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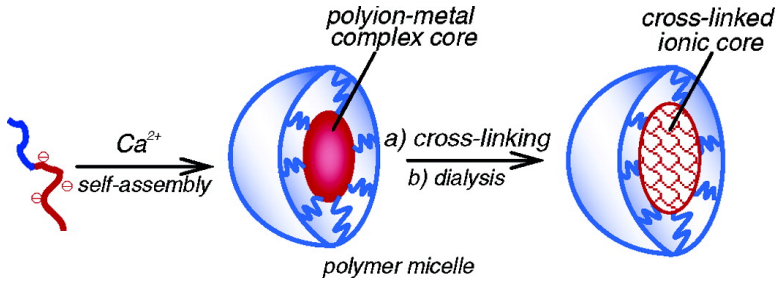

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Polymer Micelle with Cross-Linked Ionic Core

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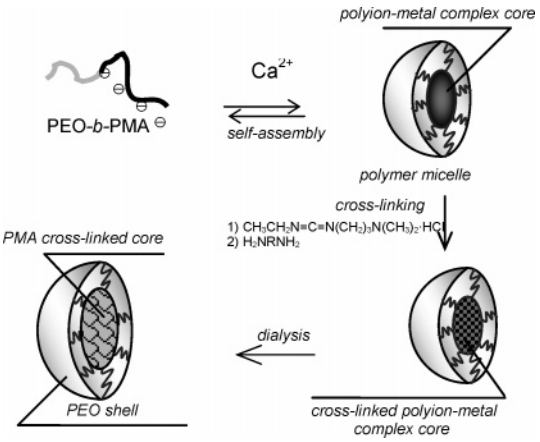
Self-assembled block copolymer micelles have attracted great attention as nanoscale carriers for delivery of low molecular mass drugs, proteins, genes,[1] and imaging agents.[2] The advantages of polymer micelles for development of novel therapeutic and diagnostic modalities include the small size and core-shell architecture leading to protection of an active agent in the core by a hydrophilic polymer shell. After administration in the body, the micelles circumvent renal excretion, display long circulation times, and extravasate into the disease sites with enhanced vascular permeability. Recently, nanofabrication of polymer micelles was significantly advanced by using block copolymers containing ionic and nonionic blocks ("*block ionomers*"). Such block copolymers react with oppositely charged species forming block ionomer complexes, which self-assemble into core-shell micelles.[3] They are unique because they allow encapsulation of charged molecules into the micelle core. However, all polymer micelles have a drawback as a delivery system because they disintegrate after dilution in the body fluids, resulting in premature drug release. Herein, we report the design of novel polymer micelles with cross-linked ionic cores that display high stability. Block ionomer complexes formed between poly(ethylene oxide)-*b*-polymethacrylate anions (PEO-*b*-PMA) and divalent metal cations were utilized as templates for the synthesis of the cross-linked micelles. Such micelles represent hydrophilic nanospheres of core-shell morphology. The core comprises a network of the cross-linked polyanions, which is surrounded by the shell of hydrophilic PEO chains.

The synthesis of the cross-linked micelles was carried out using a two-step process presented in Scheme 1. *The first step* involved self-assembly of PEO-*b*-PMA copolymers into block ionomer complexes in the presence of divalent metal ions, such as Ca^{2+} (or Ba^{2+}, Sr^{2+})[4] (see Supporting Information for details). Electrostatic neutralization of the polyion blocks by metal ions resulted in hydrophobization of these blocks and spontaneous micelle formation. Dynamic light scattering (DLS) measurements for the PEO-*b*-PMA/Ca^{2+} mixtures revealed the formation of the particles with diameters of approximately 110 nm. The low polydispersity index (<0.1) suggested a narrow particle size distribution. Spherical particles of core-shell morphology were observed by transmission electron microscopy (TEM) (Figure 1a). The contrast seen in the TEM image is enhanced by the calcium ion presence in the dispersion. Figure 1a clearly shows the high-contrast cores, surrounded by a less dense (gray colored) shells. This suggested formation of the polymer micelles with cores of PMA chains neutralized by Ca^{2+} and a PEO shell. The complex formation was completely abolished by adding an equimolar amount of a Ca^{2+} chelating agent, ethylenediaminetetraacetic acid (EDTA), into the reaction mixture.

At the second step, cross-linking of the core of the PEO-*b*-PMA/Ca^{2+} micelles was achieved using a condensation reaction between the carboxylic groups of PMA and the amine groups of 1,2-ethylenediamine in the presence of a water-soluble carbodiimide.[5] There was little change in the size of the particles in the reaction mixture; their diameter slightly increased to ca. 130 nm. This suggested that formation of cross-links was almost exclusively limited to intramicellar reactions. After completion of the reaction, Ca^{2+} ions and byproducts of the cross-linking reaction were removed by dialysis in the presence of EDTA. The resulting aqueous dispersion contained narrowly distributed particles of ca. 170 nm diameter with a net negative charge (ζ-potential, $\zeta = -19$ mV). The particles were stable and revealed no size change even upon a 100-fold dilution. They had a core-shell structure, as was confirmed using ^{1}H NMR spectroscopy (see Figure S1 in the Supporting Information). The ^{1}H NMR signals due to PMA chains (broad peaks at $\delta \sim 0.7-1.7$ for methacrylate backbone) were considerably attenuated for the cross-linked particles compared to the signals for the PEO-*b*-PMA copolymer dissolved in a 1:1 v/v D_2O/H_2O mixture. Similarly, these signals for PEO-*b*-PMA/Ca^{2+} micelles were also suppressed. For comparison, these signals were normalized with respect to the integral of the PEO signal (δ 3.7) for every sample. The normalized PMA signals for the cross-linked particles and PEO-*b*-PMA/Ca^{2+} micelles were decreased by 57 and 50%, respectively, compared to that of the free block copolymer, suggesting lower mobility and decreased solvation of the PMA chains. Clearly, for the PEO-*b*-PMA/Ca^{2+} micelles, this is consistent with the formation of a partially hydrated polyion-metal complex

Scheme 1. Synthesis of Polymer Micelles with Cross-Linked Ionic Cores



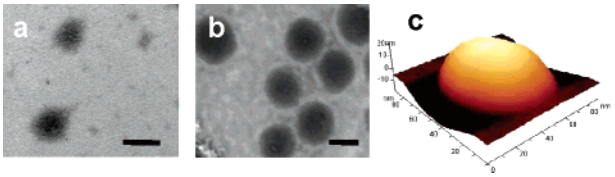


Figure 1. TEM images of (a) PEO-*b*-PMA/Ca^{2+} micelles and (b) cross-linked polymer micelles. Bar equals 100 nm. (c) Tapping-mode AFM image of a cross-linked polymer micelle in air.

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Table 1. Effect of pH on the Swelling Behavior of Cross-Linked PEO-*b*-PMA Micelles

pH	6.1	7.0	8.1	9.0
diameter, nm	169	192	230	288
ζ-potential, mV	−19.3	−27.6	−33.5	−36.8

core. Remarkably, after removal of the metal ions in the cross-linked particles, the degree of solvation and mobility of the chains remain reduced. This is in line with the core-shell architecture of cross-linked PEO-*b*-PMA micelles, that is, the ionic cross-linked PMA core with restricted mobility is surrounded by a flexible hydrophilic PEO shell.

As is seen from the TEM image obtained using uranyl acetate staining, the cross-linked micelles preserved the original spherical morphology (Figure 1b). The thickness of the PEO shell estimated from the TEM images was 7.82 ± 0.24 nm, which exceeds the theoretical value for the shell of ca. 6.2 nm calculated using a scaling scheme for the extension of the brush away from the surface.[6] This suggests that shell-forming PEO chains are essentially stretched. Atomic force microscopy (AFM) imaging provided further evidence that the cross-links within the core of the micelles stabilized their 3D structures (Figure 1c). An approximate aggregation number of 110 ± 15 copolymer chains per micelle was calculated based on the volumes determined from the analysis of the AFM images obtained in air. An equilibrium swelling ratio ($Q = V_s/V_d = 14.5$) of the cross-linked ionic core of the micelles was calculated using the volumes of the micelles in the dry (V_d) and swollen (V_s) states determined from AFM. The average number of the repeating units between the cross-links was ca. 85, as estimated using an approach for equilibrium swelling of a network in a good solvent.[7] This number is consistent with about two cross-links per each block copolymer chain in the micelle. Overall, these data suggested the formation of stable polymer micelles with cross-linked ionic cores.

The pH-induced dimensional changes of the cross-linked polymer micelles were studied by DLS (Table 1). The size and net negative charge of the cross-linked micelles increased considerably as the pH was increased. Such behavior was indicative of ionization and swelling of the network formed by the cross-linked PMA chains that compile the core of the micelle. It should be emphasized that the swelling of the micelles was completely reversible. Evidently, the protonation of the carboxylic groups upon lowering of the pH resulted in the collapse of the micelle core and a decrease in the micelle size. Furthermore, the size of the micelles was also affected by the ionic strength. Indeed, the diameters of the swollen micelles decreased from ca. 260 to ca. 210 nm as the concentration of the added salt increased from 10 to 100 mM NaCl. These data suggest that the changes in the size of the micelles are mainly governed by the decrease in gel osmotic pressure of the cross-linked ionic cores. Therefore, this micelle displayed the pH- and ionic strength-responsive hydrogel-like behavior due to the effect of the cross-linked ionic core. Such behavior may be instrumental for the design of drug carriers with controlled loading and release characteristics.

Cisplatin, a potent anticancer drug, was immobilized in the cross-linked polymer micelles by simple mixing with aqueous dispersion of polymer micelles at pH 9.[8] On the basis of the titration of unbound cisplatin using *o*-phenylenediamine as a ligand for Pt(II),[9] ca. 55% of the initial drug load was incorporated into the micelles. The density of the incorporated cisplatin in the polymer micelles allowed direct visualizing of them by TEM (a typical image obtained without negative staining is presented in Figure S2 in the Supporting Information). The net negative charge of the micelles was decreased upon their loading with cisplatin, suggesting the neutralization of the PMA segments by the added drug. This was also accompanied by a decrease of the particle size from ca. 290 to ca. 125 nm, presumably due to contraction of the cross-linked PMA core. Significantly, the drug-loaded micelles were stable in aqueous dispersions, exhibiting no aggregation or precipitation for months.

The release of the Pt(II) complexes from the polymer micelles in phosphate-buffered saline (PBS, pH 7.4, 0.14 M NaCl) was evaluated by equilibrium dialysis. About 40% of the loaded Pt(II) was released from the micelles during the first 20 h, and no burst release phase was observed. The release of the drug was accompanied with an increase of the particle size from ca. 125 to ca. 230 nm. The observed release characteristics are likely to be beneficial from the standpoint of the chemotherapeutic drug delivery in the body. By preventing the premature drug release, the micelles with the drug will be delivered in the body to the tumor, avoiding premature drug clearance. Furthermore, since PEO-coated nanoparticles avoid renal excretion,[10] we anticipate that an unwanted side effect of free cisplatin, namely, nephrotoxicity, can be also suppressed.

Thus, a new type of functional nanosystem—polymer micelles with cross-linked ionic cores—was developed using block ionomer complexes as a micellar template. The resulting micelles are hydrophilic nanospheres, which combine several key structural features that make these systems very beneficial for effective drug delivery. These are a cross-linked ionic core, a hydrophilic PEO shell, and nanoscale size. The ionic character of the core allows the encapsulation of charged therapeutic or diagnostic molecules, while the cross-linking of the core will suppress dissociation of the micelle upon dilution. Such materials are promising for further fundamental material studies and practical applications in pharmaceutical sciences.

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Supporting Information Available: Materials and Methods section; ^{1}H NMR spectra (Figure S1); TEM micrograph of cross-linked micelles loaded with cisplatin (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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