of the complexes obtained from PMMPSS−C_{12} and G5-PAMAM, synthesized at 2.5 mg/L and spin-cast from MeOH (2 mM LiBr): *w*(G5-PAMAM) = 0.2.

dodecyltrimethylammonium bromide to obtain the poly(styrenesulfonate)−brush-surfactant complex (PMMPSS−C_{12}, Figure 1) which is soluble in organic solvents such as MeOH, DMF and NMF.[12]

Experimental Part

Materials. The following chemicals have been employed in p.a. quality: methanol (Sigma-Aldrich), *N*,*N*-dimethylformamide (DMF, Fluka), lithium bromide (Sigma-Aldrich), G5-PAMAM−dendrimer (Aldrich, 5 wt % solution in methanol; the transfer into other solvents was performed by rotary evaporation and subsequent redissolution). *N*-Methylformamide (NMF, Sigma-Aldrich) was vacuum-distilled before use.

Methods. Static light scattering (SLS) was performed at 20 °C using an ALV-SP-86 goniometer, an ALV-3000 Correlator, a 25 mW Uniphase HeNe laser (λ_0 = 632,8 nm) and an ALV/High-QEAPD-Avalanche diode fiber optics detection system. Dynamic light scattering (DLS) was performed at 20 °C using ALV-SP-125−5/N-39 goniometer, an ALV-5000/EPP/60 × 0 multiple-τ realtime digital correlator, an argon-ion-laser (Beam Lock 2060, λ_0 = 514.5 nm, 500 mW, Spectra Physics) and a thermostat C25P (Haake). Measurements were performed between 30° and 150°, every 15°. Each sample was filtered with Millex-LG filters (pore size 0.2 μm, Millipore). A Nanoscope IIIa Multi-Mode instrument (Digital Instruments) was used in tapping mode for AFM pictures. The samples were taken directly out of the light scattering cuvettes and spin-cast onto freshly cleaved mica. Dialysis was performed with a Spectra/Por dialysis membrane (regular cellulose, MWCO 10 kDa).

Synthesis. PMMPSS was obtained by polymerization of polystyrene macromonomers having a polystyrene end group (M_n = 4450 g/mol, P_n = 43, MALDI-TOF mass spectrometry). Homopolymerization and fractionation[13] was performed to obtain polymacromonomers with polystyrene side chains (M_w = 4.12 × 10^6 g/mol, SLS in tetrahydrofuran, d*n*/d*c* = 0.193 mL/g).[14] Sulfonation with acetyl sulfate and subsequent neutralization with excess sodium hydroxide led to the formation of PMMPSS, which was purified by ultrafiltration.[15] Elemental analysis confirmed a degree of sulfonation of 100%. To a solution of PMMPSS in water (*c* = 1 g/L) dodecylammonium bromide was added until precipitation of the complex. Dialysis and lyophilization yielded PMMPSS−C_{12}. Elemental analysis confirmed 100% complexation.

Results and Discussion

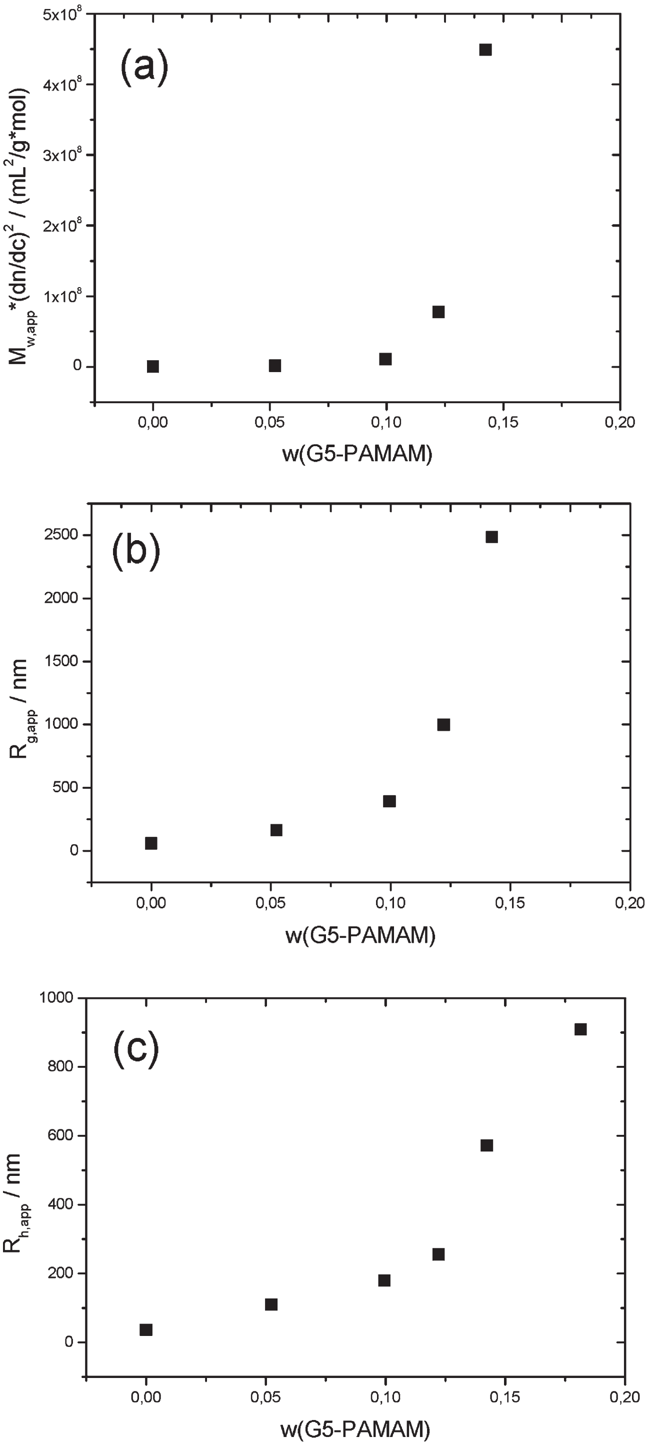

Figure 3. Scattering behavior of the complexes of PMMPSS−C_{12} and G5-PAMAM formed in MeOH: (a) concentration corrected Rayleigh ratio, (b) apparent radius of gyration, and (c) apparent hydrodynamic radius. G5-PAMAM was titrated to PMMPSS−C_{12}. The complexes were synthesized via titration of solutions of the two components with a concentration of 2.5 mg/L each.

The complexation of PMMPSS and G5-PAMAM in water leads to kinetically controlled complexes which possess a compact globular structure consisting of several PMMPSS (data not shown). The complexes were synthesized via titration of solutions of the two components. The overall polymer concentration was 0.5 mg/L.

Complexation of PMMPSS−C_{12} with G5-PAMAM in Methanol. The complexation of PMMPSS−C_{12} with G5-PAMAM in methanol with 2 mM LiBr leads to similar results. Figure 2 shows an AFM picture at a G5-PAMAM weight fraction of 0.2. Besides free PMMPSS−C_{12} a spherical aggregate can be seen.

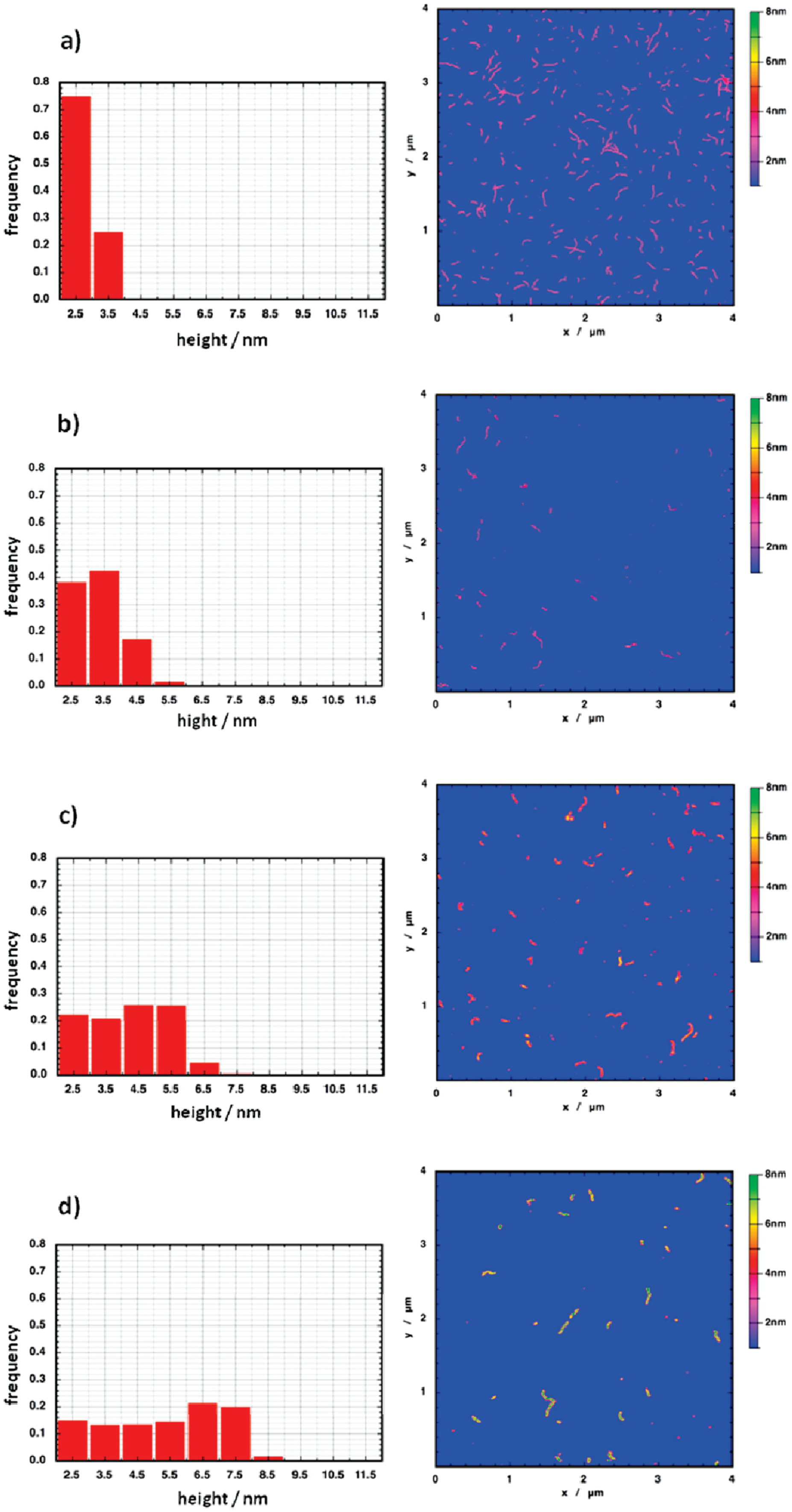

Figure 4. AFM pictures (height, right, 4 × 4 μm) of complexes between PMMPSS−C_{12} in DMF (2 mM LiBr) and the corresponding height frequencies (left): (a) *w*(PMMPSS−C_{12}) = 1; (b) *w*(PMMPSS−C_{12}) = 0.89; (c) *w*(PMMPSS−C_{12}) = 0.47; (d) *w*(PMMPSS−C_{12}) = 0.06.

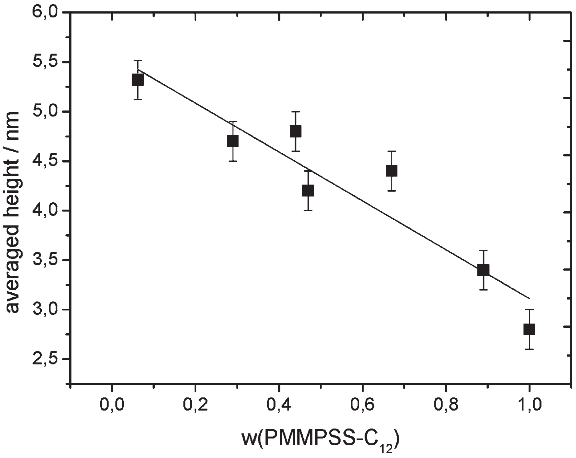

Figure 5. Average height of the complexes between PMMPSS$-$C$_{12}$ and G5-PAMAM, determined above a threshold of 2 nm.

The DLS and the SLS data of the complexes formed by addition of G5-PAMAM to PMMPSS$-$C$_{12}$ in dependence of the PMMPSS$-$C$_{12}$-G5-PAMAM-composition are illustrated in Figure 3. All LS-data were obtained at a concentration of 2.5 mg/L. The complexes were prepared directly in the cuvettes. To a solution of PMMPSS$-$C$_{12}$ (2.5 mg/L) a solution of G5-PAMAM of the same concentration was titrated. After every addition the complex solution was characterized by dynamic and static light scattering (Table 1, Supporting Information). Upon addition of G5-PAMAM the complexes grow very fast and aggregates within sizes of more than apparently 1000 nm are formed consisting of several PMMPSS$-$C$_{12}$. If the process is reversed, i.e. a G5-PAMAM-solution is added to a PMMPSS$-$C$_{12}$-solution, aggregates of more than 100 nm are formed at a PMMPSS$-$C$_{12}$ weight fraction above 0.25.

Complexation of PMMPSS$-$C$_{12}$ with G5-PAMAM in DMF. The complexation in DMF was performed similar to the complexation in methanol. All investigated compositions were prepared in different cuvettes. The overall polymer concentration was around 0.1 g/L. Both solvents have a comparable dielectric constant (DMF: 38, methanol: 33.6) but in contrast to methanol DMF is an aprotic solvent. Some of the AFM results obtained at different PMMPSS$-$C$_{12}$-G5-PAMAM-compositions are shown in Figure 4.

The complexes differ from the ones shown in Figure 2. They possess a cylindrical morphology, independent from the composition. Spherical particles arise from shorter PMMPSS$-$C$_{12}$ present due to the inherently broad size distribution. Next to each AFM picture the corresponding statistical height analysis it plotted as a histogram. These histograms show that the higher the G5-PAMAM content is the larger heights are populated until a maximum of 8.5 nm is reached. The average height in dependence of the weight fraction of the added G5-PAMAM results in a linear dependency (Figure 5). This indicates a rather controlled complex formation.

The scattering behavior of the complexes also points to a preservation of the cylindrical morphology. The radius of gyration is nearly independent of the complex composition and is found to be close to 55 nm which agrees well to the value determined for a single PMMPSS$-$C$_{12}$. The hydrodynamic radius slightly rises from 30 nm for the single PMMPSS$-$C$_{12}$ up to 37.1 nm for the complex. That means that the ρ-ratio (R_g/R_h) is slightly lower for the complexes compared to the single PMMPSS$-$C$_{12}$ which indicates an increase of the diameter of the brushes. For the complexes it lies between 1.58 and 1.69.

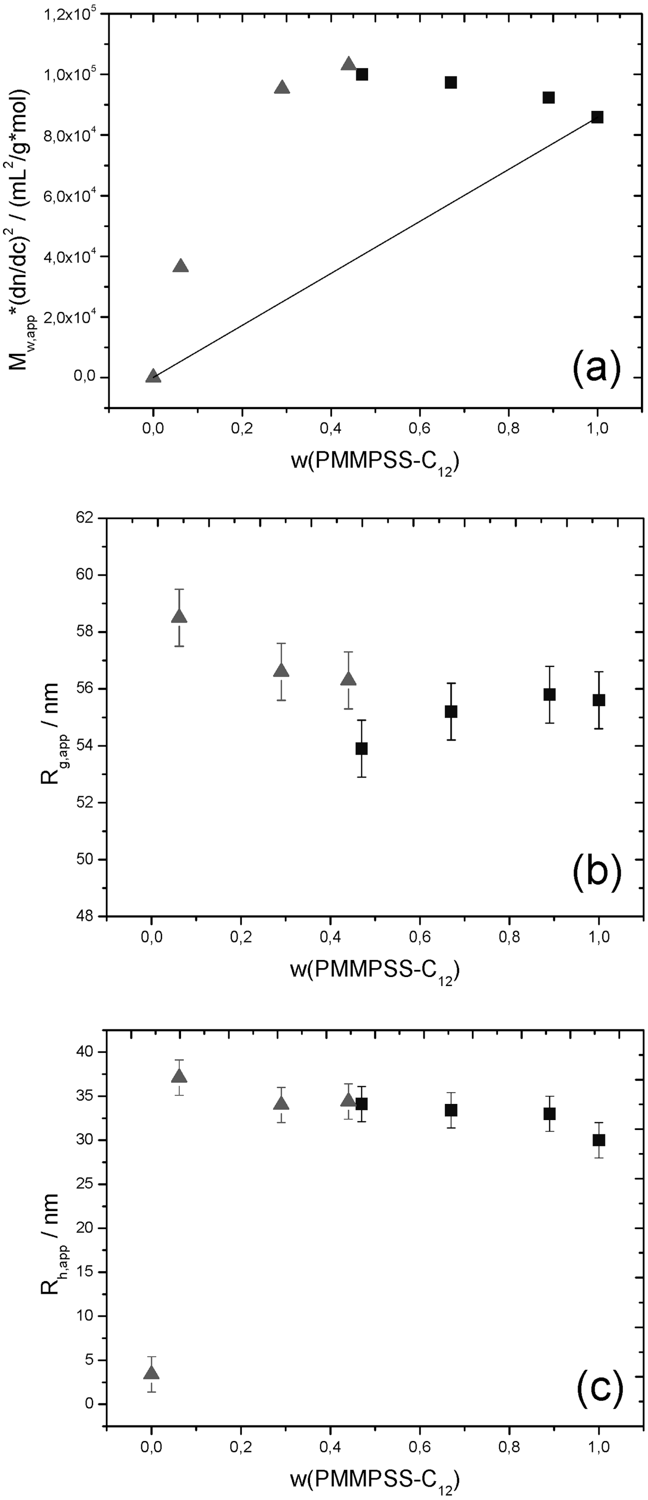

Figure 6. Scattering behavior of the complexes of PMMPSS$-$C$_{12}$ and G5-PAMAM formed in DMF: (a) concentration corrected Rayleigh ratio, (b) apparent radius of gyration, and (c) apparent hydrodynamic radius. The complexes were synthesized at a concentration of 0.1 g/L. (triangle, starting polymer solution = G5-PAMAM; square, starting polymer solution = PMMPSS$-$C$_{12}$; straight line, theoretical homopolymer mixture).

In Figure 6a, the concentration corrected Rayleigh ratio, i.e., the product of the apparent molar mass and the squared refractive index increment is shown. It is a continuous function of the composition and possesses a maximum. The comparison to the hypothetical homopolymer mixture

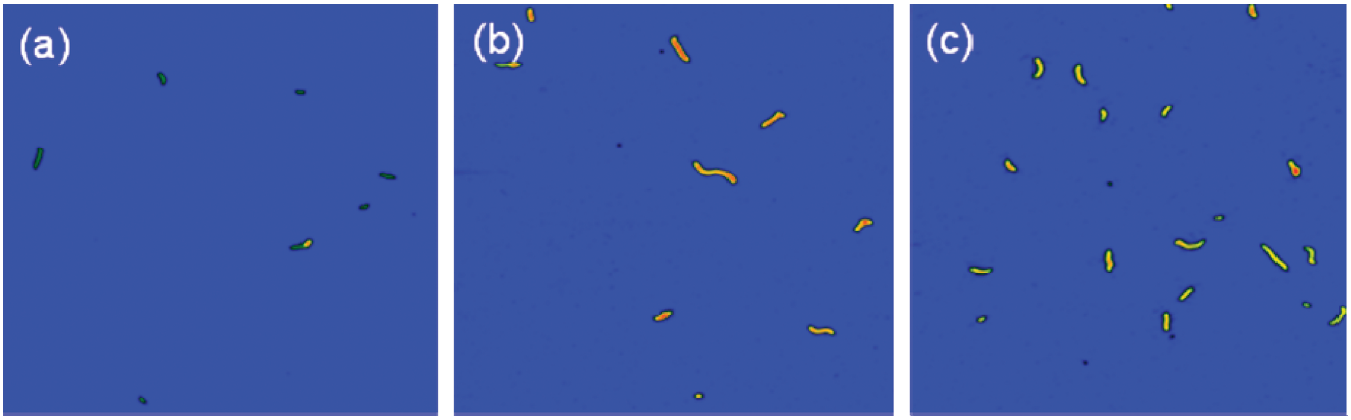

Figure 7. AFM pictures (height) of the complexes between PMMPSS$-$C$_{12}$ and G5-PAMAM in NMF: (a) w(PMMPSS$-$C$_{12}$) = 0.65, 3 μm; (b) w(PMMPSS$-$C$_{12}$) = 0.46, 2.4 μm; (c) w(PMMPSS$-$C$_{12}$) = 0.22, 2.3 μm.

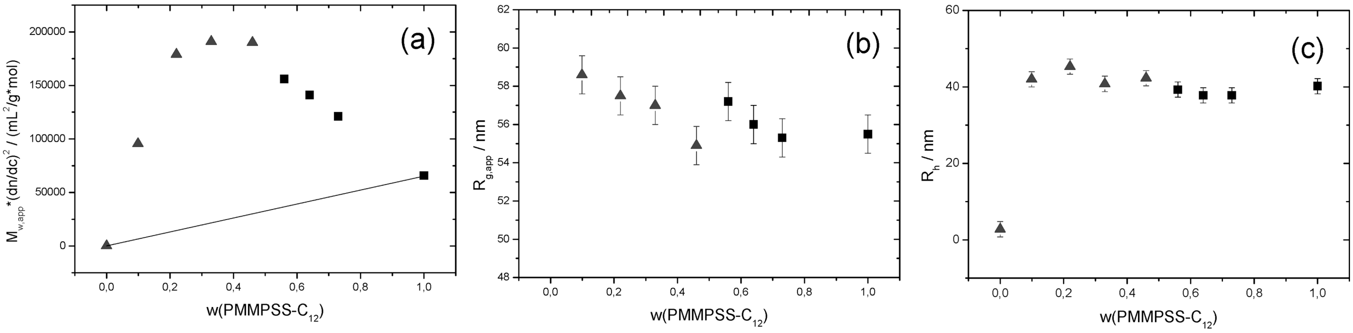

Figure 8. Scattering behavior of the complexes of PMMPSS$-$C$_{12}$ and G5-PAMAM formed in NMF: (a) concentration corrected Rayleigh ratio, (b) radius of gyration, and (c) hydrodynamic radius. The complexes were synthesized at a concentration of 0.1 g/L. (triangle, starting polymer solution = G5-PAMAM; square, starting polymer solution = PMMPSS$-$C$_{12}$; straight line, theoretical homopolymer mixture).

of PMMPSS$-$C$_{12}$ and G5-PAMAM constitutes conclusive evidence that complexes are formed.

With these results, a completely different complexation behavior is given in DMF compared to methanol, apparently without a miscibility gap. The light scattering data (Table 2, Supporting Information) indicate that cylindrical dendrimer-brush complexes are formed over the whole range of composition. Every complex consists of one PMMPSS$-$C$_{12}$. Besides it is remarkable that the size of the complexes is independent of the sequence of addition. Overall, the formed complexes seem to be equilibrium or at least near equilibrium structures.

Complexation of PMMPSS$-$C$_{12}$ with G5-PAMAM in NMF. NMF has a much higher dielectric constant in comparison to DMF (189 compared to 38) and is also an aprotic solvent.

Figure 7 shows AFM pictures of different solutions of PMMPSS$-$C$_{12}$ and G5-PAMAM with different weight fractions of PMMPSS$-$C$_{12}$. All analyzed compositions show cylindrical brushes in the size of one PMMPSS$-$C$_{12}$. No bigger aggregates point to a kinetically controlled complex formation. The overall polymer concentration is about 0.1 g/L.

In Figure 8, the scattering behavior of the complexes in dependence of the PMMPSS$-$C$_{12}$-G5-PAMAM-composition is shown. The radius of gyration of the complexes lies between 55.5 and 57.2 nm which again resembles the range of the size of one PMMPSS$-$C$_{12}$-brush (Table 3, Supporting Information). The hydrodynamic radius of the complexes determined by dynamic light scattering lies between 37.8 and 39.5 nm which is comparable to the sizes determined in DMF. The ρ-ratio is between 1.45 and 1.49. Figure 8a shows the concentration corrected Rayleigh-ratio measured by static light scattering. Similar to the complexation in DMF, it is a continuous process and the curve possesses a maximum. The comparison with the theoretical homopolymer mixture confirms that complexes are formed. Furthermore, the complexes are independent of the sequence of addition.

The concentration corrected Rayleigh-ratios possess a maximum in dependence of the polymer composition. At the maximum, the complexes are no longer able to absorb more G5-PAMAM molecules which can be interpreted that every PMMPSS$-$C$_{12}$ molecule is saturated with G5-PAMAM. If more G5-PAMAM is added, the concentration corrected Rayleigh ratio decreases because free G5-PAMAM molecules reduce the scattering intensity.

The G5-PAMAM, which possesses a degree of protonation of approximately 0.5 at pH 7 in water,[16] could be partially deprotonated by DMF and NMF in contrast to methanol, lowering the effective charges and enabling the topological control. This will be investigated more thoroughly in the future. First experiments to investigate the influence of competitively added protons to the NMF and DMF system are discussed in the following section.

Complexation of PMMPSS$-$C$_{12}$ with G5-PAMAM in NMF with Trifluoroacetic Acid (TFA). Regarding the investigated solvents for the complexation the aprotic ones result in topologically controlled complexes. To investigate the influence of competitively added protons during the complexation, the complexation of PMMPSS$-$C$_{12}$ with G5-PAMAM in NMF was executed in the presence of trifluoroacetic acid. Figure 9 shows the dynamic light scattering results (see Table 4, Supporting Information). As before, the

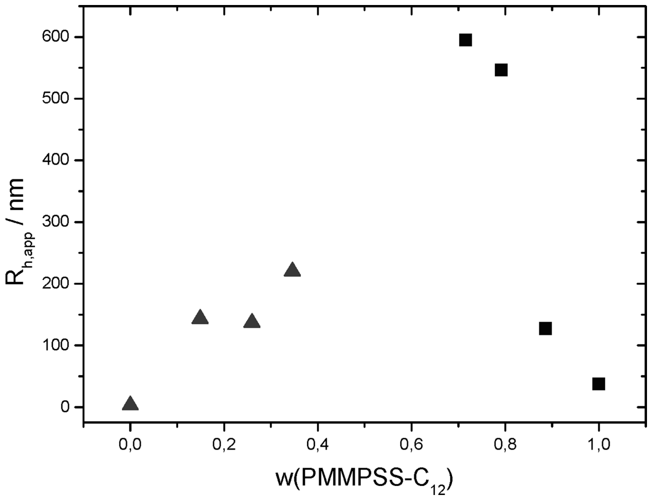

Figure 9. Scattering behavior of the complexes of PMMPSS−C_{12} and G5-PAMAM formed in NMF with trifluoroacetic acid: apparent hydrodynamic radius. The complexes were synthesized at around 0.05 g/L with a trifluoroacetic acid concentration of 0.0095 g/L. (triangle, starting polymer solution = G5-PAMAM; square, starting polymer solution = PMMPSS−C_{12}).

complexes were prepared directly in the light scattering cuvettes via titration of the two components. One half of a TFA molecule per primary amine group of the PAMAM dendrimer was added. It is obvious that the complexation behavior strongly differs from the one in NMF without TFA. The complexes grow very fast and aggregates of over 500 nm are formed consisting of several PMMPSS−C_{12} molecules. Comparable results are obtained for DMF (data not shown). These results indicate that the addition of protons promotes a rather kinetically controlled complex formation.

Conclusions

The complexes between PMMPSS−C_{12} and G5-PAMAM formed in DMF and NMF show a completely different behavior compared to the ones formed in water or methanol, where the commonly described kinetically controlled complex formation occurs. AFM and light scattering indicate cylindrically shaped complexes consisting of only one PMMPSS−C_{12}. Furthermore, both, the sequence of addition and the polymer concentration have no significant influence on the complex formation. Regarding the complexation, the effective charge density seems to be a crucial factor to determine whether the process is kinetically or thermodynamically controlled. The formation of kinetically controlled aggregates is favored in methanol as solvent system, whereas topological control is achieved in DMF and NMF, indicating the ability of the complexes to equilibrate.

The presented data support the crude schematic model Duschner et al. proposed for the complexation: when the cationic component is added to the corona of PMMPSS−C_{12}, the cylindrical morphology of the complexes is retained.

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Supporting Information Available: Tables of determined hydrodynamic radii and radii of gyration and static and dynamic light scattering results of the complexations. This material is available free of charge via the Internet at http://pubs.acs.org.

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