Transmission Electron Microscopic Study of Cross-Sectional Morphologies of Core−Corona Polymeric Nanospheres†

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ABSTRACT: Ultrathin cross sections of core−corona polymeric nanospheres, which were prepared by the free-radical polymerization of methacrylate-terminated poly(ethylene glycol) (MA-PEG) as a hydrophilic macromonomer and styrene (St) as a hydrophobic comonomer in a ethanol/water (4/1, v/v) mixed solvent, were observed by using transmission electron microscopy (TEM). Conditions of the preparation of nanosphere-embedded resins, the cutting rate by a microtome for cross-section preparation, and staining with osmium tetraoxide were optimized in order to analyze cross-sectional morphologies of the nanospheres clearly. Nanospheres prepared showed a core−corona structure in all cases. The corona layer thickness was constant at around 6 nm when we used the same macromonomer (M_n 996), even if we altered the size in the nanosphere to 133, 272, and 1530 nm by altering the monomer ratio (St/MA-PEG 20/1, 50/1, and 200/1, respectively, mol/mol). The thickness increased from 6.7 to 17 nm with an increase in the molecular weight of a macromonomer from M_n 996 to 4250, although the size decreased from 373 to 204 nm with an increase in it. TEM observations in the present study led us to have further structural understanding of core−corona nanospheres that had various technological and biomedical applications.

Introduction

Amphiphilic polymers with different surface free energies self-assemble in an aqueous phase in order to minimize an interfacial free energy, forming various morphologies such as micelles, rods, fibers, and network structures.[1] We have also studied a unique self-assembling system of amphiphilic graft polymers. During the polymerization of hydrophilic macromonomers with hydrophobic comonomers in a polar solvent in the presence of a radical initiator, generated amphiphilic copolymers self-assembled in order to form core−corona polymeric nanospheres possibly with a hydrophobic core and a hydrophilic graft polymer layer on their surfaces.[2] The size in nanospheres, the molecular weight of graft polymers, and the conversion of polymerization increased with an increase in reaction time, indicating the in-situ self-assembling process for the nanosphere formation. The nanospheres were dispersed well in an aqueous phase even at ambient temperature. An electron spectroscopy for chemical analysis (ESCA)[2] and a dynamic light scattering (DLS)[3] supported accumulation of a macromonomer component at the surface of nanospheres. From those results, we supposed the core−corona type cross-sectional morphology of the nanospheres, as is schematically shown in Figure 1. Other research groups have also studied the nanosphere preparation by a similar methodology and supposed a similar structure.[4] However, there is no research on direct observation of cross-sectional morphologies of the nanospheres.

Core−corona nanospheres have been utilized to various technological and biomedical applications, because of their possible diversity of chemical structure. Metal nanoparticles, such as silver and platinum, were stably deposited in a poly(*N*-isopropylacrylamide) corona layer of a polystyrene core nanosphere due to steric stabilization,[5] and a thermosensitive catalytic activity was performed through the hydrogenation of allyl alcohol.[5c] Sugar-binding proteins lectins were covalently conjugated to a poly(methacrylic acid) corona of the same coretype in order to capture HIV-1 gp120 and virions by the conjugated nanosphere from the aqueous phase.[6] Lactose was covalently bound to a poly(vinylamine) corona of the same coretype in order to be recognized by a lectin.[7] Nanospheres with various chemical structures were utilized as drug carriers for peptide drugs physically adsorbed on the nanosphere surfaces.[8] Although a polystyrene core−poly(vinylamine) corona nanosphere was assembled on a certain substrate, the nanosphere with a positive ζ potential hardly absorbed onto the surface of a negatively charged film.[9] In the above significant applications of core−corona nanospheres, the surface layer structures that correspond to coronas, have key roles for nanosphere functions. If we observe cross-sectional morphologies of nanospheres, further understanding of the functions will be needed. In addition, further applications might be induced from their morphologies.

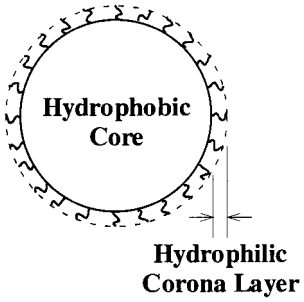

Figure 1. A schematic representation of a core−corona polymeric nanosphere.

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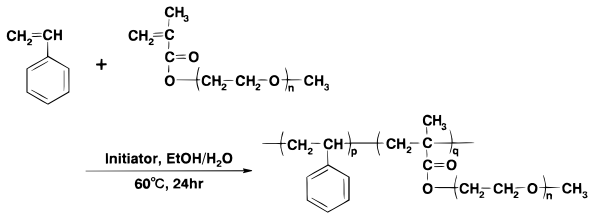
Scheme 1



Several researchers have already observed cross sections of a core−shell latex prepared by the seed polymerization, which was stained with a suitable reagent, by using transmission electron microscopy (TEM), and have showed ideal core−shell or heterogeneous structures.[10] The methodology seems to be significant in order to analyze real internal and/or surface structures of polymeric particles.

In this paper, we studied cross-sectional morphologies of polystyrene core−poly(ethylene glycol) (PEG) corona nanospheres as model ones[2d−f,j,3] with ultrathin cross sections embedded in a resin by using TEM. The present methodology and observations will be significantly applied to other types of core−corona nanospheres.

Experimental Section

Materials. Styrene (Wako Pure Chemical Ind., Japan) was distilled under reduced pressure in nitrogen. Methacrylate-terminated poly(ethylene glycol) (MA-PEG) macromonomers were kindly donated by Nippon Oil and Fats Co. (Tokyo, Japan). 2,2′-Azobis(*N*,*N*-dimethyleneisobutyramide) (VA-061) (Wako Pure Chemical Ind., Japan) was used without further purification. 2,2′-Azobis(isobutyronitrile) (AIBN) (Wako Pure Chemical Ind., Japan) was recrystallized from methanol and dried in a vacuum. Potassium peroxodisulfate (KPS) (Wako Pure Chemical Ind., Japan) was used without further purification. Ethanol was distilled before use. Milli-Q Labo provided ultrapure distilled water. A dialysis tube with the cutoff molar mass of 12 000−14 000 (Wako Pure Chemical Ind., Japan) was used after rinsing with pure water. Epok 812 (Ohken, Japan), dodecyl succinic anhydride (DDSA) (Ohken, Japan), methyl nadic anhydride (MNA) (Ohken, Japan), and tris(dimethylaminomethyl)phenol (DMP-30) (Ohken, Japan) were used by quick mixing before use. A 4% osmium tetraoxide aqueous solution (Funakoshi Co.) was used without further purification.

Nanosphere Preparation. Following our previous study,[2d−f,j] polystyrene core−PEG corona nanospheres were prepared by the free-radical copolymerization of styrene as a hydrophobic comonomer and a MA-PEG macromonomer in the presence of a radical initiator in an ethanol/water mixed solvent, as is shown in Scheme 1. Adequate amounts of styrene and a MA-PEG macromonomer were weighed into a glass tube with a suitable amount of VA-061 or AIBN (1 mol % to total monomers) and 5 mL of ethanol/water (4/1, v/v), then were degassed by freeze−thaw cycles on a vacuum apparatus, sealed off, and shook at 60 °C for 24 h. The nanosphere was purified by being dialyzed with a cellulose dialyzer for 1 day in methanol/water (1/1, v/v) and subsequently for 3 days in distilled water. The aqueous dispersion of the nanospheres obtained was lyophilized and weighed. A submicron particle analyzer (Coulter model N4SD) analyzed the size in the nanospheres. The polymerization conditions are listed in Tables 1 and 2. The nanosphere was also observed by TEM (Hitachi H700) after the nanosphere aqueous dispersion was cast on a copper mesh and dried for 12 h under reduced pressure.

To prepare a polystyrene latex particle without any coronas as a reference, the soap-free polymerization was performed as follows. Styrene and KPS (8×10^{-5} mol % to styrene) was added in distilled water and vigorously stirred at 70 °C for 7 h under a nitrogen atmosphere. The obtained particle dispersion was purified by being dialyzed for 3 days in distilled water.

TEM Observation of Cross Section. A mixture of Epok 812 (62 mL) and DDSA (100 mL) and another mixture of Epok 812 (100 mL) and MNA (89 mL) were named to A and B solutions, respectively. Adequate amounts of lyophilized nanospheres were quickly dispersed in a mixture of A and B solutions with given mixture ratios in the presence of DMP-30 as a polymerization accelerator (20 wt % to the total monomer) at ambient temperature. The dispersed solutions were hardened for 3−5 days at 30 °C. By setting the nanosphere-embedded resin to a microtome (LKB 8800 ULTROTOME III, Sweden) with a glass knife, we obtained ultrathin cross sections with less than around 0.1 μm thickness with a given cutting rate on water surface. The sections on the water surface were put on a copper mesh and dried under reduced pressure. The obtained sections were exposed to a vapor of osmium tetraoxide for a given time at a given temperature. The stained sections were observed by TEM (Hitachi H700) at an accelerate voltage of 200 kV. To measure a thickness of a corona layer from cross-sectional TEM images, the nanospheres having a similar size to normal TEM images (for intact nanospheres without cutting) were selected. For a TEM observation without staining, the nanosphere dispersion was cast on a copper mesh and dried under reduced pressure, and subsequently carbon was sputtered with 20−50 nm thickness.

Results and Discussion

Nanosphere Preparation. We have already analyzed the polymerization of wide varieties of hydrophilic macromonomers with styrene or methyl methacrylate as a hydrophobic comonomer, to prepare core−corona nanospheres with multiple functions on their surface.[2] In the present study, we selected a polystyrene core−PEG corona nanosphere with a simple chemical structure as a model one. On the basis of our previous study of the nanosphere, we synthesized them with different sizes from the same macromonomer or with different molecular weights of a grafted PEG on the nanosphere surface from the same monomer ratio.

The polymerization conditions and the characterization of the nanospheres obtained are listed in Tables 1 and 2. The size in the nanosphere increased with an increase and a decrease in the monomer ratio (St/MA-PEG) and molecular weight of MA-PEG, respectively, of which observations tended to be the same as our previous studies,[2m] indicating the nanospheres were successfully prepared in the present study. The nanospheres were utilized in order to analyze the effect of the nanosphere size and the chain length of a grafted PEG, respectively, on cross-sectional morphologies. Figure 2 shows a typical TEM observation of the nanosphere, of which the sample was normally prepared by casting of the nanosphere dispersion on a copper mesh. The nanosphere was spherical in form, and its distribution was significantly narrow. It should be noted that from the TEM image we could not recognize the internal structure of the nanosphere.

TEM Observation of Cross Section. Although there has been research on microscopic observations of cross sections of a core−shell latex,[10] conditions on a section preparation seem to be largely dependent on the nature of particles. Here, we optimized the mixture ratio of A and B solutions for a nanosphere embedding resin (see Experimental Section), a cutting rate by a microtome, and a staining condition with osmium tetraoxide for TEM observations.

We altered a mixture ratio of A and B solutions (A/B, v/v) to 4/6, 3/7, 2/8, and 1/9 and a cutting rate to 0.5, 1, 2, 5, 10, and 20 mm s^{-1}. The resin containing the nanosphere became hard with a decrease in the ratio.

Table 1. Copolymerization of MA-PEG (M_n 996) Macromonomer with Styrene[a]

	MA-PEG		St						
run	(mmol)	(mg)	(mmol)	(mg)	St/MA-PEG (mol/mol; g/g)	yield (%)	dm[b] (nm)	SD[c] (nm)	CV[d] (nm)
1	0.25	250	5	520	20/1; 2/1	85	133	16	12
2	0.10	100	5	520	50/1; 5/1	76	272	65	24
3	0.025	25	5	520	200/1; 20/1	72	1530	370	24

[a] Initiator: VA-061, 1 mol % to total monomer; polymerization time, 24 h; temperature, 60 °C. [b] dm = mean diameter. [c] SD = standard deviation. [d] CV = SD/dm, coefficient of variation.

Table 2. Copolymerization of MA-PEG Macromonomer with Styrene[a]

	MA-PEG			St						
run	M_n	(mmol)	(mg)	(mmol)	(mg)	St/MA-PEG (mol/mol; g/g)	yield (%)	dm[b] (nm)	SD[c] (nm)	CV[d] (nm)
1	996	0.088	87	8.8	913	100/1; 10/1	82	373	81	22
2	1740	0.082	140	8.2	860	100/1; 6/1	90	280	39	14
3	4250	0.068	290	6.8	710	100/1; 2.4/1	79	204	36	18

[a] Initiator: AIBN, 1 mol % to total monomer; polymerization time, 24 h; temperature, 60 °C. [b] dm = mean diameter. [c] SD = standard deviation. [d] CV = SD/dm, coefficient of variation.

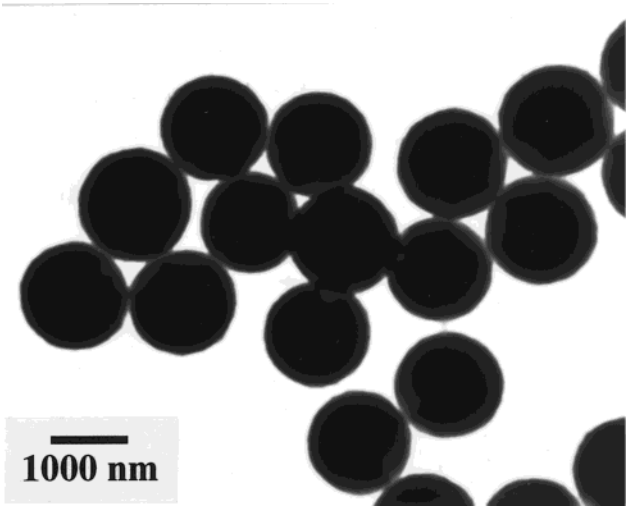


Figure 2. A TEM image of a styrene core−PEG corona nanosphere (run 3 in Table 1).

At more than a 3/7 ratio, the resins were too soft to obtain ultrathin sections at any rate. However, at less than 2/8, we successfully obtained ultrathin sections at the rate of 2 mm s^{-1} (see below and Figure 3a). At other cutting rates, the obtained sections were too thick to apply to TEM observations. At the rate of 20 mm s^{-1}, the nanosphere was surprisingly pulled out from the thick section. We found that the mixture ratio and the cutting rate were significant for preparation of ultrathin cross sections. As a consequence, we applied the ratio of 1/9 and the cutting rate of 2 mm s^{-1} to the following TEM observations.

We also altered a staining time to 5, 10, 20, 30, 45, and 60 min and a staining temperature to 20, 25, and 30 °C. At more than 20 min and 25 °C, we found enough to obtain a suitable contrast for TEM observation.

The TEM image significantly showed the nanosphere was covered with a well-stained thin layer, as is shown in Figure 3a, possibly indicating a core−corona structure of the nanosphere. In this case, the size distribution appeared to be larger than that in Figure 2. This is reasonable if we consider that the nanospheres in the resin were not placed at the same plane. On the other hand, we hardly observed such a structure without staining, although we still observed a halo, as is shown in Figure 3b. Furthermore, when we similarly prepared a cross section of a polystyrene latex that was prepared by the soap-free polymerization (see Experimental Section), we could not observe any core−corona structure, as is shown in Figure 3c. It is well-known that OsO_4 preferentially reacts with poly(diene)s, amines, hydroxyl groups, and ethers by coordinating interaction.[11] In addition, we found that a PEG solid was more strongly stained by osmium tetraoxide than a polystyrene one, possibly by easy binding to ether bonds in PEG of osmium tetraoxide. From the observations, we may say the nanosphere, which was prepared by the free-radical copolymerization of an MA-PEG macromonomer with styrene, has a polystyrene core−PEG corona structure. The nanosphere was slightly collapsed to the direction of cutting by a microtome, which resulted in an elliptical form. The quantitative analysis of a corona layer thickness in terms of a macromonomer length will be discussed in the following sections.

The corona layer on the nanospheres must be significant to induce wide varieties of functions. Metal nanoparticles seem to be sterically stabilized in the corona layer without aggregation.[5] Proteins seem to be stably conjugated in the corona layer without direct contact with a polystyrene core.[6,8] A stable dispersity of the nanosphere seems to come from the core−corona structure. On the other hand, when we prepared the nanospheres from styrene and poly(*N*-isopropylacrylamide)[2i,l] or poly(*N*-vinylisobutyramide)[2k] macromonomers with a lower critical solution temperature (LCST), the sizes in the nanospheres became small above LCST due to shrinkage of a corona layer. However, the size change was much larger than a length of the macormonomer with full extension. If the thermosensitive nanospheres have the core−corona structures similar to those presented here, the larger change might mean an induced shrinkage of a styrene core by shrinkage of a corona layer. To discuss details, further research will be needed. In next sections, we will analyze in more detail structures of core−corona nanospheres.

Particle Size Effect. We could control a particle size of the nanosphere by altering the monomer ratio between styrene and MA-PEG with the same molecular weight. Here, we observed cross sections of the nanospheres by TEM, to analyze the particle size effect on the core−corona structure.

Figure 4 shows the TEM images of the nanospheres with different sizes. In all cases, the nanospheres significantly showed a core−corona structure, indicating the structure formation was independent of a particle

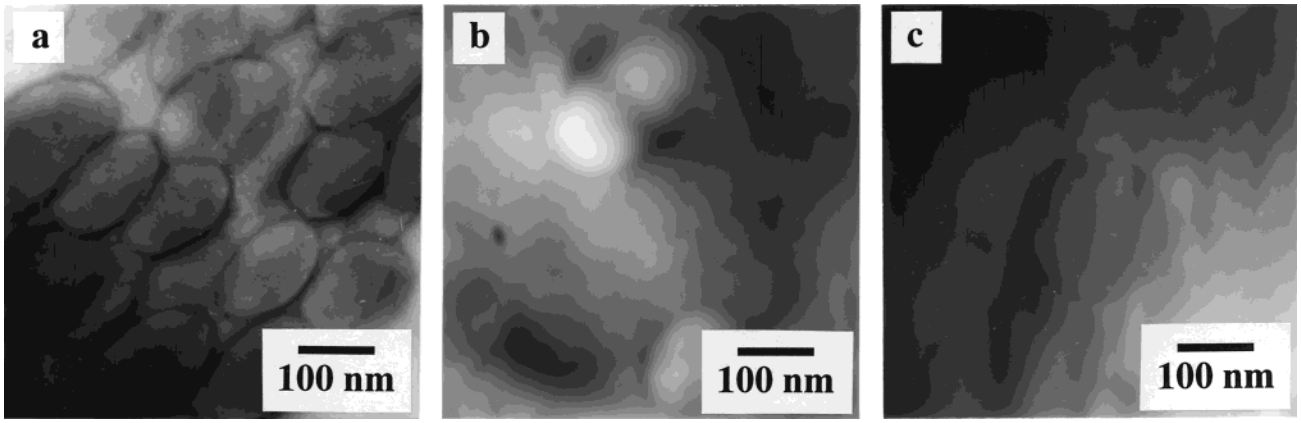


Figure 3. TEM images of ultrathin cross sections: (a) a core−corona nanosphere (run 2 in Table 1) stained with osmium tetraoxide; (b) the same nanosphere without staining; (c) a polystyrene latex stained.

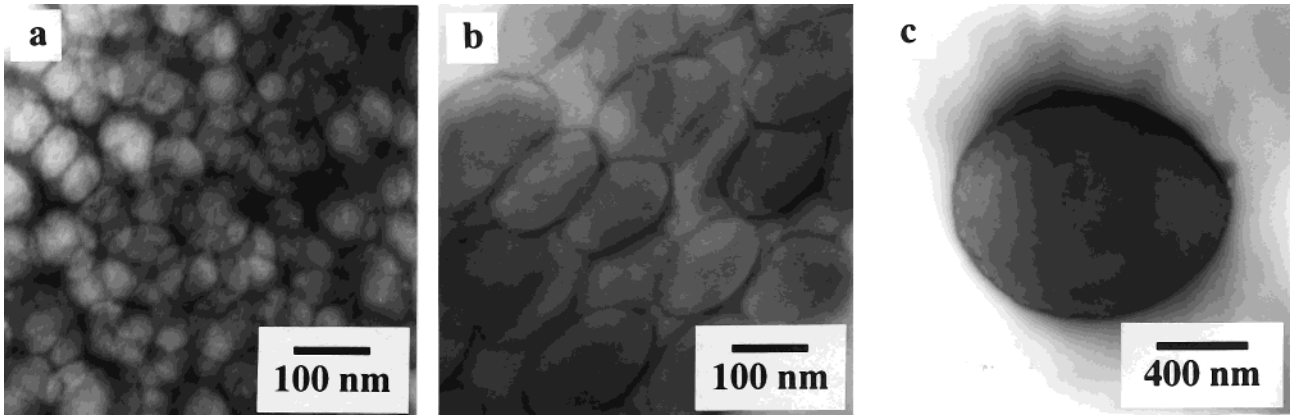


Figure 4. TEM images of ultrathin cross sections of a core−corona nanosphere stained with osmium tetraoxide: (a) run 1, (b) run 2, and (c) run 3 in Table 1.

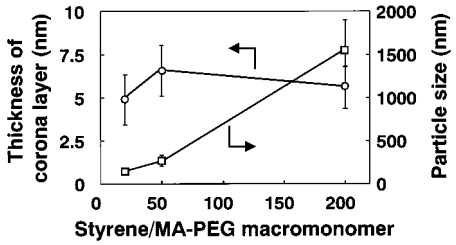


Figure 5. Dependence of the corona layer thickness and the particle size of the nanospheres in Table 1 on the monomer ratio.

size. To analyze the corona layer thickness on the nanospheres, we measured the thickness from the TEM images. We randomly selected the layer at a long axis for the ellipse nanosphere in the image and estimated the mean corona thickness; otherwise, the thickness was a large experimental error because the border between PEG and polystyrene layers at a short axis was sometimes broadened in order to be recognized well. Furthermore, we selected the nanosphere as large as possible because of an adequate estimation of the corona layer thickness. (A cross section should include a central position of the nanosphere.) Figure 5 shows the dependence of the layer thickness and the particle size on the monomer ratio. The thickness was independent of the ratio and was constant at around 6 nm, although the size increased with an increase in the ratio. This means that we will obtain the nanosphere with the constant corona layer thickness when we use a macromonomer with the same molecular weight, even if we alter the monomer ratio. We found the nanospheres even with different sizes, which were prepared from the same macromonomer, had the same corona layer thickness.

We estimated the length of the macromonomer used here with full extension from its molecular weight to be 6.7 nm, assuming the length of one ethylene glycol unit to be 0.33 nm. The thickness of the corona layer obtained from TEM images seems to be reasonable. We may say that PEG is assembled on the nanosphere with almost full extension.

Macromonomer Length Effect. We could synthesize styrene core−PEG corona nanospheres from macromonomers with different molecular weights. Here, we analyzed the molecular weight effect on a core−corona structure by TEM. As the core−corona structures were not affected by initiator species, we utilized here a series of nanospheres prepared in the presence of AIBN instead of VA-061 described above.

Figure 6 shows the TEM images of the nanospheres prepared from different macromonomers with the same monomer ratio. In all cases, the nanospheres significantly showed a core−corona structure, indicating the structure formation was independent of a molecular weight of macromonomers. To analyze details, we also estimated the layer thickness from the images by the similar procedure described in the former section. Figure 7 shows the dependence of the thickness and the particle size on the molecular weight. The thickness linearly increased with an increase in the molecular weight, although the size in the nanosphere decreased. The thickness for M_n 996, 1740, and 4250 were 6.6, 9.2, and 17 nm, respectively. We found the corona layer thickness is dependent on the molecular weight. On the

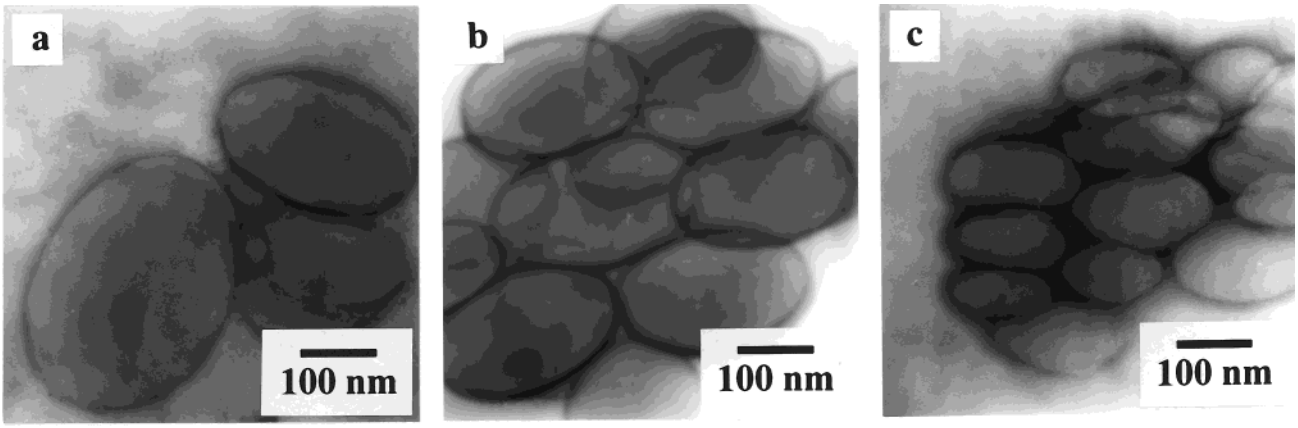


Figure 6. TEM images of ultrathin cross sections of a core−corona nanosphere stained with osmium tetraoxide: (a) run 1, (b) run 2, and (c) run 3 in Table 2.

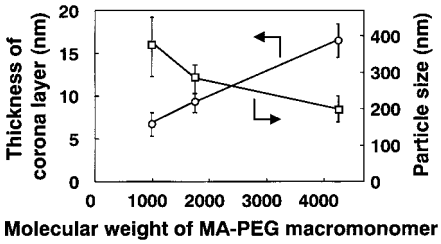


Figure 7. Dependence of the corona layer thickness and the particle size of the nanospheres in Table 2 on the monomer ratio.

other hand, a series of nanospheres in this section were prepared by AIBN as a hydrophobic radical initiator, while in the former section we used a hydrophilic KPS. We did not observe any difference in the core−corona structure and in the layer thickness with the same macromonomer. This means a radical initiator does not affect the core−corona structure and corona layer thickness.

The full lengths of the macromonomers for M_n 996, 1740, and 4250 were also estimated to be 6.7, 12, and 31 nm, respectively. The corona layer thickness with M_n 996 was almost the same to the full length, while the thickness for other larger M_n became smaller than the full length. Especially, the thickness with M_n 4250 was around half the value of the full length. This might mean the polymers in the corona layer tend to shrink more on the nanosphere surface with an increase in the molecular weight. As a consequence, we found the nanosphere prepared from macromonomers with different molecular weights also showed a core−corona structure, and the corona thickness increased with an increase in a molecular weight of macromonomers.

Conclusion

We observed ultrathin cross sections of polymeric nanospheres, which were prepared by the free-radical polymerization of a hydrophilic macromonomer and a hydrophobic comonomer in a polar solvent, by using TEM. We optimized conditions for the preparation of nanosphere-embedded resins, a cutting rate by a microtome for cross-section preparation, and staining with osmium tetraoxide. Nanospheres prepared showed a core−corona structure in all cases, supporting a structural model deprived from DLS measurement.[3] The corona layer thickness was constant when we used a macromonomer with the same molecular weight, even if we altered the size in the nanosphere by altering the monomer ratio. The thickness increased with an increase in the molecular weight of a macromonomer, although the size decreased. The core−corona structure presented here must be significant for possible applications of the nanospheres. Further research on TEM observations of other nanospheres will lead us to a detailed understanding of functions of core−corona nanospheres.

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