CHAPTER VI
pH SENSING DUALLY CORE-SHELL TYPE FLUORESCENT NANOPARTICLES

6.1 Abstract

The multiple stimuli fluorescent nanoparticles were prepared by combining thermoresponsive moieties, NIPAAM with fluorescence molecules, NVC and pH-responsive moieties, DMAEA. We prepared two ratios of block copolymer, (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ (I) and (PNIPAAM$_{62}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$, (II), to observe the effect on size and morphology of aggregate. The dynamic light-scattering studies in water indicated that the obtained block copolymers formed a nano-scale core-shell structure and changed in conformation with pH. It was found that the particle size prepared at pH 2 and 4 ranged from 160 to 200 nm and 100 to 120 nm for diblock copolymers I and II, respectively. The shorter of PNIPAAM block resulting in the smaller of particle sizes. However, for the micelles prepared at pH 7 and 10 the size ranged from 40 to 50 nm for both block copolymers I and II. It is revealed that the stimuli responsive properties significantly lie on the polymer compositions and consequently can be tuned and controlled by the design of molecular structures.

6.2 Introduction

Most of today's materials require additional modification or processing in order to exhibit the properties that would make them suitable for a particular application. The careful design of these building blocks needs integration of all the information necessary to direct their self-assembly into functional materials. There is a great potential in the generation and application of novel polymeric systems that exhibit new and advanced morphological, chemical and physical properties. During the past decade, self-organization of soft materials has shown to be valuable for the creation of a wide variety of nanostructures that can be used for applications in fields ranging from materials science to biology. The self-assembly of amphiphilic

polymers has resulted in assembles such as spheres, rods, vesicles, large compound micelles, nanofibers and nanotubes.[1-7] In solution, the formation of block copolymer aggregates of various morphologies can be controlled through variations in the copolymer composition, the initial copolymer concentration, the nature of the common solvent, the amount of water present in the solvent mixture, the temperature, the presence of additives such as ions, homopolymers, or surfactants, and the polydispersity of the corona chain.[8] In particular, polymeric micelles were developed as drug delivery systems[9, 10] and the hydrophobic core of the micelles was used as the drug-loading compartment and the hydrophilic outer shell as the stability interface. The nanosize of micelles makes them more easily ingested by cells, whilst it is difficult for the microgels to enter the cells due to the large size. Unlike micelles, vesicles can be used to encapsulate water-soluble substances such as vaccines, drugs, enzymes or vitamins within the vesicle cavity and also entrap hydrophobic substance within the membrane core.[11] In this context, the control of the micellar morphology is of great importance to obtain the desired functions and properties. Recently, the development of reversible addition-fragmentation chain transfer (RAFT)[12] polymerization has made it easy to directly synthesize polymers with desired ending functionality and low polydispersity.[13-15]

Stimuli responsive polymers are able to change their physical–chemical properties and colloidal properties in response to environmental stimuli, such as temperature, pH, magnetic field etc. and provide a variety of applications in many fields, such as drug delivery, biotechnology, and chromatography.[16, 17] Poly(*N*-isopropylacrylamide), PNIPAAM, shows lower critical solution temperature (LCST) behavior in aqueous solutions, and a sharp phase transition is observed at 32 °C in water.[18] Through the control of temperature, hydrophobic drugs can be encapsulated or released. During the circulation in body fluids, drug-loaded micelles will be subjected not only to temperature variations, but also to the variations of pH values. While the pH values of human body fluids radically change from 0.9 to 1.5 of stomach juice to 8.8 of pancreatic juice. Poly(*2*-(dimethylamino)ethyl acrylate), PDMAEA, is a pH-sensitive polymer in aqueous solution due to its pendent tertiary amine group, which can be quaternized in acidic aqueous solution.[19]

Fluorescent nanoparticles have attracted considerable interest over the past decade as valuable alternative to conventional organic fluorophores in a diversity of applications.[20, 21] Because of their small size, nanoparticles are minimally physically disruptive to the cellular environment, and, hence, fluorescent nanoparticles are nowadays widely studied as novel probes or sensors in life science and biochemical applications.[22, 23] Few examples of fluorescent nanoparticles with thermoresponsive and/or pH-responsive have been published. An amphiphilic, biotinylated PNIPAAM-*co*-*N*-(*3*-dimethylamino propyl)methacrylamide)-*block*-poly(ε-caprolactone) block copolymer was synthesized.[24] They found that the self-assembled micelles of the obtained block copolymer with loaded drug exhibited an altered drug release behavior depending on the different temperatures (27 and 37°C). Ma *et. al.* developed a new thermally responsive fluorescent copolymer based on triblock (PNIPAAM-b-polyfluorene-b-PNIPAAM) by atom transfer radical polymerization (ATRP).[25] The light-scattering studies in water indicated that the triblock copolymer formed a nano-scale core-shell structure in cold water and changed in conformation and solubility above its LCST. Fluorescein-functionalized nanoparticles by conjugation of fluorescein isothiocyanate to amine-coated crosslinked polystyrene-based were reported.[26] The fluorescent polymer nanoparticles form core-shell type architecture in the 20 nm diameter range and act as a ratiometric pH sensor with a measuring range between pH 4 and pH 8. Yang *et. al.* first reported two novel multifunctional copolymers consisting of a temperature-responsive PNIPAAM segment and a fluorescent fluorene-containing acrylic polymer segment with pH responsiveness.[27] This material provides sensitivity to the pH value because of the amine group and can be further quaternized to accommodate a sensing ability for DNA molecules. Carbazole-based compounds are attractive in scientific and industrial area due to their attractive properties, such as hole-transporting, high charge carrier, and electroluminescent properties.[28]

In this work, intelligent polymers responding to multiple stimuli were mainly prepared by combining thermoresponsive moieties, NIPAAM with fluorescence molecules, NVC and pH-responsive moieties, DMAEA. Herein, we develop a structural type of responsive fluorescent copolymer based on (PNIPAAM-*co*-poly(*N*-vinylcarbazole), PNVC)-*b*-PDMAEA. The dynamic light-scattering

studies in water indicated that the obtained block copolymers formed a nano-scale core-shell structure and changed in conformation with pH. Also it is revealed that the stimuli responsive properties significantly lie on the polymer structures and consequently can be tuned and controlled by fine design of molecular structures.

6.3 Experimental

Materials. *N*-Isopropylacrylamide (NIPAAM, Aldrich, 97%) was recrystallized from hexane. *N*-vinylcarbazole (NVC, Aldrich, 98%) and 2,2'-Azoisobutyronitrile (AIBN, Fluka, purum) were recrystallized from methanol. 2-(*N,N*-Dimethylamino)ethyl acrylate (DMAEA, 98%) and 1,4-dioxane (both Aldrich) were purified by distillation under reduced pressure. 2-{[(butylsulfanyl)-carbonothioyl]sulfanyl} propanoic acid was used as RAFT agent. MilliQ water was used in the preparation of micellar solutions. All other materials were used without further purification.

Characterization. ^{1}H NMR spectra were recorded with a Bruker Ultra Shield Avance spectrometer operating at 300 MHz. For all NMR analyses, unless otherwise stated, deuterated chloroform ($CDCl_3$) was used as the solvent with tetramethylsilane (TMS) as the internal standard. Molecular weights (M_n) and polydispersity index (*PDI*) were estimated by a Polymer Laboratories size exclusion chromatography (SEC) GPC-50 at 70°C on a system equipped with two sets of 5 μm Mixed C columns, a Waters R401 differential refractive index detector and a BIO-RAD, UV-1806 detector . The system was operated at the flow rate of 0.5 mL/min using DMF containing 0.5% (w/v) LiBr as the eluent and DMSO was used as a flow rate marker. Polystyrene standards with a molecular weight range of 6 035 000-162 g/mol were employed for calibration. Particle sizes were measured by a Malvern Instruments Zetasizer nano series dynamic light scattering (DLS). At least five measurements were made for each sample with an equilibrium time of 5 minutes before each measurement. Fluorescent emission intensities of the solutions were traced by a Varian Cary Eclipse Fluorescence spectrophotometer. The excitation wavelength was 300 nm. Surface tension was measured by using a Sigma 70 Tensiometer. A known

quantity of a concentrated solution of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA in water was added to a known volume of water using a Metrohm, motor driven piston burette 665 Dosimat to prepare the solutions for the tests. Each solution was stirred for 30 min after each addition and the surface tension was measured using a Du-Nouy ring.

Procedures

Synthesis of PNIPAAM-co-PNVC macro chain transfer agent (macroCTA). For a typical reaction procedure, 0.004 g (2.4×10^{-5} mol) of AIBN, 0.058 g (2.4×10^{-4} mol) of RAFT-C4, 0.47 g (2.4×10^{-3} mol) of NVC, 3.45 g (0.03 mol) of NIPAAM, and 5 ml of dioxane were mixed in a vial. The mixture was stirred at room temperature until all components were completely dissolved. Oxygen was removed from the solutions by bubbling nitrogen gas into the system for 20 minutes. After degassing, the polymerization vial was transferred to a heated oil bath maintained at 60°C. Polymerization was then stopped at desired times by quenching the reaction in an ice bath followed by determination of the conversion by ^{1}H NMR.

Synthesis of (PNIPAAM-co-PNVC)-b-PDMAEA copolymers. PNIPAAM-*co*-PNVC macroCTA (M_n = 14700 g/mol, *PDI* = 1.08) 0.88 g (6.0×10^{-5} mol), AIBN 0.002 g (1.2×10^{-5} mol) and DMAEA 1.09 g (7.6×10^{-3} mol) were weighed into vials containing stir bars and left to dissolve in 1.5 ml of dioxane. Oxygen was removed from the solutions by bubbling nitrogen gas into the system for 30 minutes. After degassing, the polymerization vial was transferred to a heated oil bath maintained at 60°C. The reaction was allowed to continue for 24 h after the completion of monomer feed in order to reach high conversion.

Micellization of (PNIPAAM-co-PNVC)-b-PDMAEA copolymers. Copolymers were weighed (0.01 g) and left to dissolve in 10 ml of MilliQ water to give solution with a concentration of 1 g/L. The pH of each micelle solution was adjusted to pH 2 to 10 using hydrochloric acid and sodium hydroxide. After pH adjustment, the solutions were filtered through a 0.2 micron membrane filters.

6.4 Results and Discussion

Reversible addition fragmentation chain transfer (RAFT) polymerization was utilized in the synthesis of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA as we reported previously.[29] In order to study the effect of copolymer composition, the two ratios of block copolymers were prepared with NIPAAM content 115 and 62, whilst keeping the DP of PNVC (DP = 5) and PDMAEA (DP = 125) constant yielding a well defined ($PNIPAAM_{115}$-*co*-$PNVC_5$)-*b*-$PDMAEA_{125}$, I, (M_n = 33 000, PDI = 1.18) and ($PNIPAAM_{62}$-*co*-$PNVC_5$)-*b*-$PDMAEA_{125}$ block copolymers, II, with M_n and PDI values of 26 000 and 1.24, respectively.

Copolymers were weighed (0.01 g) and left to dissolve in 10 ml of MilliQ water to give solution with a concentration of 1 g/L. The pH of each of the solutions was adjusted to pH 2 to 10 using hydrochloric acid and sodium hydroxide. After pH adjustment, the solutions were filtered through a 0.2 micron membrane filters. Particle sizes of the samples were determined by DLS (Figure 6.1). With increasing temperature, the mean diameters of the particles were observed to be nearly constant within the temperature range of 25°C to 60°C in all pH range. Below the CMT, the block copolymers should be molecularly dissolved (unimers) in solution. Instead, the mean diameters values observed below the CMT suggest that the block copolymers are not present as unimers. This suggests the presence of macromolecular assembly of the block copolymers even at temperatures below the CMT. At 25°C, which is clearly lower than CMT of pure PNIPAAM, the micelles were formed. The shift of CMT to lower temperature is attributed to the hydrophobic NVC segments. As it was reported for PNIPAAM-*b*-poly(acrylic acid) (PNIPAAM-b-PAA) diblock copolymer, the LCST of PNIPAAM is raised to 35°C when the PAA block is hydrophilic at pH 5-7 and is lowered to 29°C when the PAA is hydrophobic at pH 4.5.[30] Considering that PDMAEA block can be quaternized in acidic media, we investigated the effect of pH value on the self-organization morphologies of block copolymers in aqueous solution. In the all range of pH, both ratios of diblock copolymers could aggregate to form macromolecular self assembly by means of the hydrophobic (PNIPAAM-*co*-PNVC) group and the hydrophilic PDMAEA. The particles size was measured at various pH by DLS. It was found that the particle size

prepared at pH 2 and 4 ranged from 160 to 200 nm and 100 to 120 nm for diblock copolymers I and II, respectively. The shorter of PNIPAAM block resulting in the smaller of particle sizes. However, for the micelles prepared at pH 7 and 10 the size ranged from 40 to 50 nm for both block copolymers I and II, which is smaller than the particles prepared at pH 2 and 4. This suggests the formation of vesicles at low pH and micelles at high pH. McCormick and coworkers have reported thermally responsive vesicles from the self-assembly of thermally responsive diblock copolymers of poly[*2*-(dimethylamino)ethyl methacrylate] (PDMAEMA), which molecular structure resembles PDMAEA, and PNIPAAM in aqueous solution. However they did not seem to observe the effect of the pH on the particles morphology. At a low pH, the protonated PDMAEA chains are highly stretched due to the intramolecular electrostatic repulsions of positive charge, leading to micelles of larger size. As the pH is lowered, more amine groups of the PDMAEA block are protonated, and the size of the micelles increases. At high pH, the PDMAEA chains are deprotonated and collapsed in the shell of the micelles, leading to smaller apparent size of the micelle. Upon the increasing pH from acidic to basic pH, the morphology of diblock copolymers change form vesicles to spherical micelle.

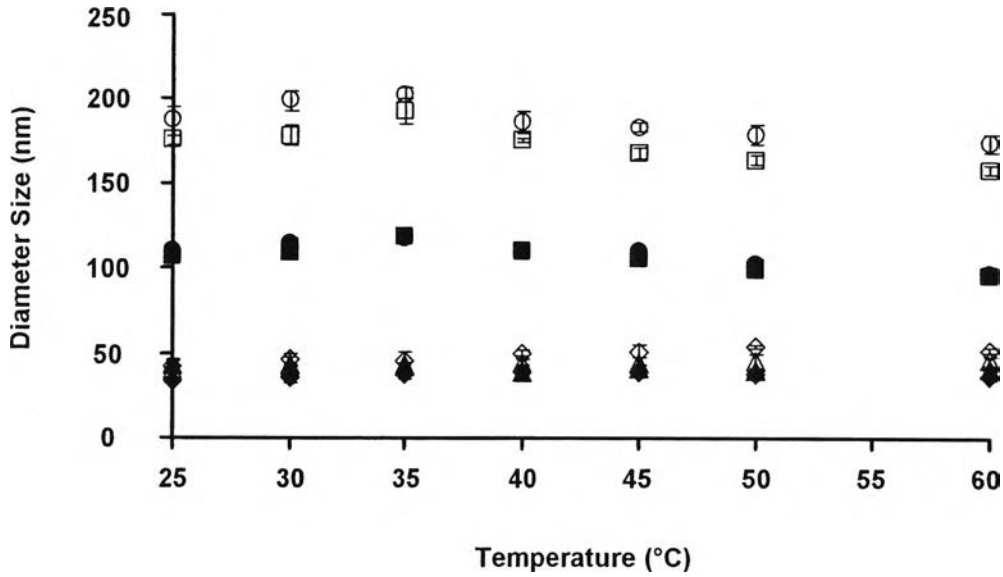

Figure 6.1 Variation of particle size with temperature for 1 g/L aqueous solutions of (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ diblock copolymers at pH 2 (○), 4 (□), 7 (Δ) and 10 (◊) and (PNIPAAM$_{62}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ diblock copolymers at pH 2 (●), 4 (■), 7 (▲) and 10 (♦).

The concentration at which the amphiphilic block copolymers form a micelle at a fixed temperature is the CMC. To test the CMC, the surface tension of aqueous solutions of amphiphilic block copolymers was measured by varying the concentrations of the block copolymer in the aqueous solutions. In the case of (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ at 25°C, pH 10, the CMC was measured to be 2.12×10^{-3} g/L, with a surface tension of 0.042 N/m as shown in Figure 6.2. The result suggests that micelles are readily formed at 25°C and confirmed previous results from DLS.

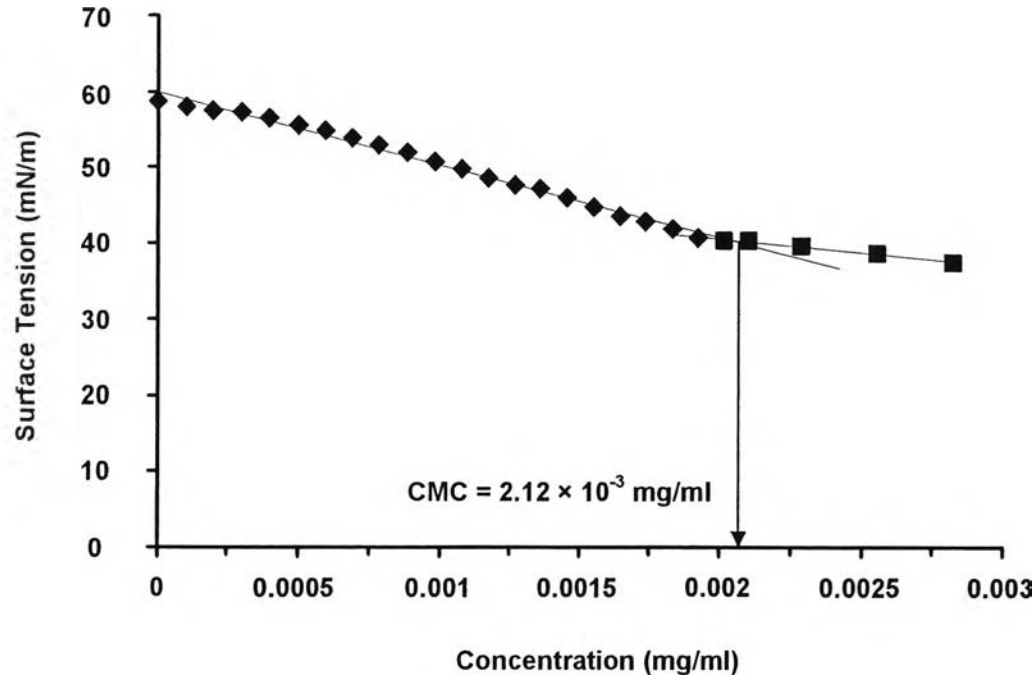

Figure 6.2 Critical Micellization Concentration (CMC) of (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ at 25 °C.

The carbazole-containing polymers were characterized in terms of their fluorescent properties, as shown in Figure 6.3. Both ratios showed characteristic fluorescence emission with a peak of 354 nm and 365 nm when excited by a 300 nm light in aqueous solution. The carbazole chromophore is known to give a partial overlap (second) and a full-overlap (normal) excimer emission at 370 and 420 nm, respectively.[31, 32] Almost the same in the peak intensities without any significant difference in the peak were observed in those block copolymers as the same amount of PNVC. Therefore, the introduction of PDMAEA segments into the (PNIPAAM-*co*-PNVC) chain did not change the fluorescence properties of PNVC.

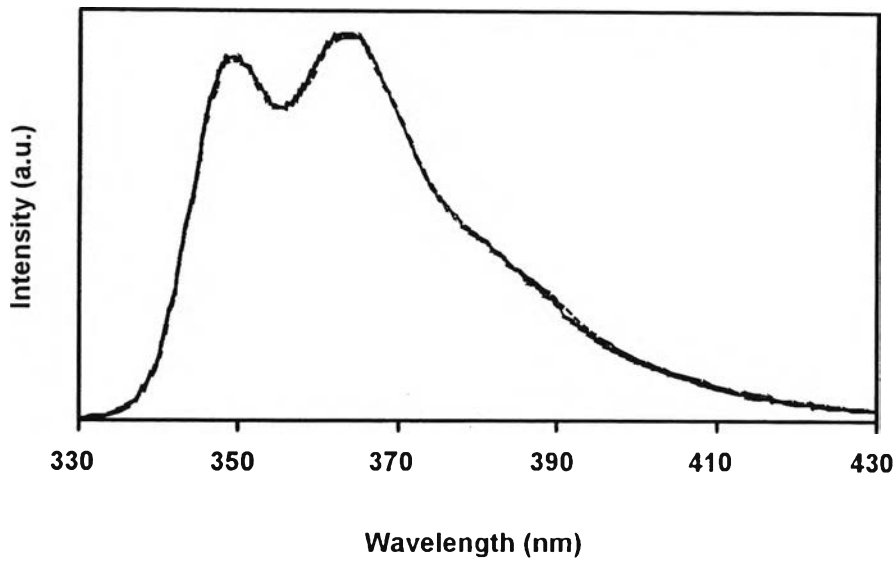

Figure 6.3 Fluorescence Spectra for 0.5 g/L aqueous solutions of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ and (PNIPAAM$_{62}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ diblock copolymers (λ_{ex} = 300 nm).

6.5 Acknowledgement

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CHAPTER VII

MOLECULAR ASSEMBLY VIA NANO-SIZE PARTICULATE MIXED MICELLE SYSTEM: A MODEL STUDY FROM CHITOSAN NANOSPHERE AND pH–THERMO-MULTI-RESPONSIVE FLUORESCENT MICELLES

7.1 Abstract

Mixed micellization behavior of two amphiphilic copolymers: chitosan grafting with phthalic anhydride and polyethylene glycol and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymer was reported. Both amphiphilic copolymers were mixed by two different methods: (1) separate mixing of each block copolymer in water solution and (2) premixing. For separate mixing, dynamic light scattering (DLS) indicates the coexistence of the individual micelles of chitosan nanosphere and multi responsive micelle of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA at pH 3-6 by negative charge of polyethylene glycol and positive charge of PDMAEA. When amphiphilic chitosan and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymer are mixed as a premixed solution, two block copolymers form micelles with mixed core of phthalic anhydride of chitosan and ($PNIPAAM_{115}$-*co*-$PNVC_{10}$) block and mixed corona of polyethylene glycol of chitosan and PDMAEA chain with pH independent. A strategy to yield stable mixed micelles in an aqueous environment from significantly different shell-forming block copolymers could, for instance, be used for application in biomedicine including drug-delivery systems (DDS).

7.2 Introduction

Complex formation between polyelectrolyte and oppositely charged amphiphilic polymer has been a subject of intense research effort for both fundamental and technological reasons.[1-5] Knowledge acquired from the study of polyelectrolyte interactions can be applied to important industrial and biological processes.[6] Continued advances in these industrial fields depend on the ability to manufacture precisely controlled building blocks (i.e., nanostructured materials)

tailor-made for specific applications in a simple manner.[7, 8] The use of mixed micelles formed from a mixture of two block copolymers with distinctly different properties can be a plausible candidate that is capable of tuning the resulting properties and meeting various requirements for specific applications.[9-12] However, the overwhelming majority of polymers differing in chemical nature are usually incompatible, although a sufficient difference in the properties between shell-forming blocks is necessary for the mixed micelles to be effective in applications.[13] The formation of stable mixed micelles requires their spontaneous formation due to strong interactions between two blocks constituting either core or shell of micelles. Traditionally, it has been achieved by (i) the cross-linking of either core or shell of premixed micelles[11] and (ii) the polymer-polymer complex formation mediated by noncovalent interactions between blocks.[12, 14, 15] Despite the improved stability of the chemical cross-linking, this approach may not be optimal in the encapsulation of a guest molecule or biodegradability. Here, we focus on a simple and versatile approach to stabilized mixed micelles through the use of noncovalent interactions that are still dynamic. Well-studied examples for mixed micelles driven by noncovalent interactions include (i) a polyelectrolyte complex between oppositely charged block ionomers,[12, 14] (ii) a hydrogen-bonding complex between a poly(methacrylic acid) and a polyether or polyol,[15] and (iii) a nucleic acid pair between block copolymers tagged with a H-bonding complementary nucleic acid.[16] In spite of the attractiveness of previous reports, their micellization behavior has largely been limited to the strong affinity between shell-forming blocks (case ii) or the micelle formation in organic medium (case iii), depending on the natures of noncovalent interactions. Thus, a strategy to yield stable mixed micelles in an aqueous environment from significantly different block copolymers is highly desirable for application in biomedicine.

A typical approach to make mixed micelles is mixing two micelle-forming block copolymers of A-B and A-C type with a common insoluble A block, which may form a micelle with a core of aggregated A block and mixed coronas of B and C chains. In many cases, the B and C chains behave differently to environmental changes, and various potential applications could be anticipated with such mixed micelles. In addition, physical chemistry of chain mixing in such a confined

geometry is also a subject of interest. However, the formation of mixed micelles is not a trivial process since the B and C chains are usually incompatible in the absence of favorable interactions.[17] Previously, our group reported the preparation of the amphiphilic chitosan nanospheres by simply grafting phthalic anhydride as a hydrophobic group and polyethylene glycol as a hydrophilic chain to obtain core–corona structured chitosan.[18] The nanospheres were formed depending on the types of the solvent whereas their self assemble phenomena are confirmed under the polar solvents. The chitosan nanospheres showed highly negative charged surface (-5 to-40 mV for pH range 4-12).[19] Recently, we reported pH- and thermo- multi-responsive fluorescent micelles of the block copolymers poly[(*N*-isopropyl-acrylamide-*co*-*N*-vinylcarbazole)-*b*-2-(dimethylamino)ethyl acrylate], (PNIPAAM-*co*-PNVC)-*b*-PDMAEA. The protonation ability of the PDMAEA chains permits the surface charge diversity by simply varying the pH.[20] The objective of this study is to explore the possibility of complexation as a noncovalent driving force between dissimilar polymer chains and form stable mixed micelle. Here, we made use of electrostatic interactions at various pHs to control the formation of the superstructure, amphiphilic chitosan and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA micelle. Such a modular concept allows producing complex superstructures with pH responsive for special applications, made from biocompatible polymers, could for instance be used as advanced drug carrier systems.

7.3 Experimental

Materials

Chitosan with deacetylation percent (%DD) of 95 with the corresponding viscosity average molecular weights (Mv) of 3.37×10^5 Da was the gift from Seafresh Chitosan (Lab) Company Limited. Phthalic anhydride, polyethylene glycol monomethyl ether (mPEG, Mn = 2000), and succinic anhydride were purchased from Fluka Chemika, Switzerland. 1-Ethyl-3-(30-dimethylaminopropyl) carbodiimide, hydrochloride (EDC) and 1-hydroxy-1H-benzotriazole, monohydrate (HOBt) were bought from TCI, Japan. *N,N*-dimethylformamide (DMF), dimethylsulfoxide (DMSO) were purchased from LabScan, Ireland. *N*-Isopropylacrylamide (NIPAAM,

Aldrich, 97%) was recrystallized from hexane. *N*-vinylcarbazole (NVC, Aldrich, 98%) and 2,2'-Azoisobutyronitrile (AIBN, Fluka, purum) were recrystallized from methanol. 2-(*N,N*-Dimethylamino)ethyl acrylate (DMAEA, 98%) and 1,4-dioxane (both Aldrich) were purified by distillation under reduced pressure. 2-{[(Butylsulfanyl)-carbonothioyl]sulfanyl} propanoic acid (RAFT-C4) was used as RAFT agents. MilliQ water was used in the preparation of micellar solutions. All other materials were used without further purification.

Characterization

Dynamic Light Scattering (DLS). Particle sizes and zeta potential were measured by a Malvern Zetasizer Nano (Malvern Instruments Ltd.) dynamic light scattering (DLS) with a detection angle of 173°, and the intensity size distributions were obtained from analysis of the correlation functions using the multiple narrow modes algorithm in the instrument software. At least five measurements were made for each sample with an equilibrium time of 5 minutes before each measurement.

Transmission electron microscopy (TEM). Samples were made by placing a drop of sample onto a carbon coated copper grid followed by addition of a drop of a staining solution (2% phosphotungstic acid). Excess solution was carefully blotted off using filter paper and samples were air dried for a few minutes before analysis. TEM images were obtained using a H-7650 Hitachi transmission electron microscope at 100 kV.

Procedures

Preparation of amphiphilic chitosan. Chitosan grafting with phthalic anhydride as hydrophobic group and polyethylene glycol as hydrophilic group was prepared as report previously.[18]

Preparation of (PNIPAAM-co-PNVC)-b-PDMAEA copolymers. (PNIPAAM-*co*-PNVC)-*b*-PDMAEA copolymers was synthesized as report previously with full characterization.[20]

Micellization of amphiphilic chitosan and (PNIPAAM-co-PNVC)-b-PDMAEA copolymers. Two different mixing methods were used: separation and premixing to prepare mixed micelle. In the separation method, the solutions of amphiphilic

chitosan and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA copolymers in distilled water were prepared separately at a concentration of 1.0 mg/mL each. The two solutions were mixed together and leave for overnight. In the premixing method, amphiphilic chitosan and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA copolymers are dissolved in DMF at a mass ratio of 1:1. The mixture was dialyzed using a dialysis tubing cellulose membrane (12,400 molecular weight cut off) against distilled water to obtain the mixed micelle in white colloidal solution form. The pH of each solution was adjusted ranging from pH 3 to 10 using hydrochloric acid and sodium hydroxide.

7.4 Results and Discussion

The series of the solutions of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{106}$ diblock copolymer and amphiphilic chitosan (1 g/L) at pH 3-10 were mixed by separation methods with 1:1 ratio. The colloidal solutions were analyzed by dynamic light scattering, DLS, to evaluate the particle size at each pH. The size was averaged from at least five measurements. Figure 7.1 shows the plot of diameter size of chitosan nanosphere, copolymer micelle, and mixed micelle with zeta potential by separation method at pH range 3-10. The particle sizes of copolymer micelles are in the range of 40-60 nm whereas chitosan nanospheres are in the range 1500-2000 nm. After separated mixing at pH 4-5, the mixed micelles are bigger than the size of chitosan nanosphere implying the complexation of copolymer micelle and chitosan nanosphere. However, the particle sizes of chitosan nanosphere and mixed micelle are nearly the same for pH 3 and 6-10 implying no interaction of nanosphere and micelle. These phenomena can be explained by the zeta potential of chitosan nanosphere, polymer micelle and mixed micelle system as shown in Figure 7.2. Chitosan nanosphere showed positive charged surface for pH 3 and negative charged for pH range 4-12 from polyethylene glycol. For copolymer micelle, at low pH, the PDMAEA chains are protonated, leading to dispersion of positive charge around the surface of micelle. As this pH is further decreased, the amine groups of the PDMAEA block are further protonated, thus resulting in an increase in positive charge. In contrast, at high pH, the PDMAEA blocks are deprotonated, which leads to the collapse of the shell of the micelle, and as a result, the surface charges are

negative. Therefore, the polyelectrolyte complexation of negative charge chitosan nanosphere and positive charge copolymer micelle specifically occurs in the pH range 4-5. The higher amount positive charge of copolymer micelle than the negative charge of nanosphere leads to the net positive zeta potential of mixed micelle at pH 4-5.

The DLS results were confirmed by TEM imaging. Figure 7.3 shows TEM images of particles obtained at pHs 4 (Figure 7.3a and 7.3b) and 10 (Figure 7.3c and 7.3d). Figure 7.3a shows particle of sizes around 1.5 μm, with a dark inner layer of thickness *ca.* 1 μm. This morphology suggests the formation of mixed micelle from chitosan nanosphere surrounded by a lot of polymer micelles with the size 40-50 nm as shown the enlarge picture in Figure 7.3b. On the other hand, Figure 7.3c shows a clear image of particles with sizes 150 nm, thus implying the chitosan nanosphere at pH 10.[19] In the same time, polymer micelles with the size 40-50 nm were founded in other areas (Figure 7.3d). Figure 7.3c and 7.3d confirmed the non-forming of mixed micelle at high pH.

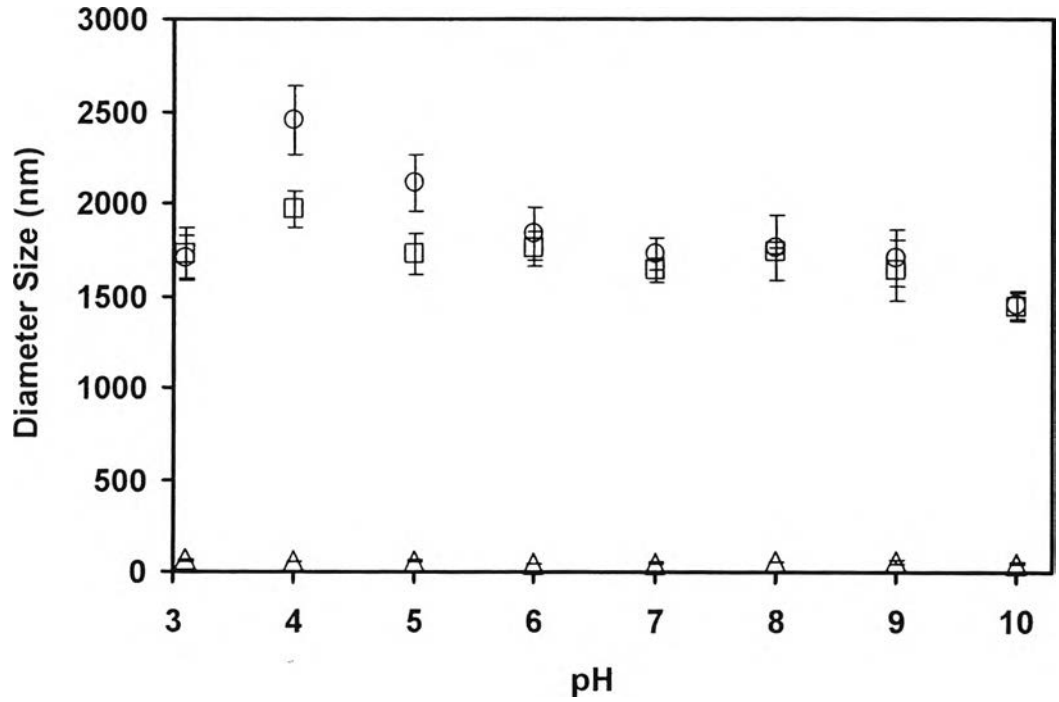

Figure 7.1 Particle size with pH for chitosan nanosphere (□) copolymer micelle (△), and mixed micelle (○) prepared by separation method.

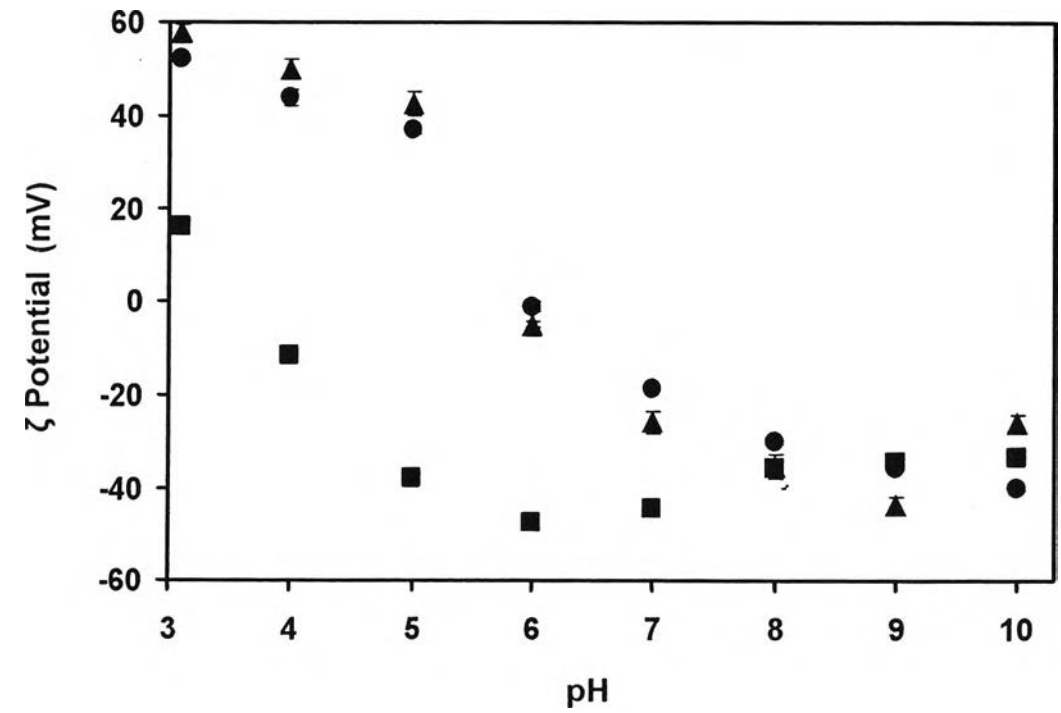

Figure 7.2 Zeta potential with pH for chitosan nanosphere (■) copolymer micelle (▲), and mixed micelle (●) prepared by separation method.

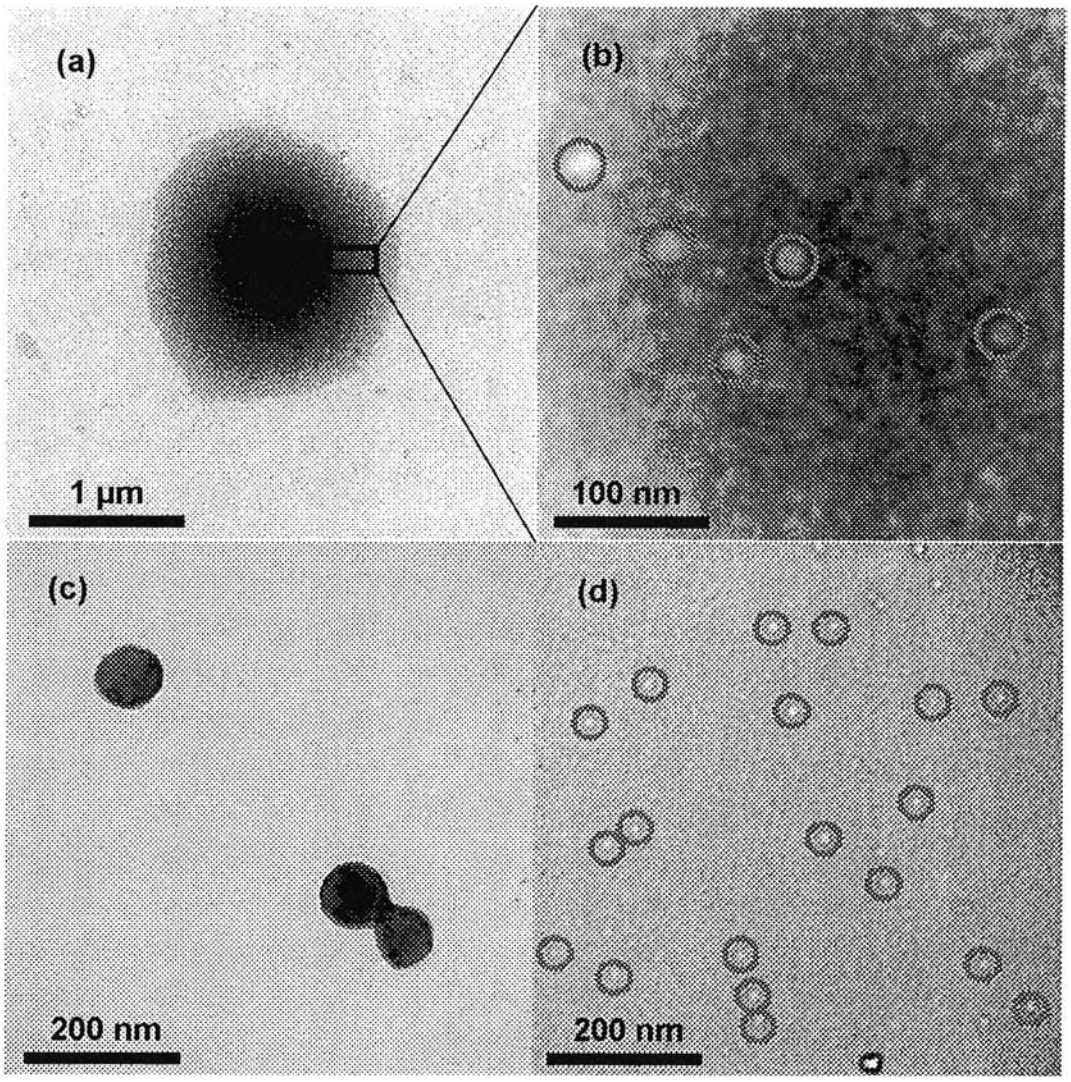

Figure 7.3 TEM images of mixed micelle prepared by separation method (a) pH 4 (b) enlargement of a (c) and (d) pH 10 after negative staining.

The aggregate formation was also found to depend on the formation condition. By premixing method, mixed micelles were prepared by mixing of chitosan amphiphilic polymer and (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{106}$ diblock copolymer in DMF and, then, dialysis against water to form macromolecular structure. The results of diameter size of chitosan nanosphere, copolymer micelle, and mixed micelle by premixing method at pH range 3-10 were shown in Figure 7.4. DLS analyses clearly show that the sizes of the mixed micelles for all pH range are in the range 300-400 nm which smaller than chitosan nanosphere (1600-5200 nm) and copolymer micelle (600-850 nm). This implied the hybrid core-shell formation of amphiphilic chitosan and (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{106}$ diblock copolymer, with the hydrophobic phthalic anhydride and (PNIPAAM$_{115}$-co-PNVC$_{10}$) block forming the core and hydrophilic polyethylene glycol and PDMAEA chain forming the corona of the micelle. The zeta potential of chitosan nanosphere, copolymer micelle, and mixed micelle were shown in Figure 7.5. The zeta potentials of mixed micelle are positive in pH range 3-5 when polymer micelle shows positive surface charge. For pH 6-10, the mixed micelles show the negative surface charge. However, there is no effect of the linear decreasing of zeta potential on forming the mixed micelle when the pHs were varied from 3-10. The size of mixed micelles not significant change upon varying pHs. TEM images of the mixed micelles prepared by premixing method (Figure 7.6) confirm the DLS analysis, by showing spherical micelles with diameter 250 nm at pH 4 (Figure 7.6a) and 10 (Figure 7.6b), respectively. The images show the dark spot spreading on the mixed micelle. This implied the (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{106}$ diblock copolymer chain was mixed in the mixed micelle with mPEG grafted phthaloylchitosan chain. The particle sizes estimated from TEM were smaller than those obtained by DLS, as TEM imaging reflects the conformation of the particles in their dry state, while the DLS reflects the size of the particles with their shell fully hydrated. There is no significant difference in terms of size and shape of mixed micelle at pH 4 and 10. The results confirmed the independent of forming stable mixed micelle on pH.

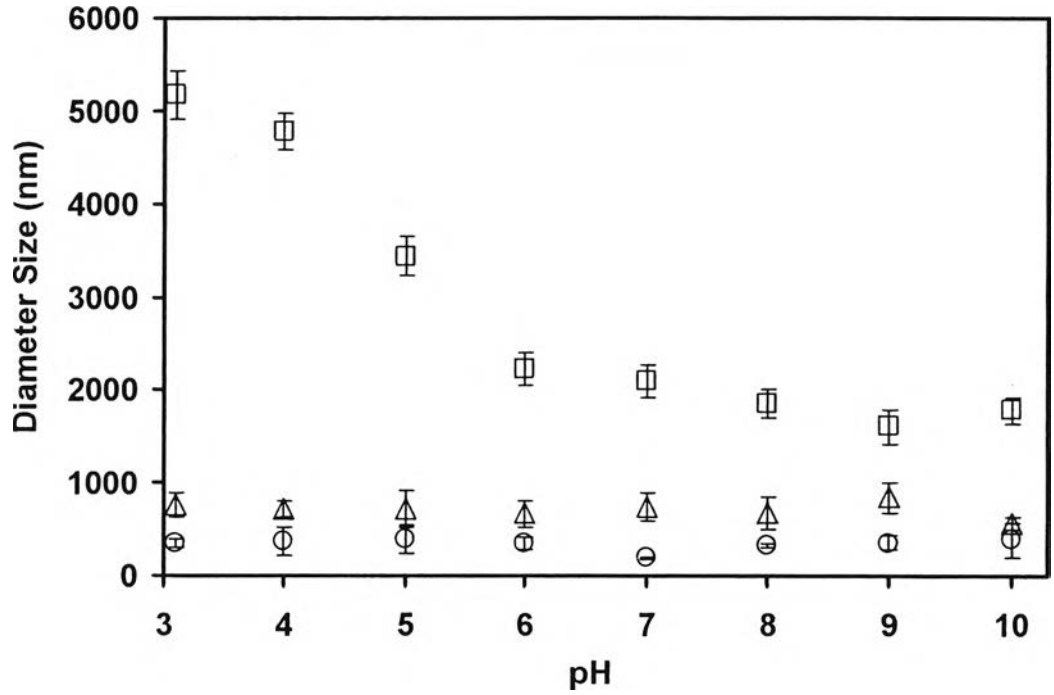

Figure 7.4 Particle size with pH for chitosan nanosphere (□) copolymer micelle (△), and mixed micelle (○) prepared by premixing method.

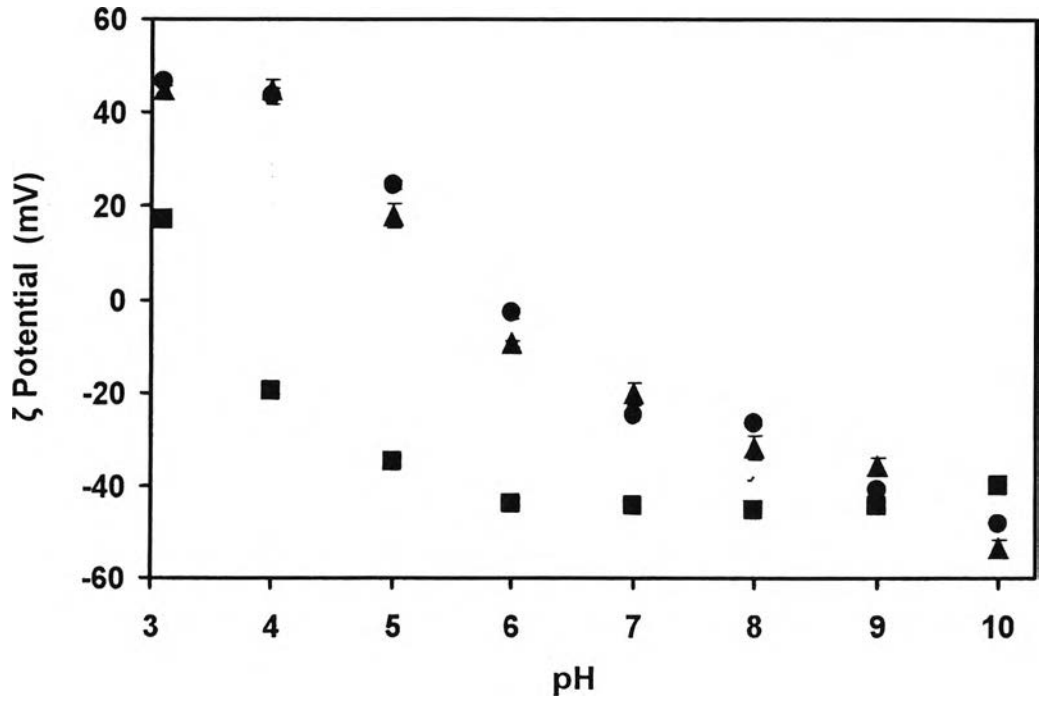

Figure 7.5 Zeta potential with pH for chitosan nanosphere (■) copolymer micelle (▲), and mixed micelle (●) prepared by premixing method.

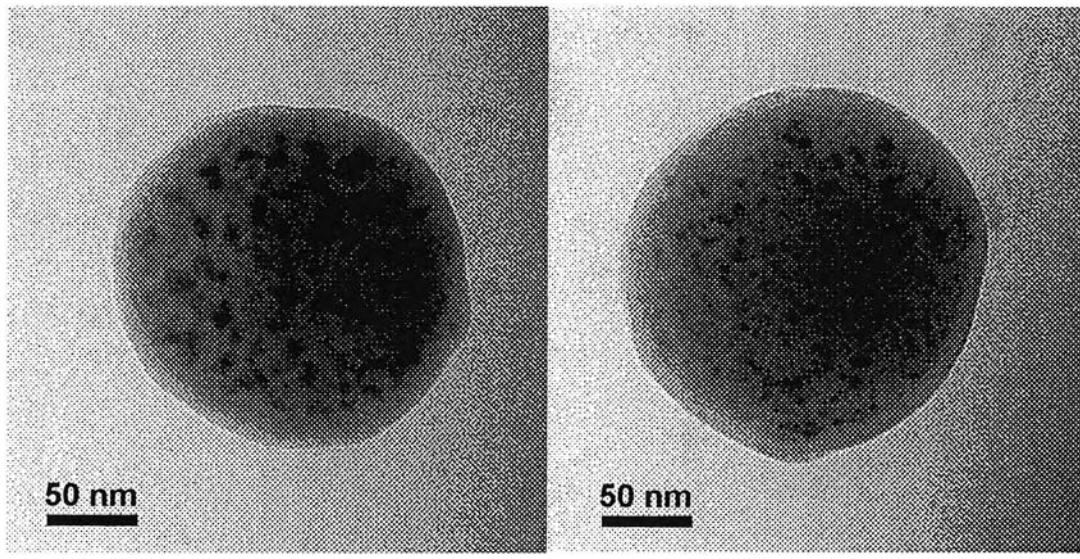

Figure 7.6 TEM images of mixed micelle prepared by premixing method (a) pH 4 and (b) pH 10 after negative staining.

7.5 Conclusions

In summary, chitosan grafting with phthalic anhydride and polyethylene glycol and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymer form mixed micelles separately, two kinds of micelles consisting of each block copolymers were formed. The results indicate that the two separate micelles form mixed micelle in the specific pH range 4-5. When two copolymers form mixed micelle with a premixed solution, the diameter sizes are in the range 300-400 for pH 3-10. The diameter size of mixed micelles which much lower than sizes of chitosan nanosphere and multi responsive micelle indicates the hybrid micelle structure for all pH range 3-10.

7.6 Acknowledgement

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