Triblock Nanospheres and Their Use as Templates for Inorganic Nanoparticle Preparation

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Received January 31, 2000. Revised Manuscript Received April 26, 2000

A polyisoprene-*block*-poly(2-cinnamoylethyl methacrylate)-*block*-poly(*tert*-butyl acrylate), PI-*b*-PCEMA-*b*-P*t*BA, sample with 370 isoprene, 420 CEMA, and 550 *t*BA repeat units forms spherical micelles in tetrahydrofuran (THF)/hexanes (HX) with 65% volume fraction of HX. The micelles consist of a PI corona, a solvent-insoluble PCEMA shell, and P*t*BA core. Their structure is locked in by photo-cross-linking the PCEMA shell to yield nanospheres. The nanospheres were made water-dispersible by hydroxylating the PI double bonds. The core was made compatible with inorganic species by removing the *tert*-butyl groups of P*t*BA. The feasibility of using such nanospheres as templates for inorganic nanoparticle preparation was demonstrated by incorporating iron oxide magnetic particles into the cores.

I. Introduction

We report, in this paper, the preparation of water-soluble triblock nanospheres and their use as templates for preparing iron oxide magnetic particles. Such nanospheres are derived, following Scheme 1, from spherical micelles of polyisoprene-*block*-poly(2-cinnamoylethyl methacrylate)-*block*-poly(*tert*-butyl acrylate), PI-*b*-PCEMA-*b*-P*t*BA (Chart 1), with $n = 370$, $m = 420$, and $l = 550$. The spherical micelles with soluble PI coronas, insoluble PCEMA shells and P*t*BA cores were prepared in THF/HX with a HX volume fraction of 65%.

This research is novel in several aspects. First, the water-dispersible nanospheres with a hydrophilic core and corona and a cross-linked hydrophobic shell are structurally new and have never been prepared previously. Their relatives would be diblock vesicles prepared and observed by Zhang and Eisenberg,[1] by us,[2] by Jenekhe et al.,[3] and by Discher et al.[4] Alternatively, they may be viewed as relatives of the zwitterionic shell-cross-linked micelles prepared by Butun et al.[5] More closely they resemble cross-linked diblock vesicles prepared by us.[6,7] They are different from the cross-linked vesicles because a vesicle contains a polymer-free solvent pool in the center. The vesicles also have a much larger core and wider core size distribution.

Second, the study of ABC triblock copolymer micelles is still relatively new. Despite many reports on diblock copolymer micelle formation in block-selective solvents[8] and those on the ABC triblock morphologies in bulk,[9] there have been only several experimental studies of triblock micelles in block-selective solvent.[10,11]

Third, the triblock copolymer nanospheres should be useful as nanoreactors or templates for making inorganic nanoparticles including metals, semiconductors, or inorganic oxides. This potential has been demonstrated by forming cubic γ-Fe_2O_3 nanoparticles in the cores. There have been many reports on the preparation of inorganic nanoparticles in the core of diblock micelles,[12,13] in diblock solids,[14] in the water pool of reverse micelles,[15] or vesicles.[16] The mesophasic structures of diblocks have also been used as templates for the

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[1] (a) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728. (b) Zhang, L.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777.

[2] (a) Ding, J.; Liu, G. *Macromolecules* **1997**, *30*, 2408. (b) Ding, J.; Liu, G.; Yang, M. *Polymer* **1997**, *38*, 5497.

[3] Jenekhe, S. A.; Chen, X. L. *Science* **1998**, *279*, 1903.

[4] Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C.-M.; Bates, F. S.; Discher, D. E.; Hammer, D. A. *Science* **1999**, *284*, 1143.

[5] Butun, V.; Lowe, A. B.; Billingham, N. C.; Armes, S. P. *J. Am. Chem. Soc.* **1999**, *121*, 4288.

[6] Ding, J.; Liu, G. *J. Phys. Chem.* **1998**, *102*, 6107.

[7] Ding, J. Liu, G. *Chem. Mater.* **1998**, *10*, 537.

[8] For reviews see, for example: (a) Tuzar, Z.; Kratochvil, P. *Surf. Colloid Sci.* **1993**, *15*, 1. (b) Brown, R.; Masters, A. J. Price, C.; Yuan, X. F. In *Comprehensive Polymer Science*; Allen, G., Bevington, J. C., Booth, C., Price, C., Eds.; Pergamon Press: Oxford, 1989; Vol. 2. (c) Liu, G. *Curr. Opinion Colloid Interface Sci.* **1998**, *3*, 200.

[9] See, for example: (a) Breiner, U.; Krappe, U.; Thomas, E. L.; Stadler, R. *Macromolecules* **1998**, *31*, 135. (b) Zheng, W.; Wang, Z.-G. *Macromolecules* **1995**, *28*, 7215.

[10] (a) Patrickios, C. S.; Forder, C.; Armes, S. P.; Billingham, N. C. *J. Polym. Sci., Part A: Polym. Chem.* **1997**, *35*, 1181. (b) Kriz, J. Massar, B.; Plestil, J.; Tuzar, Z.; Pospisil, H.; Doskociloca, D. *Macromolecules* **1998**, *31*, 41. (c) Chen, W. Y.; Alexandridis, P.; Su, C. K.; Patrickios, C. S.; Hertler, W. R.; Hatton, T. A. *Macromolecules* **1995**, *28*, 8604. (d) Yu, G.-E.; Eisenberg, A. *Macromolecules* **1998**, *31*, 5546.

[11] Stewart, S.; Liu, G. *Chem. Mater.* **1999**, *11*, 1048.

[12] (a) Seregina, M. V.; Bronstein, L. M.; Platonova, O. A.; Chernyshov, D. M.; Valetsky, P. M.; Hartmann, J.; Wenz, E.; Antonietti, M. *Chem. Mater.* **1997**, *9*, 923. (b) Klingelhofer, S.; Heitz, W.; Greiner, A.; Oestreich, S.; Forster, S.; Antonietti, M. *J. Am. Chem. Soc.* **1997**, *119*, 10116. (c) Antonietti, M.; Wenz, E.; Bronstein, L.; Seregina, M. *Adv. Mater.* **1995**, *7*, 1000. (d) Watson, K. J.; Zhu, J.; Nguyen, S. T.; Mirkin, C. A. *J. Am. Chem. Soc.* **1999**, *121*, 462.

[13] (a) Moffitt, M.; Vali, H.; Eisenberg, A. *Chem. Mater.* **1998**, *10*, 1021. (b) Moffitt, M.; McMahon, L.; Pessel, V.; Eisenberg, A. *Chem. Mater.* **1995**, *7*, 1185.

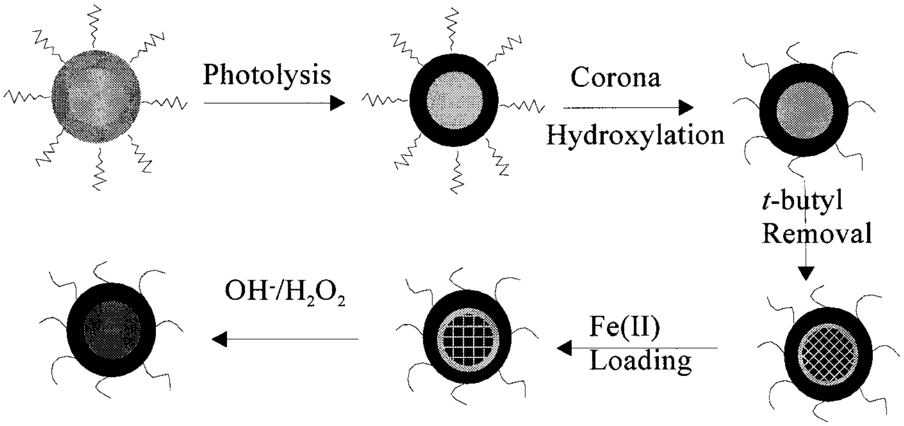
[14] (a) Ng, C. C. Y.; Schrock, R. R.; Cohen, R. E. *J. Am. Chem. Soc.* **1992**, *114*, 7295. (b) Yue, J.; Sankaran, Cohen, R. E.; Schrock, R. R. *J. Am. Chem. Soc.* **1993**, *115*, 4409. (c) Cohen, R. E.; Clay, R. T.; Ciebien, J. F.; Sohn, B. H. In *The Polymeric Materials Encyclopedia*; Salmone, J. C., Ed.; CRC Press: Bocca Raton, 1996; p 4143

[15] See, for example: (a) Sager, W. F. C. *Curr. Opin. Colloid Interface Sci.* **1998**, *3*, 276. (b) Menger, F. M.; Tsuno, T. *J. Am. Chem. Soc.* **1990**, *112*, 6723.

[16] Igumenova, T. I.; Vasil'tsova, O. V.; Parmon, V. N. *J. Photochem. Photobiol. A. Chem.* **1996**, *94*, 205.

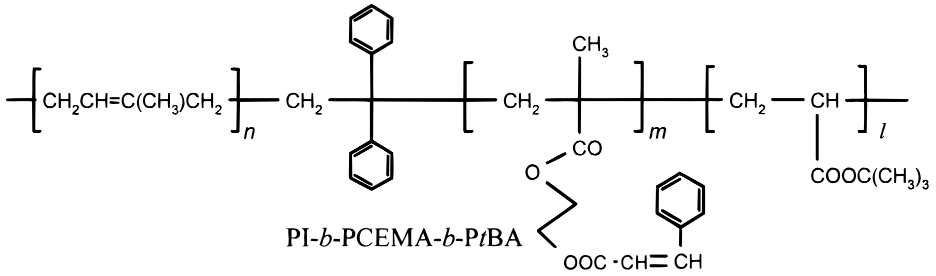
10.1021/cm0000705 CCC: $19.00

Scheme 1. Preparation of Water-Dispersible Magnetic Nanoparticles[a]



[a] Photolysis cross-links the PCEMA shell (gray to dark). The PI corona chains are made water-soluble by hydroxylating the double bonds (wavy lines to free-hand lines). The core is made inorganic compatible by removing the *tert*-butyl groups (light gray to gridded pattern). Soaking the nanospheres in aqueous $FeCl_2$ enables proton exchange by Fe^{2+} (slant to vertical grids) and the Fe^{2+} ions are precipitated and oxidized to yeild cubic γ-Fe_2O_3 magnetic particles using NaOH and H_2O_2 (last step).

Chart 1



preparation of mesoporous aluminosilcate[17] and semiconductor[18] solids. The advantage of using the triblock copolymer nanospheres or dendrimers[19] for templating the preparation of inorganic nanoparticles lies in the stability of the template. Its chemical stability enables the preparation of nanoparticles according to the size of the template and inorganic nanoparticle fusion should be negligible during the preparation.

The magnetic nanoparticles produced are attracted by a magnet and are water-dispersible. Because of their small size, the particles should be superparamagnetic[20] like those prepared previously in polymer films,[21] in polymer gel particles,[22] in microemulsion droplets,[23] in lipid vesicles,[24] or in water or oil under controlled formation conditions.[25] They should have many applications such as in immunoassay,[26] in water reclamation,[27] or in probing the local viscoelasticity of living cells.[28] The limiting factor may lie in their production cost.

II. Experimental Section

Polymer Synthesis and Characterization. The precursor to PI-*b*-PCEMA-*b*-P*t*BA was synthesized by anionic polymerization as described previously.[10,29] Isoprene was polymerized in a small amount of hexane at room temperature for 1 day using *sec*-butyllithium as the initiator. After it had reacted, an aliquot was taken to characterize the PI block. Then 1,1-diphenylethylene was added to convert the PI anions to the less reactive 1,1-diphenylethylene anion. THF was distilled into the polymerization flask by cryodistillation. The second monomer, 2-(trimethylsilyloxy)ethyl methacrylate (TMS−HEMA) was added and polymerized at −78 °C. Once the TMS−HEMA was reacted, *tert*-butyl acrylate was added to form the third block. The polymerization was terminated with the addition of methanol. The methanol also helps the hydrolysis of the TMS−HEMA block to poly(2-hydroxyethyl methacrylate) (PHEMA). A subsequent reaction with cin-

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(17) Templin, M.; Franck, A.; Du Chesne, A.; Leist, H.; Zhang, Y.; Ulrich, R.; Schadler, V.; Weisner, U. *Science* **1997**, *278*, 1795.

(18) Braun, P. V.; Osenar, P.; Stupp, S. I. *Nature*, **1999**, *380*, 325.

(19) (a) Zhao, M.; Sun, L.; Crooks, R. M. *J. Am. Chem. Soc.* **1998**, *120*, 4877. (b) Balogh, L.; Tomalia, D. A. *J. Am. Chem. Soc.* **1998**, *120*, 7355. (c) Zhao, M.; Crooks, R. M. *Angew. Chem., Int. Ed.* **1999**, *38*, 364.

(20) Leslie-Pelecky, D. L.; Rieke, R. D. *Chem. Mater.* **1996**, *8*, 1770.

(21) (a) Ziolo, R. F.; Giannelis, E. P.; Weinstein, B. A.; O'Horo, M. P.; Ganguly, B. N.; Mehrotra, V.; Russell, M. W.; Huffman, D. R. *Science* **1992**, *257*, 219. (b) Winnik, F. M.; Morneau, A.; Mika, A. M.; Childs, R. F.; Roig, A.; Molins, E.; Ziolo, R. F. *Can. J. Chem.* **1998**, *76*, 10. (c) Yamaguchi, K.; Sato, T.; Kato, Y.; Inoue, M.; Fujii, T. *Mater. Sci. Forum* **1998**, *287−288*, 483. (d) Liu, G.; Ding, J.; Hashimoto, T.; Saijo, K.; Winnik, F. M.; Nigam, S. *Chem. Mater.* **1999**, *11*, 2233. (e) Jarjayes, Fries, P. H.; Bidan, G. *J. Magnet. Magnet. Mater.* **1994**, *137*, 205.

(22) (a) Winnik, F. M.; Morneau, A.; Ziolo, R. F.; Stover, H. D.; Li, W. H. *Langmuir* **1995**, *11*, 3660. (b) Kroll, E.; Winnik, F. M.; Ziolo, R. F. *Chem. Mater.* **1996**, *8*, 1594.

(23) (a) Lopez-Quintela, M. A.; Rivas, J. *Curr. Opin. Colloid Interface Sci.* **1996**, *1*, 806. (b) Lopez-Perez, J. A.; Lopez-Quintela, M. A.; Mira, J.; Rivas, J.; Charles, S. W. *Phys. Chem. B.* **1997**, *101*, 8045.

(24) (a) Mann, S.; Skarnulis, J.; William, R. J. P. *J. Chem. Soc. Chem. Commun.* **1979**, 1030. (b) Yaaco, I. I.; Nunes, A. C.; Bose, A. *J. Colloid Interface Sci.* **1995**, *171*, 73.

(25) (a) Lefebure, S.; Dubois, E.; Cabuil, V.; Neveu, S.; Massart, R. *J. Mater. Res.* **1998**, *13*, 2975. (b) Matijevic, E. *Prog. Colloid Polym. Sci.* **1996**, *101*, 38.

(26) (a) Sutor, J. J. US Patent 5,648,124, 1997. (b) Muller-Schelte, D.; Brunner, H. *J. Chromatogr. A* **1995**, *711*, 53. (c) Li, X.; Sun, Z. *J. Appl. Polym Sci.* **1995**, *58*, 1991. (d) Kondo, A.; Fukuda, H. *J. Ferment. Bioengin.* **1997**, *84*, 337.

(27) Kolaris, L. O.; Anderson, N. J.; Bolto, B. A.; Chin, C. T.; Priestley, A. J. *Water Supply* **1994**, *13*, 253.

(28) Bausch, A. R.; Moller, W.; Sackmann, E. *Biophys. J.* **1999**, *76*, 573.

Table 1. Characteristics of the Polymers

The PI block				The triblock			
d*n*/d*c* (mL/g)	LS $\bar{M}_w$ (g/mol)	$\bar{M}_w/\bar{M}_n$ GPC	1,4-PI[a]	n	m	l	$\bar{M}_w/\bar{M}_n$ GPC
0.101	25×10^3	1.06	93%	370	420	550	1.16

[a] The degree of 1,4-addition was determined from NMR.

namoyl chloride in pyridine converted the PHEMA to the desired PCEMA.

The PI block and the triblock were characterized by GPC, NMR, and light scattering (LS). The characterization results are summarized in Table 1. The symbol d*n*/d*c* in Table 1 denotes specific refractive index increment of the PI block determined in cyclohexane for LS analysis. The sample was monodisperse as revealed by GPC analysis.

Micelle Preparation and Cross-Linking. The triblock was dissolved in THF (Fisher), a solvent that is good for all three fo the blocks. Micelles, at the final concentration of 1.0 mg/mL, were formed by adding HX (Fisher), selectively good for the PI block, to the THF solution. The micelle solution was then irradiated with UV light from a 500-Watt mercury lamp, which had passed through a 260 nm cutoff filter. Vigorous stirring was rendered, and the temperature was controlled at 20 °C throughout the photolysis. The conversion of the PCEMA double bond was monitored, using a Perkin-Elmer Lamba Array 3840 Instrument, from the absorbance decrease of the PCEMA group at 274 nm, where other groups absorbed negligibly.

Micelle Functionalization and Characterization. Once cross-linked, the polyisoprene block was hyroxylated to give poly(2,3-dihydroxyisoprene) (PHI) following a method adaptated[5] previously from the literature.[30] To 16 mL of 90% formic acid (0.376 mol, Fisher) at room temperature were added 4 mL of acetic anhydride (0.042 mol, Fisher), 4 mL of 30% hydrogen peroxide (0.038 mol, Aldrich), and 0.10 mL of concentrated sulfuric acid. The mixture was stirred for 5 min before 20 mL of it was taken and mixed with 100 mL of 1.0 mg/mL, cross-linked nanosphere solution (0.180 mmol of isoprene units). The mixture was allowed to react for 8 h. The solution was concentrated to 1/10 its original volume using a rotary evaporator, and the nanospheres were precipitated into iced water. The nanospheres were filtered and added to 20 mL of a 1 N sodium hydroxide (Fisher) solution made from mixing equal amounts of a 2 N sodium hydroxide solution in water and tetrahydrofuran. The mixture was allowed to react 18 h at room temperature before the nanospheres were precipitated by rotor evaporating most of the solvent. The precipitate was washed with water. As the ionic species were removed, the nanospheres became progressively more soluble in water and the aqueous dispersion was added into acetonitrile (Fisher) to precipitate poly(2,3-dihydroxyisprene) (PHI) shelled nanospheres. The reaction was confirmed using ^{1}H NMR, FTIR, and solid-state ^{13}C NMR.

The cleavage of the *tert*-butyl groups was achieved in dry dichloromethane with iodotrimethylsilane (Aldrich). The hydroxyl groups of the PHI corona also reacted with iodotrimethylsilane, thus enough excess had to be added to react with the hydroxyl and the P*t*BA groups. The nanospheres were dried and added to dichloromethane to form a slurry. Three mole equivalence, relative to P*t*BA, of iodotrimethylsilane was added. The reaction was allowed to go for 2 h at room temperature. After centrifugation, the dichloromethane was decanted and the polymer was washed three more times with dichloromethane to remove excess iodotrimethylsilane. In the final time the sample was dried and then dissolved in water/methanol, which was added dropwise to acetonitrile to precipitate the nanospheres. Cleavage of the *tert*-butyl group was verified using solid-state ^{13}C NMR and FTIR.

Iron Oxide Formation. The nanospheres were dissolved in water at 6 mg/mL. To 2.5 mL of the nanosphere solution was added 2.5 mL of a saturated solution of iron(II) chloride. The nanosphere/iron solution was stirred for 24 h. The sample was centrifuged at 14 × 10^3 rpm for 10 m to settle the nanospheres. The supernatant which contained excess Fe(II) was decanted. The nanospheres were rinsed three times by dispersing them in pH 2 water and precipitating them out via centrifugation. A final wash was performed with distilled water (pH 7). The nanospheres were then redissolved in 2.5 mL of neutral water, to which a solution of 1 N NaOH was added dropwise until the pH equaled 10. At this point 2.5 mL of hydrogen peroxide was added, and the mixture was stirred 18 h to oxidize the iron(II) to Fe_2O_3. After oxidation the sample was again centrifuged and washed three times to remove excess base and H_2O_2. The loading and oxidation step were repeated two more times to ensure adequate loading of the core. The orange color of the solution containing the loaded nanospheres became darker with each additional loading step.

TEM Studies. TEM images were obtained using a Hitachi H-7000 instrument operated at 100 kV. TEM samples were obtained by aspirating a fine mist of a dilute solution (~0.1 mg/mL) of the polymer micelles or nanospheres onto a carbon-coated copper grid using a home-built device.[31] The samples, with the exception of the iron-loaded nanospheres, were then placed in a vial with osmium tetraoxide (Aldrich) vapor for >4 h to stain the double bonds.

TEM specimen of the nanospheres containing Fe_2O_3 had to be prepared differently. When prepared by the traditional method, no or very few nanoparticles could be detected by TEM presumably because the particles left the grids due to the attraction by magnets in instrument. To prepare such a specimen, an aqueous solution of PHI homopolymer and the nanospheres at the ratio of 20:1 and a total concentration of 1 mg/mL was sprayed on carbon-coated copper grid. The sample was sprayed thicker than usual so that a thin polymer film formed on the grid. Such a thin film was then studied without further staining.

Dynamic Light Scattering Measurements. Dynamic light scattering (DLS) measurements were performed with a Brookhaven model 9025 instrument using an argon ion laser operated at 488 nm. Solutions at a concentration of ~0.1 mg/mL were centrifuged at 14 × 10^3 rpm for 5 min, and the supernatant was used for such measurements. DLS data were analyzed following the method of cumulants.[32] The viscosity used for 65% THF/HX was measured using a Ubbelhode sidearm viscometer and was found to be 0.304 cP. The viscosities for hexanes and water were taken as 0.326 and 1.002 cP, respectively.[33]

NMR and Fourier Transform Infrared Spectroscopy Measurements. Solution ^{1}H NMR measurements were carried out on a Bruker ACE-200 instrument using the delay time of 45 s between successive pulses. Solid-state ^{13}C NMR measurements were performed on an AMX-300 instrument using a 4 mm BL4 solid probe with the CP21ev program. All spectra were obtained at 8 kHz and repeated at 13 kHz to identify spinning sidebands. FTIR data was collected on a Galaxy Series 4030 instrument (Mattson) using WinFIRST V2.10 software. All FTIR spectra were obtained in the solid state. The samples were prepared by blending potassium bromide with ~1% polymer (w/w). The powder was then compressed into a pellet using an evacuable die.

III. Results and Discussion

The sequence of Scheme 1 is used for presenting experimental evidence, demonstrating our success in

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(29) Liu, G.; Ding, J.; Stewart, S. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 835.

(30) (a) Swern, D.; Billen, G. N.; Findley, T. W.; Scanlan, J. T. *J. Am. Chem. Soc.* **1945**, *67*, 1786. (b) Swern, D. *Org. React.* **1953**, *7*, 378. (c) Swern, D. *J. Am. Chem. Soc.* **1947**, *69*, 1692.

(31) Ding, J.; Liu, G. *Macromolecules* **1999**, *32*, 8413.

(32) Koppel, D. E. *J. Chem. Phys.* **1972**, *57*, 4814.

(33) Weast, R. C. *CRC Handbook of Chemistry and Physics*, 56th ed.; CRC Press: Boca Raton, FL, 1975.

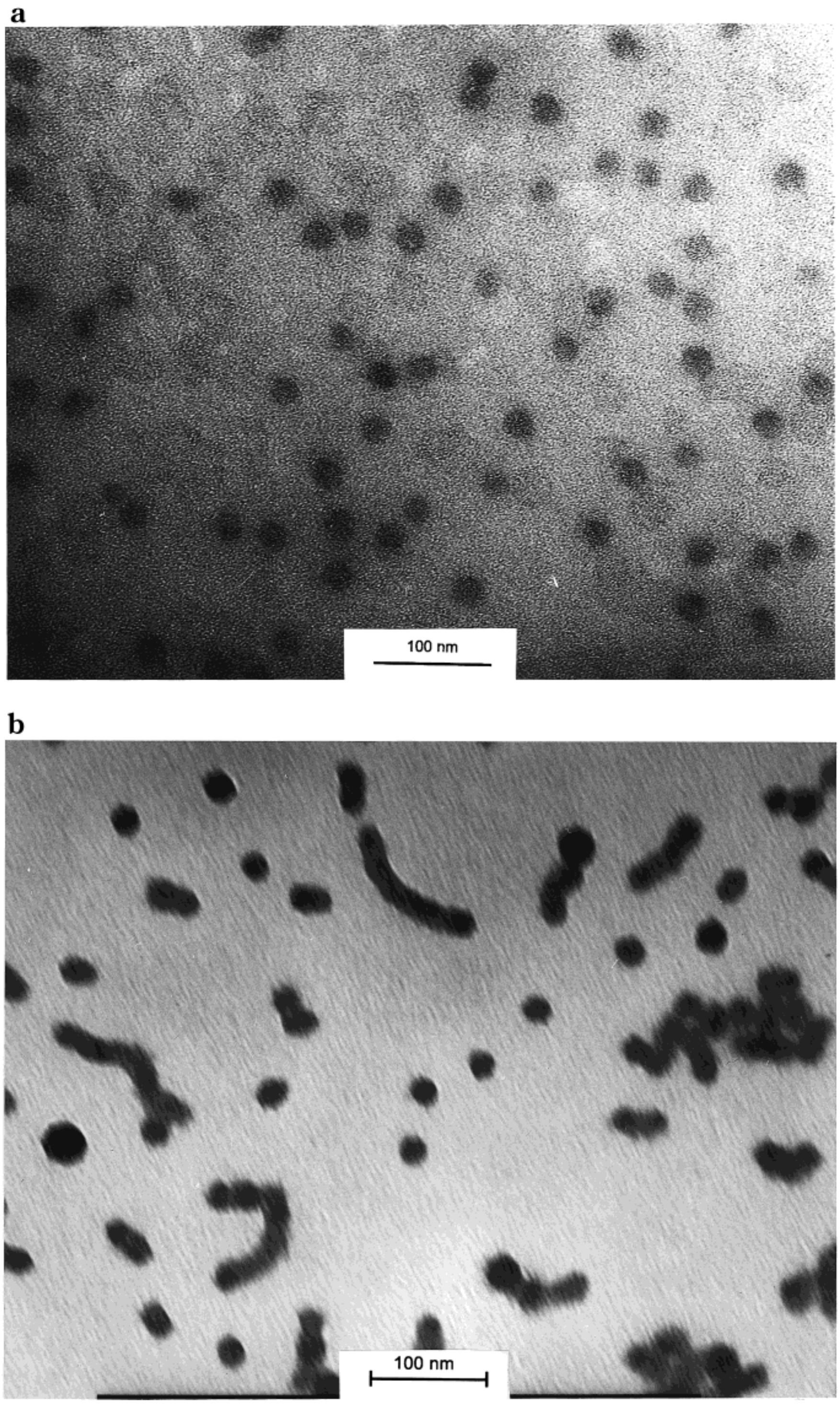

Figure 1. TEM images of PI-*b*-PCEMA-*b*-P*t*BA micelles sprayed from THF/HX with 65% (a) and 97% HX (b).

each stage for the preparation of triblock nanospheres and water-dispersible magnetic nanoparticles. Nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR) results are used to elucidate the chemical reactions occurring. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) are used to reveal potential particle size or morphology changes at different reaction stages.

Spherical Micelle Formation. Of the PI, PCEMA, and P*t*BA blocks, only PI was soluble in HX. We anticipated the formation of micelles with PI corona, PCEMA shell, and P*t*BA core in THF/HX with predominantly HX. We demonstrated the formation of micelles in different THF/HX mixtures using TEM. Illustrated in Figure 1 are the TEM images of micelles formed in THF/HX with 65% and 97% HX, respectively. Monodisperse spherical micelles are formed in THF/HX with 65% HX and spherical micelles coexist with "pearl-necklace-shaped"^{1} micelles in THF/HX with 97% HX. The pearl-necklace-shaped particles formed presumably due to the aggregation of the spherical micelles with a decrease in the solvation power of the THF/HX mixture. A comparison of TEM images of micelles formed in THF/HX with the HX volume fractions of 95%, 93%, 80%, 70%, and 65% revealed that the micelle size distribution appeared the most narrow in THF/HX with 65% HX.

Because of this, micelles used for subsequent studies were all prepared in this solvent composition. The diameter, d_m, of the micelles sprayed from THF/HX with 65% HX as determined by TEM in Figure 1a is ~21 nm. Since the specimen was stained with OsO_4 which reacts with the double bonds of PI and PCEMA, the diameter must reflect the overall size of the micelles.

The same micelles were also characterized by dynamic light scattering. The hydrodynamic diameter (d_h) thus determined was 86 ± 1 nm, which is substantially larger than the d_m value. The different d_m and d_h values suggest that the micelles were substantially swollen in THF/HX with 65% HX.

Because of the small size and the aggregation state of the micelles, our TEM could not be used to demonstrate the layered structure at this stage. No other effort was made to obtain this structure information, because this will become evident later as the structural details of the nanospheres at different stages are revealed.

Micelle Cross-Linking. Photo-cross-linking of the PCEMA block of the 65% HX sample occurs due to the dimerization of the PCEMA double bonds of different chains to give cyclobutane.[34] The conversion of the double bonds was monitored by a decrease in their UV absorbance at 274 nm. A double-bond conversion of 40% was used in this study to lock in the micellar structure. A plot of CEMA conversion as a function of UV irradiation time is not shown here because PCEMA cross-linking is well-established and has been used extensively in the past in our laboratory to lock in various block copolymer mesophasic structures for nanostructure preparation.

The occurrence of the cross-linking reaction was also demonstrated by our solid-state ^{13}C NMR results shown in Figure 2 the PI-*b*-PCEMA-*b*-P*t*BA triblock and the cross-linked micelles. The NMR spectra look essentially the same after PCEMA cross-linking, suggesting the structural integrity of the polymer during this process. A decrease in the relative intensities of the peaks of the alkenyl carbon atoms at 118 and 148 ppm relative to that of the phenyl peak at 130 ppm alludes to the disappearance of some of these carbons due to cross-linking.

The cross-linking of the micelles could also be judged visually. The triblock dissolved in a wide range of solvents such as chloroform, THF, and toluene and formed clear molecular solutions. The irradiated micelles only dispersed in such solvents to yield dispersions with a bluish tinge. Compared in Figure 3 are the solution ^1H NMR spectra of the triblock and its cross-linked micelles. According to peak assignments of Figure 3, signal is observed of most protons of the triblock. In contrast, peaks from PI and only one peak from P*t*BA are seen of the cross-linked micelles. The reduced relative intensity of the *tert*-butyl proton peak and the complete disappearance of the PCEMA proton peaks suggest the reduced segmental mobility of P*t*BA and PCEMA blocks. This is reasonable, because these blocks form the shell and core of the nanospheres. The PI signals remain, because PI forms the corona of the nanospheres.

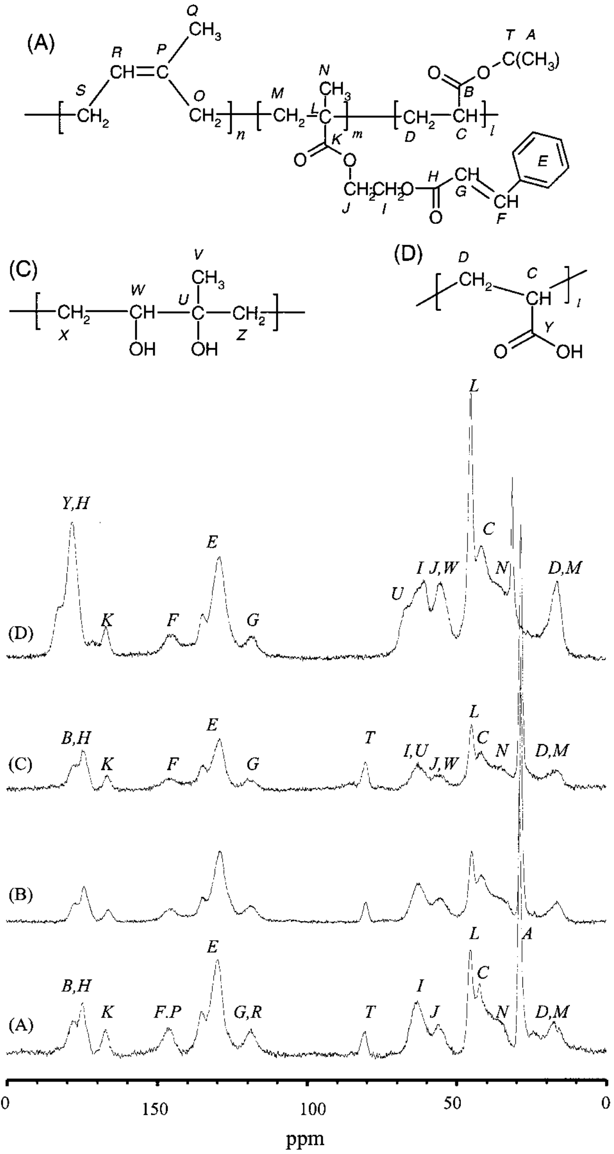

Figure 2. Comparison of solid-state ^{13}C NMR spectra for (A) PI-*b*-PCEMA-*b*-P*t*BA polymer, (B) PI-*b*-PCEMA-*b*-P*t*BA nanospheres, (C) PHI-*b*-PCEMA-*b*-P*t*BA nanospheres, and (D) PI-*b*-PCEMA-*b*-PAA nanospheres.

The *tert*-butyl signal did not disappear completely probably due to the swelling of the core by $CDCl_3$. The surprising result has been the observed distortion of the relative intensities of protons of different PI units. The intensity of some proton peaks of the *trans*-1,4 (15 molar % [35]) and 3,4-added (4%) units seems to increase drastically relative to that of the *cis*-1,4 units (81%). This is probably due to the decreased mobility of the protons of the *cis*-1,4 units relative to those of the other microstructures. While we do not have a definitive explanation to this or to the question why only some of the protons for the 3,4-added or *trans*-1,4 units are seen, the intensity pattern is the same at the delay time of 15 or 45 s between magnetization pulses of the NMR experiment.

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[34] (a) Kato, M.; Ichijo, T.; Ishii, K.; Hasefawa, M. *J. Polym. Sci., Part A: Polym. Chem.* **1971**, *9*, 2109. (b) Guillet, J. E. *Polymer Photophysics and Photochemistry − An Introduction to the Study of Photoprocesses in Macromolecules*; Cambridge University Press: Cambridge, UK, 1985.

[35] Morton, M. *Anionic Polymerization: Principles and Practice*; Academic Press: New York, 1983; p 150.

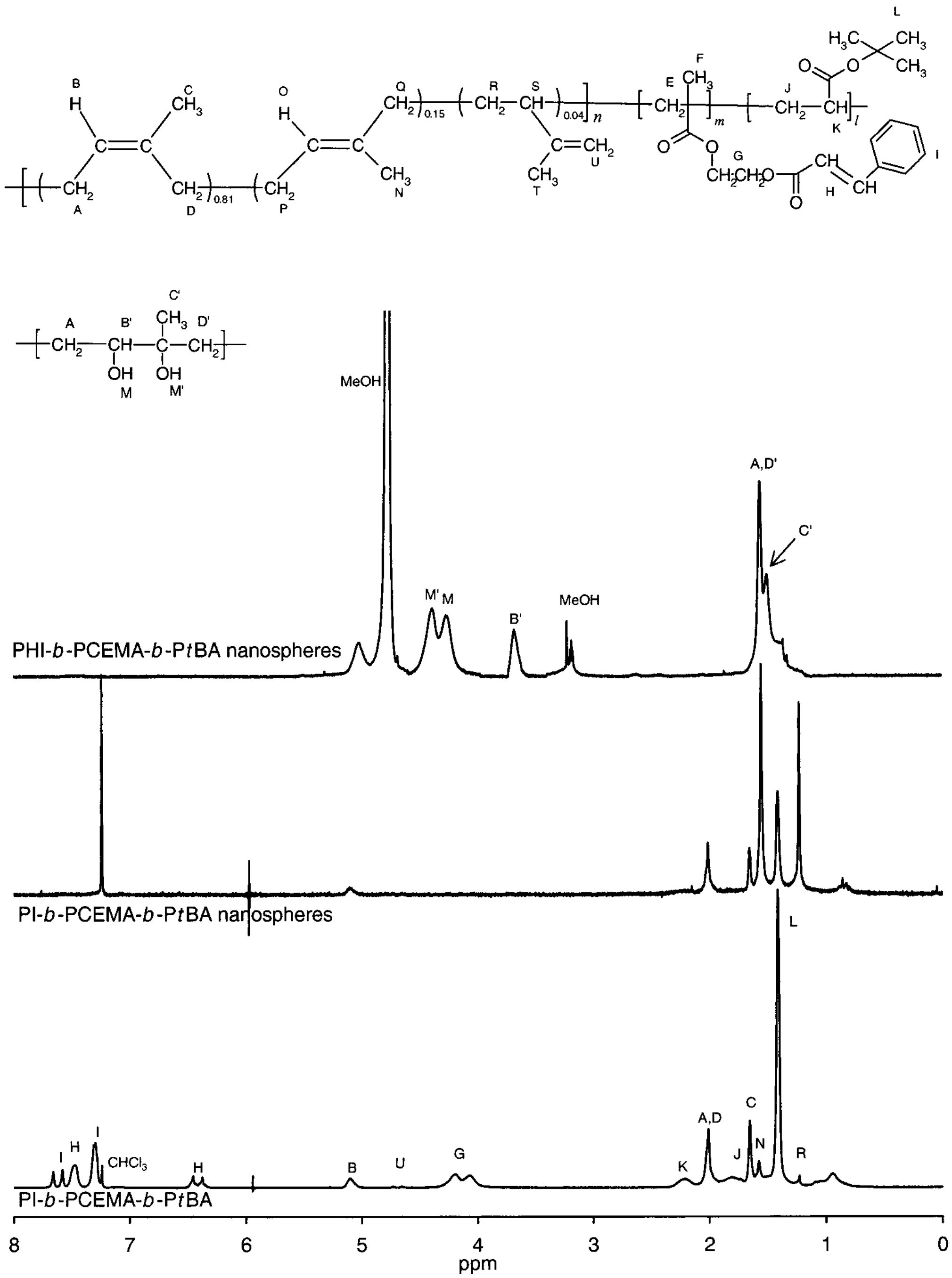

Figure 3. Comparison of ^{1}H NMR spectra of PI-*b*-PCEMA-*b*-P*t*BA (a), PCEMA-cross-linked nanospheres (b), and PI-hydroxylated nanospheres (c).

We also performed DLS and TEM to check the morphologies of the cross-linked particles. DLS performed in THF/HX with 65% HX after PCEMA cross-linking yielded a hydrodynamic diameter of 87 ± 1 nm (Table 2), which is the same as the value of 86 ± 1 nm found for the un-cross-linked micelles. This suggests that the cross-linking of PCEMA did not change but only locked in the micellar structure, resembling what was observed in previous cases.[36] The d_m value measured by TEM increased to ~26 nm (Table 2). Since the size of the sample (particles imaged on one picture) probed by TEM is small, we caution the over-interpretation of this seemingly higher d_m value. On the other hand, a higher d_m value would be reasonable, because the cross-linked micelles may not shrink as much as the un-cross-

Table 2. TEM (d_M, nm) and Hydrodynamic (d_h, nm) Diameters of the Nanospheres at Different Stages

reaction stage	d_M (nm), spray solvent	d_h (nm), solvent
micelles	21, THF/HX = 35/65	86, THF/HX = 35/65
after PCEMA cross-linking	26, THF/HX = 35/65	87, THF/HX = 35/65
after PI hydroxylation	25, THF	57, THF
after *tert*-butyl removal	15, H_2O/CH_3OH = 50/50	89, H_2O
after Fe_2O_3 loading	~10, H_2O	90, H_2O

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[36] (a) Tao, J.; Stewart, S.; Liu, G.; Yang, M. *Macromolecules* **1997**, *30*, 2738. (b) Guo, A.; Tao, J.; Liu, G. *Macromolecules* **1996**, *29*, 2487.

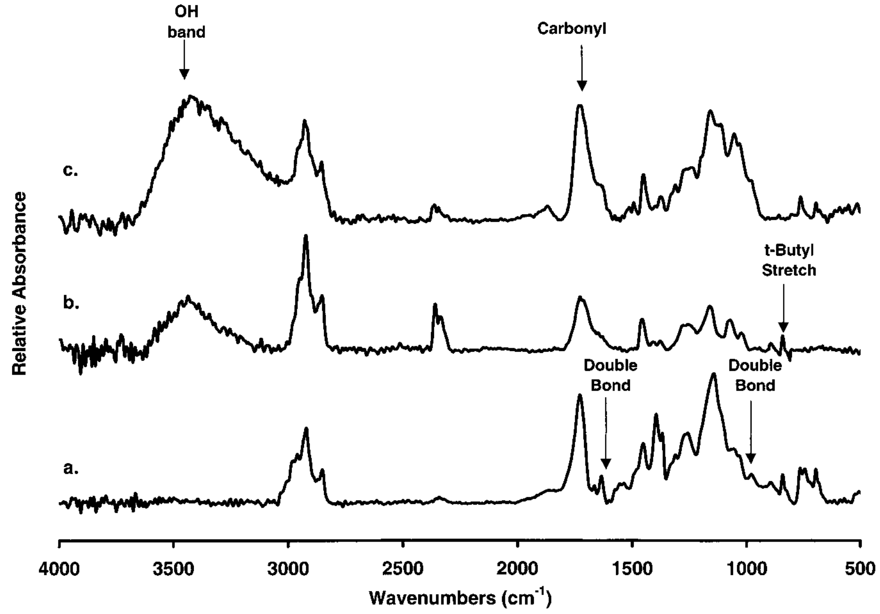


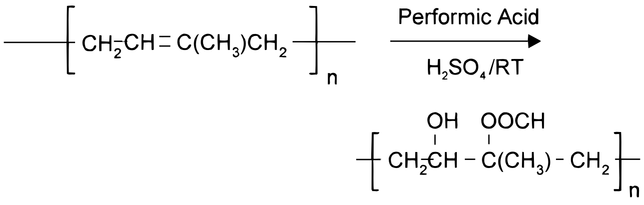
Figure 4. FTIR spectra of the nanospheres after PCEMA cross-linking (a.), after PI hydroxylation (b.), and after *tert*-butyl group removal (c.).

linked ones when dried over carbon-coated copper grid after spraying from the THF/HX mixture.

Hydroxylation of the PI Corona. The polyisoprene block was hydroxylated to give poly(2,3-dihydroxyisoprene) (PHI) following a method adapted[5] previously from the literature.[27] This method involved a sequence of reactions. The first involves the in-situ formation of performic acid from formic acid and hydrogen peroxide. Acetic anhydride was used to consume the water present in formic acid and hydrogen peroxide:

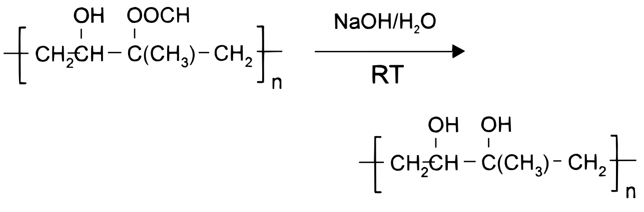
$$\mathrm{HCO_2H + H_2O_2 \xrightarrow[\text{room temperature}]{\text{acetic anhydride}} HCO_3H + 2CH_3COOH}$$

Concentrated sulfuric acid was used as a catalyst. The reaction between performic acid and the double bond of PI is supposed to yield an oxirane initially, which is not stable and may be further converted into a hydroxy formoxy derivative:



$$\left[\mathrm{CH_2CH{=}C(CH_3)CH_2}\right]_n \xrightarrow[\mathrm{H_2SO_4/RT}]{\text{Performic Acid}} \left[\mathrm{CH_2\overset{OH}{C}H-\overset{OOCH}{C}(CH_3)-CH_2}\right]_n$$

Hydrolysis of the formyl ester in 1.0 M aqueous sodium hydroxide removes the formyl group to yield PHI:



$$\left[\mathrm{CH_2\overset{OH}{C}H-\overset{OOCH}{C}(CH_3)-CH_2}\right]_n \xrightarrow[\mathrm{RT}]{\mathrm{NaOH/H_2O}} \left[\mathrm{CH_2\overset{OH}{C}H-\overset{OH}{C}(CH_3)-CH_2}\right]_n$$

The occurrence of the above reactions was supported by FTIR and NMR evidence. Shown in Figure 2 is a comparison between solid-state ^{13}C NMR spectra of the nanospheres before and after the hydroxylation reactions. The overall spectrum changed little after the reactions, suggesting the overall integrity of the polymer backbone after the reactions. The occurrence of the reactions is indicated by a further decrease in the relative intensity of peaks at 118 and 148 ppm. Also, the shape of the peaks in the 55–65 ppm range has changed somewhat. More convincing evidence was gathered from a solution ^{1}H NMR study with results shown in Figure 3. The proton peaks have changed completely and the positions of all the new peaks are in agreement with what are expected of the product except one at 5.1 ppm. This peak may suggest the survival of some PI double bonds. Such an assignment is, however, questionable, because the other proton peaks in such an isoprene are not seen. Thus, side reactions other than those depicted may have also occurred.

The hydroxylation of the PI double bonds was also confirmed by our FTIR results shown in Figure 5. After the hydroxylation reaction, an OH stretching band at 3400 cm^{-1} appeared at the partial cost of the double-bond stretching band at 1620 cm^{-1} and the complete consumption of =C–H out-of-plane bending vibration at 980 cm^{-1}. The peak at 1620 cm^{-1} is only partially gone, as PCEMA double bond absorbs there as well.

Morphology and Properties of the Hydroxylated Nanospheres. An indirect but the most visual evidence for the hydroxylation of nanospheres is their dispersion in water or methanol. They also disperse in THF containing a small amount of NaOH. Illustrated in Figure 5 is a TEM image of the nanospheres after PI hydroxylation. The integrity of the nanospheres is evidently retained. The diameter of the nanospheres is ~25 nm, which is comparable to that observed before hydroxylation (Table 2) and suggests that the observation of PHI layer by TEM probably due to staining of the residual isoprene double bonds by OsO_4. Because of the small sample size, we again caution the over interpretation of the d_m values. A direct comparison of

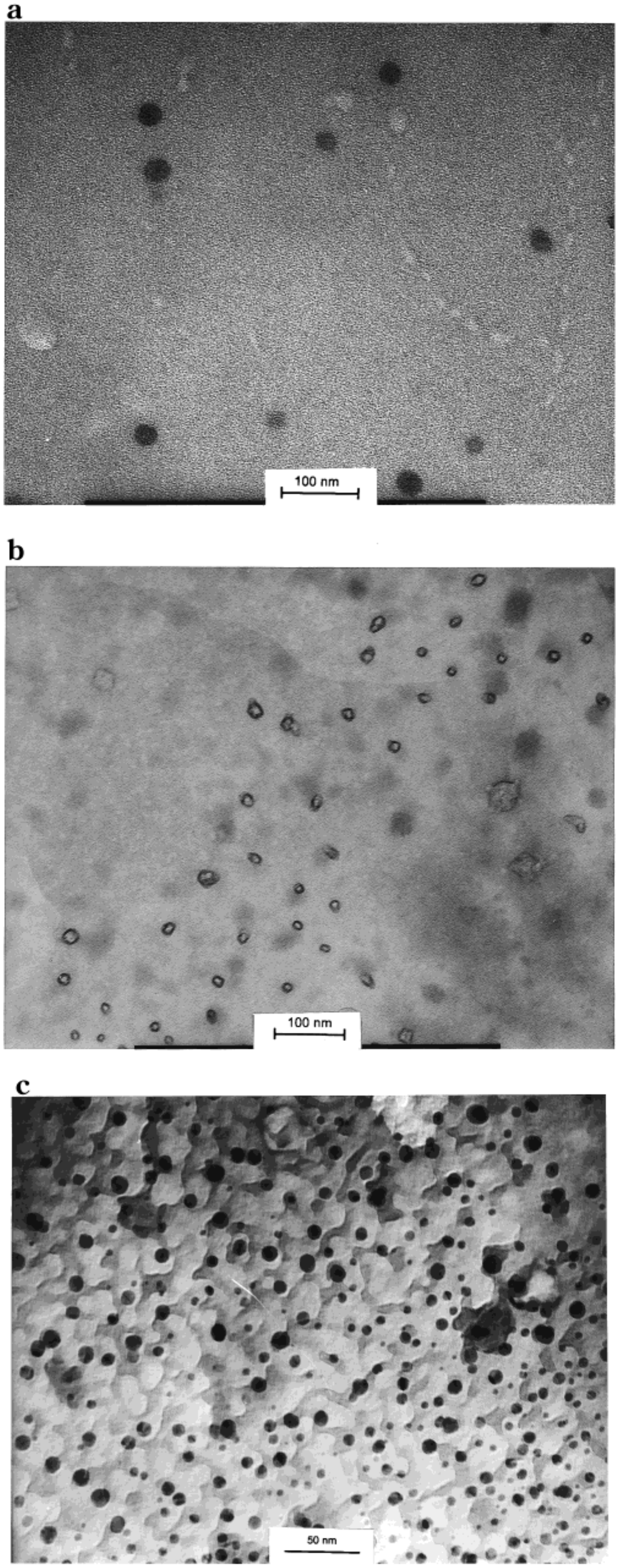


Figure 5. TEM images of PI-*b*-PCEMA-*b*-P*t*BA nanospheres at each stage in the synthesis: (a) after PCEMA cross-linking and PI hydroxylation (stained with OsO_4 overnight), (b) after removal of *tert*-butyl groups (stained with OsO_4 over a weekend), and (c) after Fe_2O_3 loading (no staining).

the d_m values is further cautioned against here because the PCEMA-cross-linked and the hydroxylated nanospheres were sprayed from different solvents.

A dynamic light scattering experiment was performed in THF with a few drops of an aqueous NaOH solution. The hydrodynamic diameter of the nanospheres is 57 nm. This is substantially larger the d_m value of 25 nm but smaller than d_m of the nanospheres before hydroxylation determined in THF/HX. This may be due to the fact that PHI does not swell significantly in THF. The insignificant swelling of the PHI chains in THF can be judged from the observation that the nanospheres with a PHI corona did not dissolve in THF and dissolved only upon the addition of an aqueous NaOH solution.

Cleavage of the *tert*-Butyl Group. The hydrolysis was carried out in two steps, as depicted in Scheme 2. The hydroxylated nanospheres were treated with excess trimethylsilyl iodide to effect the conversion of the *tert*-butyl groups into trimethylsilyl groups.[37] The trimethylsilyl groups then hydrolyzed in either water or methanol. These reactions should occur effectively as they have been used previously by us to prepare water-soluble nanospheres[38] or thin films with nanochannels.[39]

Evidence for their occurrence in this particular system was gathered from FTIR, NMR, and TEM studies. Illustrated in Figure 2 is a comparison between the ^{13}C NMR spectra of the nanospheres before and after the *tert*-butyl removal procedure. The quantitative removal of the *tert*-butyl groups is unambiguously confirmed by the disappearance of the peaks at 29 and 81 ppm. It is also supported by the shift in the position of the P*t*BA ester carbon from ~173 to ~176 ppm.

A comparison between the FTIR spectra of the nanospheres before and after the trimethylsilyl iodide and methanol treatment is shown in Figure 4. The disappearance of *tert*-butyl rocking peak at ~845 cm^{-1} and the increase in the hydroxyl group band at ~3500 cm^{-1} also render support for the removal of the *tert*-butyl group.

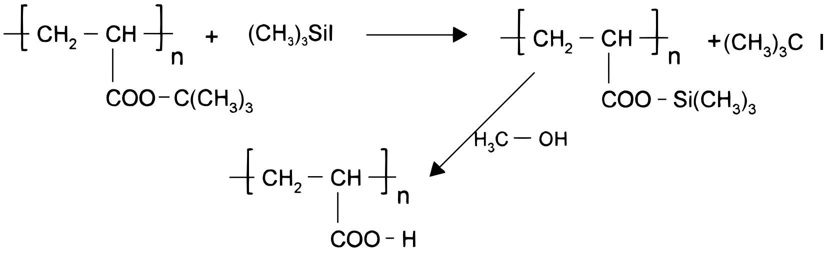
No appreciable change in dispersibility in different solvents was noticed of the nanospheres before and after *tert*-butyl group removal. This is understandable, because the corona, which governs the dispersibility of the nanospheres, is unaffected. A dynamic light scattering study of the sample yielded a d_h value of 89 nm. This value is same as those of the micelles or nanospheres determined in THF/HX with 65% HX. The increase relative to that determined in THF is due to the fact that the PHI corona solubilizes better in water than in THF.

Illustrated in Figure 5 is a TEM image of the nanospheres after *tert*-butyl group removal. The nanospheres have a light core. Since OsO_4 does not stain PAA, this clearly suggests that PAA forms the core of the nanosphere. The diameter, d_m, of the particles including the dark shell is ~20 nm. Once again we caution the over-interpretation of the d_m value due to the different specimen preparation conditions and the small size of the sample.

We do not know the exact reason the PAA cores in Figure 5 appear distinguished. It may be due to the better phase separation between PCEMA segments and PAA chains than that between PCEMA segments and P*t*BA chains.

Iron Oxide Formation in the Cores. A literature method[19] was followed for preparing iron oxide in the cores of the nanospheres. The method involved (a) the

Scheme 2. Hydrolysis of *tert*-Butyl Groups



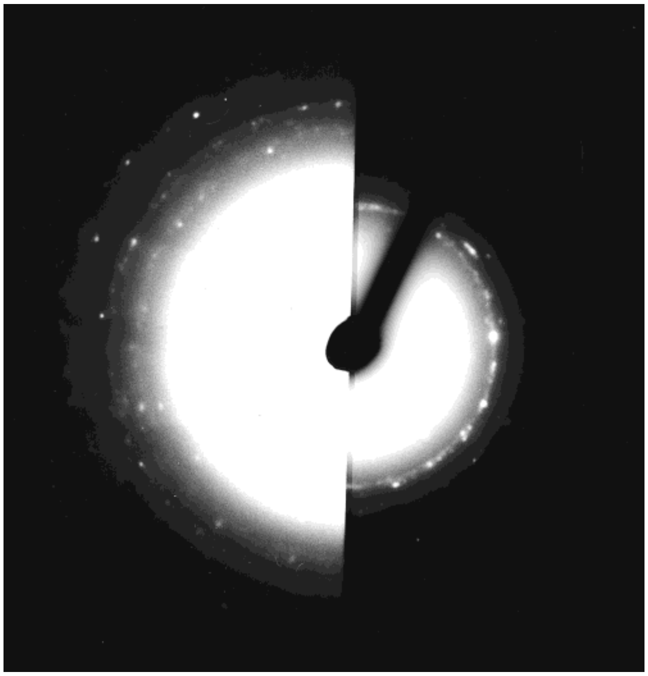
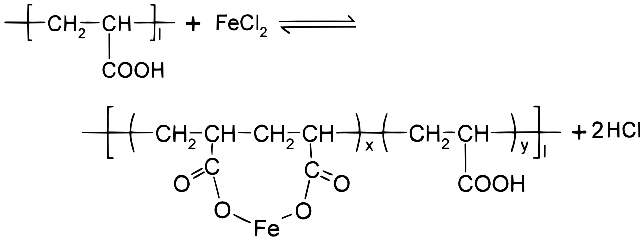


Figure 6. Electron diffraction pattern of the iron-oxide-containing nanospheres.

exchange of the PAA protons by Fe^{2+}, (b) the addition of NaOH to precipitate out the Fe^{2+} ions in the core, and (c) the oxidation of Fe(II) to Fe(III). The ion exchange reaction was achieved by stirring the functionalized nanospheres with $FeCl_2$:



where $x + y = 100\%$ and Fe^{2+} does not necessarily bind to adjacent acrylic acid groups of the same chain. Instead, the acrylic acid groups can be from different chains or the same chain but are not adjacent in the chain sequence. Since this method has been used by several groups, no effort was made to quantify the degree of reaction or to increase x.

The formation of iron oxide inside the nanosphere cores after treating the Fe(II)-loaded nanospheres with NaOH and H_2O_2 was concluded from the electron diffraction pattern shown in Figure 6. The electron diffraction pattern exhibits five distinguishable halos. By comparing the positions of halos with those observed for a gold standard,[40] we obtained the interplanar distances, d_{hkl}, as listed in Table 3. The agreement between our d_{hkl} values and the literature values[41] is excellent. We also compared the relative intensities of the different halos to those reported and these comparisons suggest that the particles formed in the cores are γ-Fe_2O_3 cubic crystals.

Table 3. Interplanar Spacings Fe_2O_3 Particles and Their Comparison with Literature Values for γ-Fe_2O_3 (Cubic-Maghemite)

d_{hkl} (Å) (exp)	d_{hkl} (Å)	Miller indices: hkl
2.52	2.52	311
2.20	2.23	321
1.65	1.61	511, 333
1.49	1.48	440
1.26	1.27	533

The TEM result of Figure 5 also indicated the formation of Fe_2O_3 in the nanosphere cores. Since the specimen was not stained before viewing and the nanospheres were embedded in a homo-PHI matrix, the polymer encapsules should be invisible and the dark spherical domains must represent the Fe_2O_3 particles. The particles have diameters ranging from ~4 to ~16 nm. The value ~16 nm is in agreement with the nanosphere core size and suggests the control offered by these templates over the sizes for inorganic particles prepared.

Properties of the Magnetic Nanoparticles. The TEM image of Figure 5 clearly shows that the nanospheres did not aggregate due to Fe_2O_3 loading. This was also confirmed by a DLS study. After Fe_2O_3 impregnation, the hydrodynamic diameter of the nanospheres was 90 nm, which was the same as that determined after *tert*-butyl removal. As expected, the Fe_2O_3-loaded nanospheres remained dispersible in water and such dispersion was stable in water for tens of minutes without appreciable precipitation. The particles however settled close to the poles of a U-shaped laboratory magnet within minutes. This is clearly illustrated in Figure 7where a picture is shown of two samples with one standing next to the U-shaped magnet and the other

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(37) Jung, M. E.; Lyster, M. A. *J. Am. Chem. Soc.* **1976**, *99*, 968.

(38) (a) Henselwood, F.; Liu, G. *Macromolecules* **1997**, *30*, 488. (b) Wang, G.; Henselwood, F.; Liu, G. *Langmuir* **1998**, *14*, 1554. (c) Henselwood, F.; Wang, G.; Liu, G. *J. Appl. Polym. Sci.* **1998**, *70*, 397.

(39) (a) Liu, G.; Ding, J.; Herfort, M.; Bazett-Jones, D. *Macromolecules* **1997**, *30*, 1851. (b) Liu, G.; Ding, J. *Adv. Mater.* **1998**, *10*, 69.

(40) Misell, D. L.; Brown, E. B. *Electron Diffraction: An Introduction for Biologists* Elsevier, Amsterdam, 1987.

(41) (a) Joint Committee on Powder Diffraction. *Mineral Powder Diffraction File Data Book*; Pennsylvania, 1980; Vol. 1. (b) Zhang, L.; Eisenberg, A. *Science* **1995**, *268*, 1728. (c) Zhang, L.; Yu, K.; Eisenberg, A. *Science* **1996**, *272*, 1777.

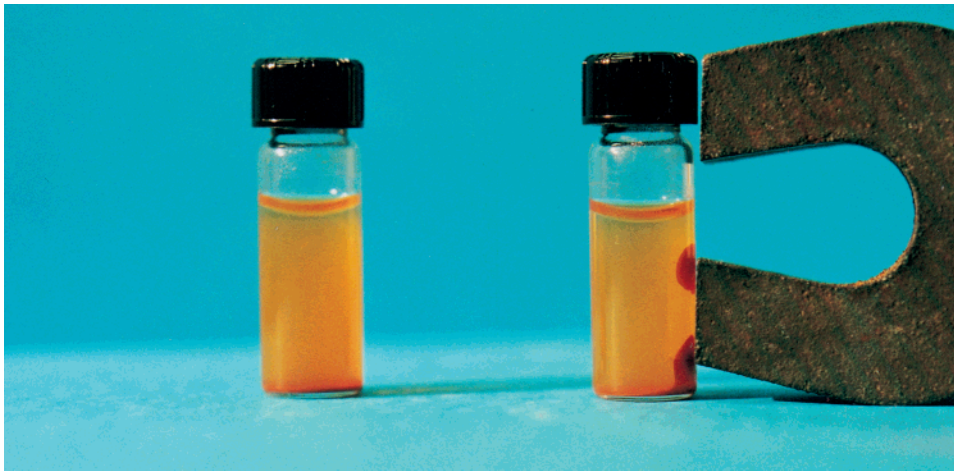

Figure 7. Photograph showing the attraction of the magnetic nanoparticles by a laboratory magnet (right). The right is the result of a control experiment, indicating less settling of the nanoparticles at the bottom of the vial due to gravitational force 6 h after shaking the bottles.

free standing. This picture was taken 6 h after the magnet was placed beside the nanosphere dispersion.

The properties of particles have not been characterized by SQUID magnetometry. The particles are, however, expected to be superparamagnetic, because they are far smaller than the critical size of 160 nm for exhibiting bulk magnetic properties.[20] They are definitely not ferromagnetic as judged from their dispersibility in water.

Acknowledgment. NSERC of Canada is acknowledged for financially sponsoring this research. R.U. also thanks NSERC, the Killam Foundation, and the University of Calgary for graduate scholarships and teaching assistantship. Sean Stewart synthesized and characterized the triblock copolymer used. Qiao Wu obtained the ^{13}C NMR data. Their contributions are gratefully acknowledged.

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