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Nanostructures

Hierarchical Self-Assembly in Solutions Containing Metal Ions, Ligand, and Diblock Copolymer[**]

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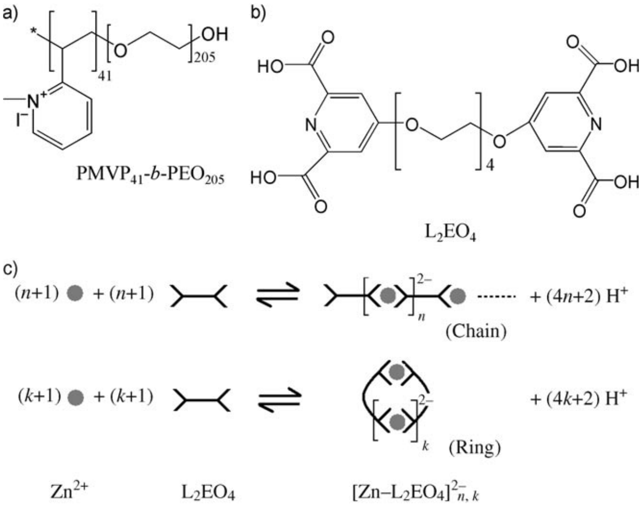
Complex coacervate core micelles (C3Ms) are self-assembled nanoparticles formed by mixing aqueous solutions of a polyion- neutral diblock copolymer and an oppositely charged polyelectrolyte.[1] Before mixing, none of the constituent molecules are capable of forming self-assembled structures. It is only upon mixing that a driving force for micellization arises, namely, electrostatic interaction between the charged polymers. The micellar core, composed of an electrostatic complex, is stabilized against unbound growth by electroneutral segments of the diblock copolymer. These kinds of micelles have been denoted as "polyion complex micelles" (PIC micelles) by Harada and Kataoka,[2] or "block ionomer complexes" (BICs) by Kabanov et al.[3] Since the driving force for micellization is the tendency of the oppositely charged polyelectrolytes to form a complex coacervate phase, the term "complex coacervate core micelles" (C3Ms) was introduced by us.[1] Charged biomolecules can also be used as homopolyelectrolytes to for C3Ms with diblock copolymer. In this way, C3Ms have great potential as nanoscale carriers for the delivery of charged compounds such as DNA and proteins.[4–6]

Herein we address the question as to whether it is possible to fabricate self-assembled objects such as C3Ms with reversible supramolecular polymers, which are self-assembled chains themselves. Such chains are typically responsive: their chain length is not fixed but can adjust to variables such as temperature, concentration, etc. Water-soluble, charged supramolecular polymers indeed exist—namely, in the form of coordination polymers. The purpose of this study is to show that it is the responsiveness of these polymers which enables the formation of a new kind of hierarchical self-assembled nanostructures.

In general, a coordination complex is formed by metal–ligand interaction;[7–9] chain formation becomes possible when an organic molecule having two such ligands is mixed with metal ions. Supramolecular polyelectrolytes based on coordination bonds have been reported, but most of them are either not water soluble[10–13] or they carry only a few charges per unit length of chain.[13–15] Also, many metal/ligand combinations form rather long-lived, nearly permanent bonds.[16] However, the metal–bisligand system developed by Vermonden et al.[8,9] is water soluble, has high charge density, and responds quickly to changes in concentration and composition, as expected for a truly reversible system.

The diblock copolymer used in the current study is poly(2-vinyl-*N*-methylpyridinium iodide)-*b*-poly(ethylene oxide) ($PMVP_{41}$-*b*-PEO_{205}) (Scheme 1 a), which is obtained by quaternization of poly(2-vinylpyridine)-*b*-poly(ethylene oxide) (PVP_{41}-*b*-PEO_{205}). The supramolecular coordination polymer which Vermonden et al. used was prepared by mixing equimolar amounts of zinc nitrate and a bisligand compound based on pyridine-2,6-dicarboxylic acid groups connected at the 4-position of the pyridine ring by four ethylene oxide spacers (L_2EO_4, Scheme 1 b)[8] (where the



Scheme 1. Structure of a) $PMVP_{41}$-*b*-PEO_{205} diblock copolymer, b) L_2EO_4, and c) illustration of the formation of a Zn-L_2EO_4 (1:1) coordination polymer.

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notation "C4" was used instead of "L_2EO_4"). In aqueous solution, both "oligomers" with small rings and "polymers" with long chains are possible structures when an L_2EO_4 and zinc ions are mixed in a 1:1 ratio (Scheme 1 c), but the fractions of L_2EO_4 molecules in the rings and the chains as well as the average length of the chains depend on the concentration. At L_2EO_4 concentrations less than 10 mM, most L_2EO_4 molecules exist in the form of small rings.[8] As each coordination center of the Zn-L_2EO_4 complex has two negative charges overall ($4COO^- + Zn^{2+}$), the Zn-L_2EO_4 coordination polymer can be considered as a negatively charged polyelectrolyte with a charge density of about two elementary charges per monomer.

Mixing dilute solutions of PMVP$_{41}$-*b*-PEO$_{205}$ and Zn-L_2EO_4 at different molar ratios results in solutions that are all clear to the naked eye. However, the measured scattered light intensity depends strongly on the composition, thus indicating the formation of mixed self-assembled nanostructures. We express the compositional variations in terms of the mixing fraction $f-$; for a system with positive and negative chain molecules this is defined by Equation (1),

$$f- = \frac{[-]}{[-]+[+]} \tag{1}$$

where $[-]$ and $[+]$ are the molar concentrations of charges for each of the chains. From Figure 1 a it is clear that the scattered intensity at 90° is a maximum at the stoichiometric mixing fraction $[+]=[-]$ (that is, $f-=0.5$; Figure 1 a, Δ). It can also be seen that the scattered intensity is low for all the mixing ratios of the bisligand with the diblock copolymer (without Zn^{2+} ions, Figure 1 a, ■) or of Zn^{2+} ions with diblock copolymer (without L_2EO_4), namely, of L_2EO_4/PMVP$_{41}$-*b*-PEO$_{205}$ or of Zn/PMVP$_{41}$-*b*-PEO$_{205}$ mixtures. This finding indicates that no self-assembled structures are present in those mixtures. However, addition of L_2EO_4 to the mixture of Zn^{2+} ions and PMVP$_{41}$-*b*-PEO$_{205}$ (Figure 1 a, ●) or addition of Zn^{2+} ions to the (L_2EO_4 + PMVP$_{41}$-*b*-PEO$_{205}$) mixture results in the observation of a similar intensity maximum (at 1:1 mole ratio of Zn^{2+} ions to L_2EO_4, at which the value of $f- = [L_2EO_4]/[Zn^{2+}]+[L_2EO_4]$ in the whole systems is also 0.5). If the mixing ratio of Zn^{2+} ions to L_2EO_4 is not unity, the light intensity is also low. An example of the latter is also shown in Figure 1 a for the case of $Zn^{2+}:L_2EO_4 = 1:2$, at which small L_2EO_4-Zn-L_2EO_4 clusters are mainly formed, so that no scattering peak is observed (Figure 1 a ▲).

At this stage we can conclude that self-assembled objects are formed in solutions containing three species: polycation-neutral diblock copolymer PMVP$_{41}$-*b*-PEO$_{205}$, bisligand L_2EO_4, and Zn^{2+} ions, and that optimal formation of the structure requires both $[Zn^{2+}]=[L_2EO_4]$ and $f-=0.5$. The fact that the light intensity changes upon variation of $f-$, and the occurrence of a maximum of the scattered intensity (independent of the order of mixing) are both indications of the reversibility of these self-assembled structures.

More information on the size and structure of these self-assembled species was obtained from dynamic light scattering (DLS) and from transmission electron microscopy (TEM) studies. DLS measurements revealed that the self-assembled species are quite homodisperse particles with a hydrodynamic radius of approximately 25–27 nm. This is shown by the size probability distributions obtained by a CONTIN analysis (Figure 1 b).

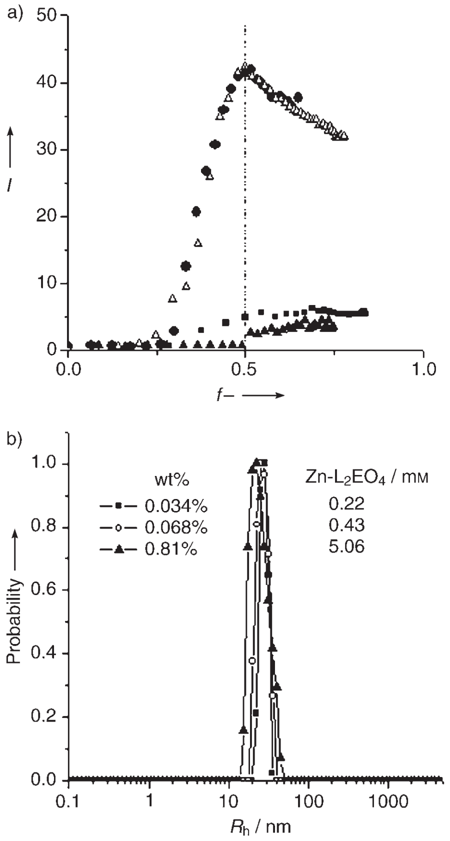

Figure 1. a) Variation of the scattered light intensity *I* with the mixing fraction $f-$, on addition of the Zn-L_2EO_4 coordination polymer to a solution of PMVP$_{41}$-*b*-PEO$_{205}$ (△), L_2EO_4 to PMVP$_{41}$-*b*-PEO$_{205}$ (■), L_2EO_4 to the mixed solution of (Zn^{2+} + PMVP$_{41}$-*b*-PEO$_{205}$) (●), and L_2EO_4-Zn-L_2EO_4 to PMVP$_{41}$-*b*-PEO$_{205}$ (▲). b) Distribution of the hydrodynamic radius (R_h) from dynamic light scattering (DLS) measurements by using CONTIN analysis for the Zn-L_2EO_4/PMVP$_{41}$-*b*-PEO$_{205}$ mixture of different concentrations at $f-=0.5$. Both the total concentration (wt%) and the molar concentration of Zn-L_2EO_4 are shown. All the experiments were done at 298 ± 0.1 K.

Spherical objects were observed by both conventional TEM and cryo-TEM. In both methods the contrast is enhanced compared to normal C3Ms because of the presence of zinc ions. The average radius of the species determined from both the TEM and cryo-TEM studies is about 10 nm (Figure 2). As the hydrodynamic radius obtained from dynamic light scattering is about 25–27 nm, we conclude that with cryo-TEM we observe the complex coacervate cores of the micelles, and that the micellar corona has a thickness of 15–17 nm. The shape of the cores observed with cryo-TEM is seen to be not perfectly spherical but irregular, which is probably due to "frozen-in" shape fluctuations. We interpret this as an indication that the core is very dynamic, as expected for a reversible system.

A significant feature of Figure 2 is the dense hexagonal packing of the spheres. We believe that this dense regular

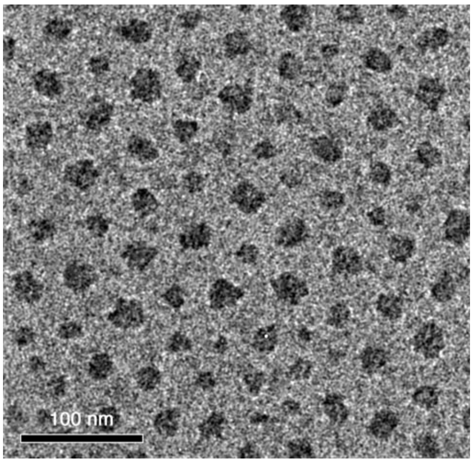

Figure 2. Cryo-TEM image of 0.34% (wt%) Zn-L_2EO_4/$PMVP_{41}$-*b*-PEO_{205} mixtures at $f- = 0.5$.

packing is induced by the sample preparation process, as was also found for self-assembly in an ionic liquid.[17] The solution itself, of course, is a dilute dispersion of independent micelles. However, the mean separation of about 54 nm in Figure 2 is an indication that the average radius of these micelles is about 27 nm, which agrees well with the hydrodynamic radius obtained from DLS studies.

A narrow distribution of micelles still exists after dilution of the mixture from 8 mg mL^{-1} to 0.2 mg mL^{-1}, thus indicating that no secondary structures are formed from C3Ms in the wide concentration range studied.

It may seem surprising that C3Ms are formed at all with Zn-L_2EO_4 coordination polymers at concentrations as low as 0.2–6.0 mM. It was found previously that at such a low concentration the average degree of polymerization of the Zn-L_2EO_4 system is only about two, and Zn-L_2EO_4 forms predominantly small rings.[8] Furthermore, investigations on covalent polymers demonstrated that C3Ms cannot be formed from "oligoelectrolytes" with such a low degree of polymerization.[18] Indeed, no C3Ms were formed at a Zn^{2+}/L_2EO_4 mixing ratio of 1:2 (see Figure 1 a, ▲). At this mixing ratio, short coordination complexes with ends of $L_2EO_4^{4-}$ ions, instead of coordination polymers with long chains, are the dominant species. On the basis of this result one might argue that the rings of 1:1 Zn-L_2EO_4, which are equally small, would not form C3Ms with the diblock copolymer. However, it should be kept in mind that reversible coordination polymers are capable of responding to the conditions. In the present case, the formation of C3Ms and an increase in the length of the coordination polymers go hand in hand. The increase in the length of the coordination polymer is associated with the high local concentration inside the complex coacervate cores of the C3Ms, and is further promoted by the interactions with the oppositely charged PMVP blocks, while the increase in the length of the coordination polymer simultaneously enables the formation of C3Ms (Figure 3). This is a beautiful example where the responsiveness of a reversible supramolecular polymer is essential for the observed result.

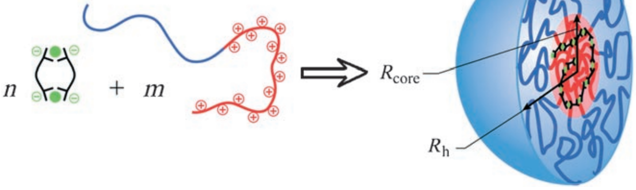

Figure 3. Illustration of the formation of complex coacervate core micelles in the Zn-L_2EO_4/$PMVP_{41}$-*b*-PEO_{205} mixed system. For simplicity, only one Zn-L_2EO_4 chain is illustrated in the core.

Besides Zn^{2+} ions, L_2EO_4 coordination polymers with Ni^{2+}, Co^{2+}, Nd^{3+}, and La^{3+} ions were also found to form C3Ms with $PMVP_{41}$-*b*-PEO_{205}; we expect that any multivalent metal ion that can form coordination polymers with L_2EO_4 can be incorporated into C3Ms. The formation of C3Ms with reversible coordination polymers as one of the components constitutes an entirely new type of cooperativity and of hierarchical self-assembly. Such self-assembly is promising for further fundamental material studies and practical applications in pharmaceutical and environmental sciences.

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