Communications to the Editor

Spontaneous Formation of Vesicles from Complexes of Block Ionomers and Surfactants

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We have shown that the complexes formed in aqueous solutions by block copolymers containing ionic and nonionic water-soluble segments (block ionomers) and oppositely charged single-tail surfactants spontaneously arrange in small vesicles. This structure is unprecedented for polymer−surfactant complexes. It was observed for a variety of cationic surfactants, differing in the length of the aliphatic radical and size of the headgroup. The formation of vesicles by the block ionomer complexes demonstrates that the opportunities for the control of morphology in these systems are much broader than has been previously considered. The unique self-assembly behavior, the simplicity of the preparation, and the wide variety of available surfactant components make these systems promising in addressing various theoretical and practical problems, particularly in pharmaceutics, where block copolymers and polyelectrolyte complexes are already being intensively investigated as drug and gene delivery systems.[1,2]

The diblock copolymer of poly(ethylene oxide) and poly(sodium methacrylate) (PEO-*b*-PMANa) was reacted with various single-tail cationic surfactants.[3] The resulting solutions are either optically transparent or slightly opalescent for all of the systems studied. This is in marked contrast to the mixtures of homopolymer PMANa and cationic surfactants, which precipitate under the same conditions.

The complex particles are negatively charged for compositions containing the block ionomer in excess.[4] The addition of increasing amounts of surfactant results in the ζ-potential increase due to the neutralization of the anionic units of the block ionomer by the surfactant cations. For all of the systems studied, at the equivalency of the carboxylate group and surfactant concentrations, the particle net charge equals zero (i.e., the complexes are stoichiometric). Diameters of the stoichiometric complex particles range from 85 to 120 nm (Table 1). Subsequent measurements with these samples show no change in ζ-potential and size of the complexes for at least several weeks. Since these systems are quite stable in solution and have sizes close to those of block copolymer micelles,[5] they appear to be micelle-like aggregates in which insoluble polyion−surfactant parts are stabilized in aqueous media by PEO chains.

Table 1. Effective Diameters of the Stoichiometric Complexes Formed between PEO-*b*-PMANa and Cationic Surfactants[15]

surfactant	diameter, nm	surfactant	diameter, nm
DDTAB	94	C_{12}PyCl	85
TDTAB	96	C_{16}PyBr	96
CTAB	125		

The morphology of these aggregates was investigated by electron microscopy using negative staining and freeze fracture techniques.[6] The images are very similar for all block ionomer and surfactant pairs. The complex particles are spherical as it is seen in typical micrographs presented in Figure 1. Images obtained using uranyl acetate staining are characteristic of those commonly observed with small phospholipid vesicles (Figure 1a). Vesicular structures are also clearly seen in freeze fracture electron micrographs (Figure 1b). When negative staining with ammonium molybdate is used, the "bagel"-like structures are formed (Figure 1c). Similar structures have been previously reported from unilamellar phosphatidylcholine/phosphatidic acid liposomes stained with ammonium molybdate[7] and are attributable to collapsed or flattened vesicles.

Fluorescent dye entrapment experiments using 5,6-carboxyfluorescein (CF) and calcein suggest that the particles of the block ionomer complexes efficiently encapsulate and retain hydrophilic molecules. For instance, Figure 2 shows the release of CF from the vesicles after their disintegration with Triton X-100.[8] The

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(1) Kwon, G.S.; Kataoka, K. *Adv. Drug. Deliv. Rev.* **1995**, *16*, 295−309.
(2) Kabanov, A. V.; Felgner, P. L.; Seymour, L. W., Eds.; *Self-assembling complexes for gene delivery. From laboratory to clinical trial*. John Wiley & Sons: New York, 1998.

(3) PEO-*b*-PMANa was synthesized by anionic polymerization as described before (Kabanov, A. V.; Bronich, T. K.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *Macromolecules* **1996**, *29*, 6797−6802). The segment lengths were 176 and 186 repeating units for PEO and PMANa, respectively. The following surfactants were used: dodecyltrimethylammonium bromide (DDTAB), tetradecyltrimethylammonium bromide (TDTAB), cetyltrimethylammonium bromide (CTAB), dodecylpyridinium chloride (C_{12}PyCl), and cetylpyridinium bromide (C_{16}PyBr). The complexes were prepared by mixing PEO-*b*-PMANa and surfactant aqueous solutions at room temperature in the absence of added electrolyte. In all cases, the final concentration of the PEO-*b*-PMANa carboxylate groups was 0.6 mM, the [surfactant]/[carboxylate group] ratio varied from 0.1 to 1.0, pH 8.4 to 8.6. No additional mechanical agitation commonly used for vesicle preparation was applied.

(4) Particle ζ-potential was measured as previously described (Bronich, T. K.; Kabanov, A. V.; Kabanov, V. A.; Yu, K.; Eisenberg, A. *Macromolecules* **1997**, *30*, 3519−3525).

(5) Khougaz, K.; Astafieva, I.; Eisenberg, A. *Macromolecules* **1995**, *28*, 7135−7147.

(6) For the negative staining experiment, a drop of sample solution was settled on a Formvar-coated EM grid for 1 min. Excess sample was wicked away with filter paper, and a drop of staining solution (aqueous 1% uranyl acetate or 1% ammonium molybdate) was allowed to contact the sample for 1 min. The samples were analyzed using a Philips 410 TEM microscope. For freeze fracture, samples were loaded in winged double replica holders and frozen by plunging into liquid propane. After freezing, the samples were fractured in a Balzers 301 freeze fracture apparatus at −110 °C, followed by platinum/carbon replication. Replicas were washed in 1:1:1 mixture of acetic: nitric:sulfuric acids, rinsed in distilled water, and picked up on 200-mesh copper grids. The images of the replicas were recorded using a Hitachi H-600 TEM microscope.

(7) Johnson, S. M.; Bangham, A. D.; Hill, M. W.; Korn, E. D. *Biochim. Biophys. Acta* **1971**, *233*, 820−825.

(8) Stoichiometric complexes prepared in the presence of the dye (10 mM CF or 0.1 mM calcein) were separated from nonentrapped dye by gel penetration chromatography on Sephadex G-25 equilibrated with 0.01 mM solutions of the surfactant. The vesicles disintegrated in the presence of Triton X-100 (0.16%) as shown by photon correlation spectroscopy. This was accompanied by the release of CF in the external solution as evidenced by an increase in fluorescence resulting from the dye dilution (de la Maza, A.; Parra, J. L.; Leal, J. S. *Langmuir* **1992**, *8*, 2422−2426). In the calcein experiment, addition of $CoCl_2$ to the calcein-containing vesicles resulted in quenching of the dye fluorescence ($\lambda_{ex} = 490$ nm and $\lambda_{em} = 520$ nm) in the external solution by Co^{2+} ions (Oku, N.; Kendall, D. A.; Macdonald, R. C. *Biochim. Biophys. Acta* **1982**, *691*, 332−340). The residual fluorescence corresponding to calcein entrapped in the vesicles (2% of the initial value) quenched after vesicle disintegration by Triton X-100.

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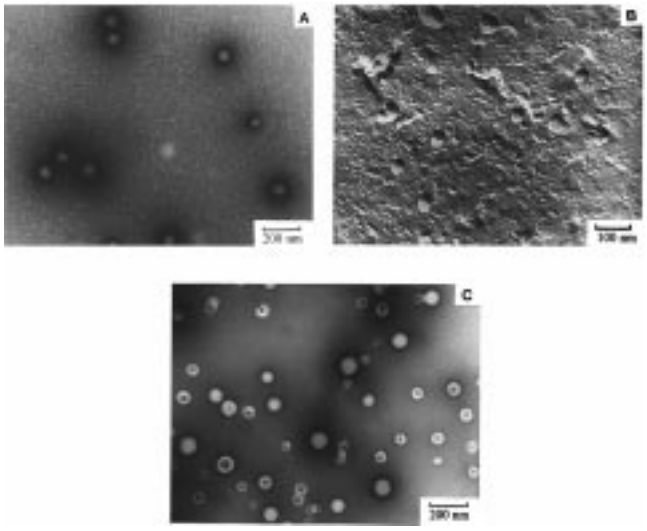

Figure 1. Microphotographs of stoichiometric PEO-*b*-PMANa and C_{16}PyBr complexes ([COONa] = [C_{16}PyBr] = 0.6 mM) obtained using (A) uranyl acetate staining, (B) freeze fracture, and (C) ammonium molybdate staining. Same morphology was observed with all pairs of block ionomer and surfactants listed in Table 1.

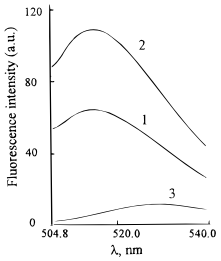

Figure 2. Fluorescence emission spectra of CF (λ_{ex} = 495 nm) in PEO-*b*-PMANa and C_{16}PyBr vesicles (1), after vesicle disintegration with Triton X-100 (2), and in C_{16}PyBr (0.8 mM) micelles (3).

maximum of the fluorescence emission of the vesicle-entrapped CF is observed at the same wavelength (517.2 nm) as that of the free dye in aqueous solution. In contrast, there is a significant shift in emission maximum for CF solubilized in the cationic surfactant micelles (528.4 nm). This suggests that CF in the vesicles localizes in an aqueous environment rather than in the hydrophobic domains formed by the surfactant radicals. The experiments using calcein provide further evidence of formation of the vesicles with internal aqueous volume.[8]

Vesicles from amphiphilic diblock copolymers of polystyrene and poly(acrylic acid) (PS-*b*-PAA) have recently been described.[9] The PS segments formed the walls in such vesicles, and the wall thickness varied from 18 to 22 nm, depending on the PS block length among other factors. The impact of the hydrophilic PAA shell on the vesicle wall thickness was negligible (0.3 nm) because the PAA chains were very short. In the case of the block ionomer complexes, the length of the hydrophobic segments was comparable with those of the PS segments, while hydrophilic PEO segments were significantly longer than the PAA segments (180 vs 8). Nevertheless, the thickness of the walls of the block ionomer vesicles was only 12.5 ± 0.03 nm, which is evidently due to the different structure of the wall compared to that in the PS-*b*-PAA vesicles.[10] It is known that the period of the lamellar structures formed by swollen polyelectrolyte−surfactant complexes is about 4 nm.[11] On the other hand, the thickness of the PEO shell attached to lipid bilayers in Stealth liposomes approximate 4 nm.[12] It is likely that the block ionomer vesicles are composed of bilayers from polymethacrylate anion-bound surfactant with a shell from "grafted" PEO chains stabilizing the complexes in aqueous media. The estimated thickness of such structure is ca. 12 nm, which is consistent with the experimental value.

In conclusion, the block ionomer−surfactant complexes belong to a novel class of polymer materials, exhibiting combined properties of block copolymers and polyelectrolyte complexes. In such complexes, the surfactant ionic headgroups bind to the oppositely charged units of polyion segment, while the surfactant tails segregate into hydrophobic domains.[4] In contrast to regular polymer−surfactant complexes which precipitate,[11] these materials spontaneously form small vesicles of remarkably low polydispersity. The vesicles are formed under conditions of electroneutrality, a situation which is significantly different when compared to that of vesicles from single-tailed cationic and anionic surfactants, where an excess of one of the components is required.[13] Vesicle formation in the present block ionomer system most probably is a result of steric repulsion and the lyophilizing effects of PEO chains preventing stacking of individual lamellae and of closing of the dispersed lamellae into spheres to avoid contact of the edges with water. This self-assembly mechanism is very different from that involved in vesicle formation from most other material. This includes most Stealth liposomes, in which the steric repulsion is normally introduced after the vesicles have been formed or which require additional energy input. This work suggests that the vesicle morphology in the block ionomer complexes is thermodynamically stable. There are only a few studies in which spontaneous formation of PEO-grafted liposomes from double-tail amphiphiles has been observed,[14] and therefore, the present observation of spontaneous vesicle formation in a completely different system is of both theoretical and experimental significance.

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(9) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728−1731.

(10) The wall thickness was determined using 46 measurements randomly collected from four ammonium molybdate TEM images using the Image Pro Plus program (Media Cybernetics).

(11) In the solid state, regular complexes organize into lamellae consisting of alternating layers of polymer chains separated by layers of surfactant molecules (Khandurina, Yu. V.; Dembo, A. T.; Rogacheva, V. B.; Zezin, A. B.; Kabanov, V. A. *Polym. Sci. U.S.S.R.* **1994**, *36*, 189−194.).

(12) McIntoch, T. J.; Kenworthy, A. K.; Needham, D. *Stealth Liposomes*, Lasic, D., Martin, F., Eds.; CRS Press: Boca Raton, 1995; pp 63−71. The thickness of PEO layers in Stealth liposomes was compared at PEO:lipid molar ratio ca. 1% which is close to the PEO:surfactant ratio in the studied complexes.

(13) Kaler, E. W.; Murthy, A. K.; Rodriguez, B. E.; Zasadzinski, J. A. *Science* **1989**, *245*, 1371−1374.

(14) Szleifer, I.; Gerasimov, O. V.; Thompson, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 1032−1037.

(15) Effective diameters were determined by photon correlation spectroscopy using a "ZetaPlus" ζ-potential analyzer (Brookhaven Instrument Co.) equipped with the multiangle option. The sizing measurements were performed in aqueous solutions ([COONa] = [surfactant] = 0.6 mM) at 25 °C at a detection angle of 90°. Polydispersity indexes were less than 0.1, indicating narrow size distribution.