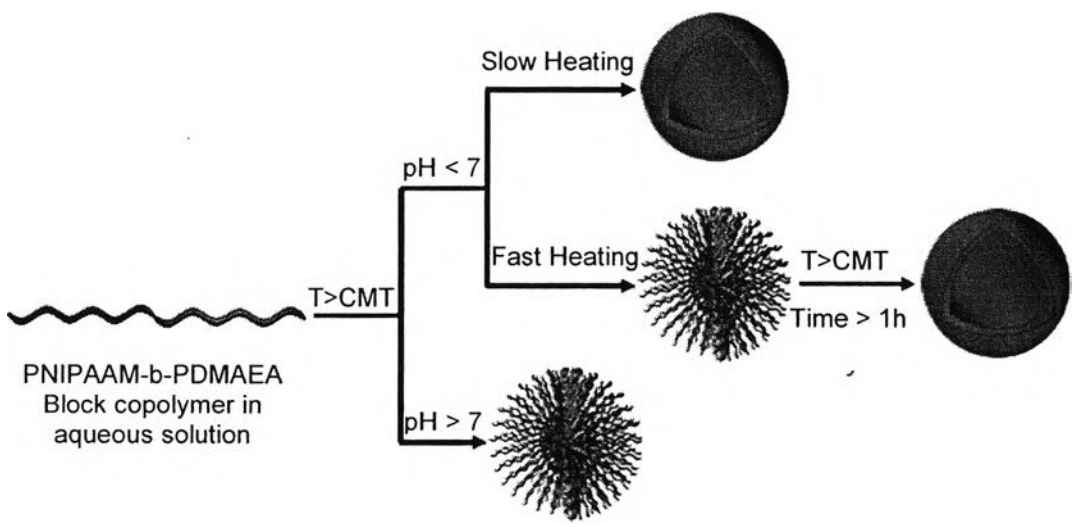
CHAPTER V
CONTROL OF BLOCK COPOLYMER MORPHOLOGY: AN EXAMPLE OF SELECTIVE MORPHOLOGY INDUCED BY SELF ASSEMBLY FORMATION CONDITION

5.1 Abstract

An example case of selective morphology by simply varying pH and heating profile based on a diblock copolymer, i.e. poly(*N*-isopropylacrylamide) (PNIPAAM) and poly[*2*-(dimethylamino)ethyl acrylate] (PDMAEA) is reported. A variation of pH induces an aggregation of the block copolymers in either micelles or vesicles. In a subsequent step, temperature variation triggers the formation of vesicular structures. This demonstrates that not only the temperature but also the heating rate tunes the nanostructures from micelles to vesicles.

Keywords : block copolymers, responsive, micelles, vesicles

5.2 Graphical Abstract



5.3 Introduction

The rapid progress of nanotechnology in medical fields such as nanomedicine and biotechnology are calling for better defined and finely tuned nanostructures. A practical way to engineer nanostructure via bottom-up approach is to apply the self-assembly of polymers[1,2] In the case of molecular assembly of the block copolymers, at a given concentration, the copolymers with hydrophobic block and hydrophilic block self-organize their interactions into a variety of nanostructures of well-defined morphology to form spheres, vesicles, rods and lamellae.[3-6] Vesicle morphology is especially interesting as it mimics the structure of cells, with a hollow hydrophilic core separated from its surrounding by a membrane formed by a bilayer of amphiphiles.[7,8] This unique structure makes vesicles ideal candidates for encapsulation of functional hydrophilic substances such as vaccines, drugs, enzymes or vitamins within the vesicle cavity, and for the entrapment of hydrophobic substances within its membrane; their applications range from cosmetics to drug delivery.[9] Recent advances in polymer synthesis develop unique vesicle structures which are responsive to the environment. Among the various environmental parameters, pH[10] and temperature[11-13] are simple and effective to control the assembly systems. Chécot et al. showed that polybutadiene-*b*-poly(L-glutamic acid) (PB-*b*-PGA) diblock copolymer vesicles responded to pH or to ionic strength with a change in their hydrodynamic radius.[14] Lecommandoux and coworker demonstrated the formation of 'schizophrenic' vesicles, which could be reversibly produced in moderate acidic or basic aqueous solutions from polypeptide diblock copolymers.[15] Armes and coworkers reported a new type of shape-persistent polymeric vesicles with pH-tunable membrane permeability. These structures were formed by a self-assembly of either a pH-responsive of hydrolytically self-cross-linkable copolymer, poly(ethylene oxide)-*block*-poly[*2*-(diethylamino)ethyl methacrylate-*stat*-3-(trimethoxysilyl)propyl methacrylate], [PEO-*b*-P(DEA-*stat*-TMSPMA)], in THF/water mixtures or a biocompatible block copolymer produced form the polymerization of *2*-

(methacryloyloxy)ethyl phosphorylcholine (MPC) and *2*-(diisopropylamino)ethyl methacrylate (DPA).[16,17]

Recently, McCormick and coworkers first reported the structure adopted by block copolymers of *N*-(3-aminopropyl)-methacrylamide hydrochloride, AMPA, and *N*-isopropylacrylamide, which exist as a unimer in aqueous solution, and self-assemble into vesicles when the solution temperature is increased.[18] The self assembly relies on the thermoresponsive poly(*N*-isopropylacrylamide) (PNIPAAM), which undergoes a coil-globular transition and switches form hydrophilic to hydrophobic when the temperature reaches its lower critical solution temperature (LCST).[19] The same group reported the use of a similar system, based on the block copolymers of poly[*2*-(dimethylamino)ethyl methacrylate] (PDMAEMA) and PNIPAAM to encapsulate gold nanoparticles.[20] PNIPAAM was also associated to poly(ethylene oxide) (PEO) by Discher and coworkers,[9] and to poly(sodium 2-acrylamido-2- methylpropanesulfonate) (PAMPS) as reported by Maci et al.[21] show vesicular structure with thermoresponsive manner.

Since the nature of aggregation phenomena is governed by subtle changes in defined factors, it is possible to induce a transition between aggregate structures by slight perturbations of any given system.[22,23] For instance, Schilli et. al.[24] have indicated the formation of large aggregates at pH 4.5 and micelles at pH 5-7 with temperature above LCST from PNIPAAM-*b*-polyacrylic acid, PAA. Aggregation formation can be influenced by the properties of the building block (e.g. pH and temperature tuning[25-27]) including the method and/or conditions of preparation.[28,29] For example, McCormick and coworkers reported that variations in morphology from micelles, worm-like micelles and vesicles could be obtained from the block copolymer PDMAEMA-*b*-PNIPAAM, by changing the balance of the hydrophobic / hydrophilic segments in the copolymer.[30] Soo and Eisenberg demonstrated that the variables that control the formation of block copolymer aggregates include the copolymer composition, the initial copolymer concentration, the nature of the solvent, the amount of water present in a solvent mixture, the temperature, the presence of additives such as ions, homopolymers, or surfactants, and the polydispersity of the polymeric chains forming the corona.[22] Such a

wealth of factors influencing aggregation makes these systems very complex, and it is therefore desirable to develop systems for which the vesicle morphology can be controlled by simple means.

Herein, we proposed an example of temperature-induced molecular assemblies of block copolymers of PNIPAAM and poly[*2*-(dimethylamino)ethyl acrylate], PDMAEA, synthesized via the RAFT process, for which the morphology can be controlled by simply varying the pH and heating profile. In this system, PNIPAAM shows a variation in hydrophilicity depending on the system temperature, and PDMAEA changes its conformation from random coil to extended chains at a specific pH. This combination of variations in morphology is attractive, as it is a simple route[31] to obtaining vesicle structures.

5.4 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 (RAFT-C4) was synthesised as reported previously.[32,33] MilliQ water was used in the preparation of micellar solutions. All other materials were used without further purification.

Characterization

Nuclear Magnetic Resonance (NMR). Proton NMR spectra were recorded with a Bruker Ultra Shield Avance spectrometers operating at 300 MHz. For all NMR analyses, unless stated otherwise, deuterated chloroform ($CDCl_3$) was used as the solvent with tetramethylsilane (TMS) as the internal standard.

Size Exclusion Chromatography. Molecular weights (M_n) and polydispersity index (PDI) were estimated by size exclusion chromatography (SEC) at 70°C on a system equipped

with two sets of Polymer Laboratories 5 μm Mixed C columns with both the differential refractive index detector (Waters, R401) and UV detector (BIO-RAD, UV-1806). 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 MW range of 6 035 000-162 g/mol were employed for calibration.

Dynamic Light Scattering (DLS). Particle size measurements were carried out by dynamic light scattering (DLS) using a Malvern Instruments Zetasizer nano series instrument 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 100kV.

Methods

Synthesis of PNIPAAM macro chain transfer agent (macroCTA). The PNIPAAM macroRAFT agent was synthesis following a procedure previously reported by Perrier and coworkers.[34,35] 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, 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), deoxygenated with nitrogen gas (20 min), and then immersed in a heated oil bath 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.

M_n (determined by GPC) = 26 300 g/mol; PDI = 1.08; M_n (determined by NMR = 12 950 g/mol.

^{1}H NMR (δ, ppm): 1.1-1.3 (C**H**-C**H$_2$**), 1.2 (6H, s, (C**H$_3$**)$_2$), and 4.1 (1H, s, NH-C**H**-(CH$_3$)$_2$).

Synthesis of PNIPAAM-b-PDMAEA copolymers. PNIPAAM macroCTA (M_n = 13000 g/mol, PDI = 1.06) 0.78 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 vial containing stir bars and left to dissolve in 1.5 ml of dioxane. Oxygen was removed from the solutions by bubbling nitrogen gas into 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.

M_n (determined by GPC) = 47 000 g/mol; *PDI* = 1.12; M_n (determined by NMR = 28 000 g/mol.

^{1}H NMR (δ, ppm): 1.2 (6H, s, C**H$_3$**), 1.4-2.1 (CH-C**H$_2$**-C**H**-C**H$_2$**-C**H**-C**H$_2$**-C**H**), 2.26 (6H, s, (C**H$_3$**)$_2$, 2.6 (2H, t, CH$_2$-C**H$_2$**-N(CH$_3$)$_2$, 4.1 (1H, s, NH-C**H**-(CH$_3$)$_2$), and 4.2 (2H, t, O-C**H$_2$**-CH$_2$).

Micellization of PNIPAAM-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 of the micelle 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.

5.5 Results and Disscussion

Reversible addition fragmentation chain transfer (RAFT) polymerization[36-38] was used to synthesize diblock copolymers from the thermally responsive NIPAAM and pH-responsive DMAEA. RAFT polymerization is one of the most versatile techniques to produce a wide range of functional polymeric architectures.[31,39,40] The PNIPAAM homopolymer was synthesized in dioxane at 60°C using *2*-{[(butylsulfanyl)-carbonothioyl]sulfanyl} propanoic acid as the RAFT agent and AIBN as the initiator (M_n=13 000 g/mol, *PDI*=1.06). The prepolymer obtained was employed as a

macromolecular chain transfer agent for RAFT polymerization of DMAEA. The polymerization gave a well-defined $PNIPAAM_{115}$-*b*-$PDMAEA_{106}$ diblock copolymer of molecular weight M_n = 28 000 g/mol with PDI of 1.12.

A series of the solutions of $PNIPAAM_{115}$-*b*-$PDMAEA_{106}$ diblock copolymer (1 g/L) at pH 2, 4, 7 and 10 were prepared. The colloidal solutions were analyzed by dynamic light scattering, DLS, to evaluate the particle size (Figure 1) at each pH. The size was averaged from at least five measurements, and was found to have a narrow distribution, which implies the stability of the particles at each pH. At pH 10, sizes around 5 nm with large PDIs were observed for temperature varying from 25 to 50 °C, thus implying the block copolymers exist as a unimer in the solution. However, when the temperature is increased above 50°C, DLS revealed an increase in size to 50 nm, with a drop in PDI, which reflects the formation of micelles. Usually, the LCST (lower critical solution temperature) is used to define the temperature for which the polymer shows a phase transition. In our case, since only a section of the block copolymer, i.e., PNIPAAM, responds to such a phase transition, and this transition leads to the formation of aggregates, the critical micelle temperature (CMT) is more appropriate to describe the aggregate or micelle formation. In the present study, a CMT of 50°C was observed at pH 10. A similar trend is observed at pH 7, with a CMT of 40-45 °C. For pHs 2 and 4, the CMT is measured at 30-35 °C, which is close to the LCST of PNIPAAM (32 °C). These results are in good agreement with reports indicating that the LCST of PNIPAAM increases when the pH is raised above pH 7.[41] It is noteworthy that at all pHs, after the aggregates are formed they tend to decrease in size. This phenomena is due to either a decrease in the aggregation number or the further dehydration of the PNIPAAM blocks.[42]

DLS reveals that at the temperatures above CMT, the particle sizes are in the range of 180 - 270 nm for pHs 2 and 4, whereas they are in the range of 40 - 70 nm for pHs 7 and 10 (Figure 1). As observed in other studies,[18] a diameter range of 180 to 270 nm for the aggregates in acidic solution suggests the formation of vesicular rather than micellar structures due to the fact that the size is much larger than the block copolymer

contour length (60 nm). Indeed, Li et al[20] have shown that PDMAEMA$_{73}$-*b*-PNIPAAM$_{99}$ block copolymers formed vesicles with an average diameter of 200 nm above the phase-transition temperature in aqueous solution, although their studies did not include the effect of pH on diameter size and morphology. Moreover, the very low polydispersities obtained for the large aggregates (PDI < 0.05) suggests further the formation of vesicles.

The DLS results were confirmed by TEM imaging. Figure 2 shows TEM images of particles obtained at pHs 2 and 10. Figure 2a shows particle of sizes around 200 nm, with a dark outer layer of thickness *ca.* 60 nm. This morphology suggests the formation of vesicular structures.[†] On the other hand, figure 2c shows a clear image of particles with sizes ranging from 40 to 50 nm, thus implying the formation of micellar structures. At low pH, the protonated PDMAEA chains are extended, due to the electrostatic repulsions of the quaternized amino pendant groups, leading to vesicles with larger sizes (200 nm). As this pH is further decreased, the amine groups of the PDMAEA block are further protonated, thus resulting in an increase in size of the vesicles. 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 particle size is significantly deceased. A similar observation was reported by Chen et al. for aggregates consisting of a polystyrene core and poly(ethylene glycol)/PDMAEMA mixed corona prepared at pH 4.0 and 9.2.[43]

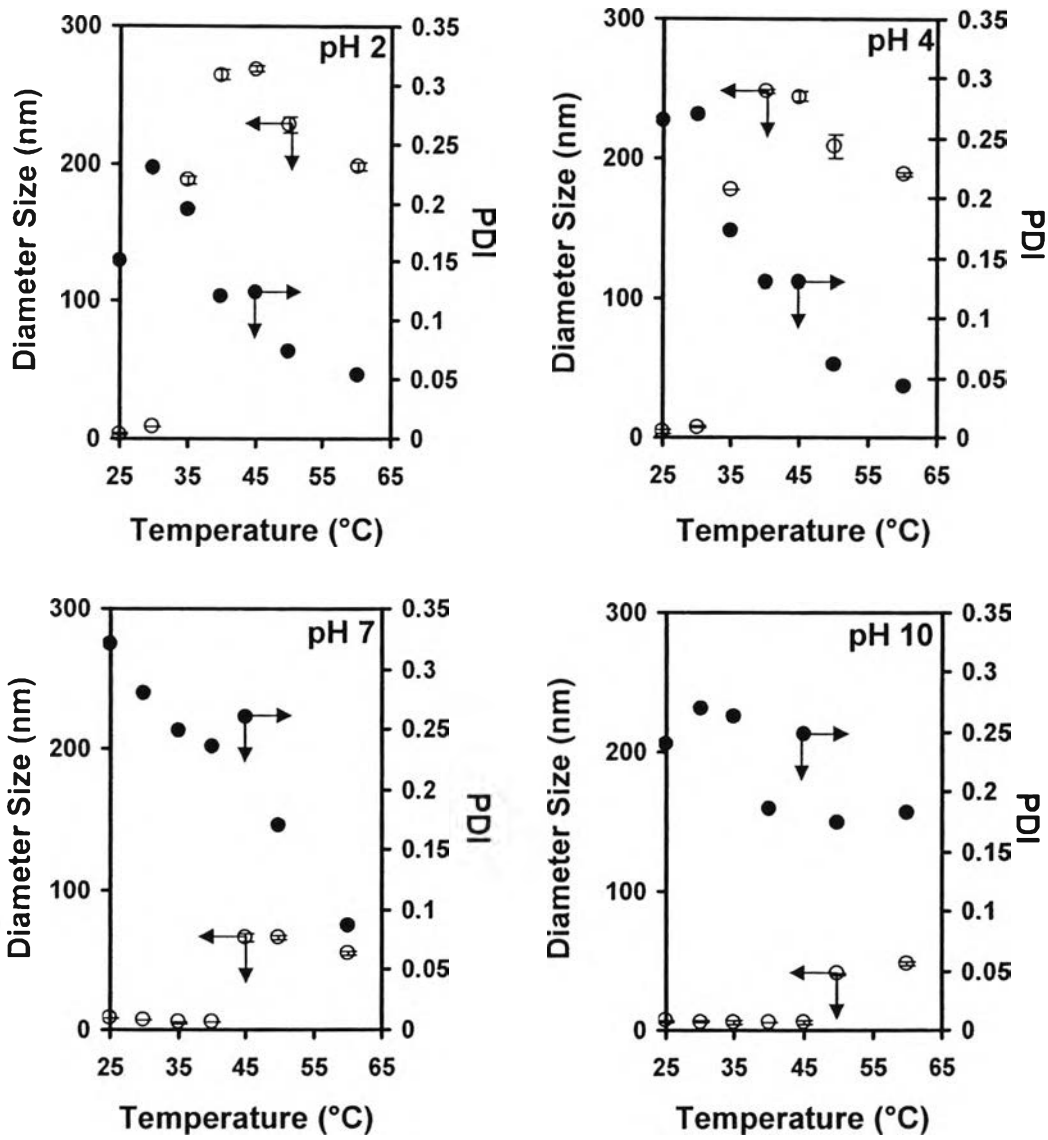


Figure 5.1 Particle size and polydispersity index (PDI) with temperature for 1 g/L aqueous solutions of $PNIPAAM_{115}$-*b*-$PDMAEA_{106}$ diblock copolymers at pH 2, 4, 7 and 10.

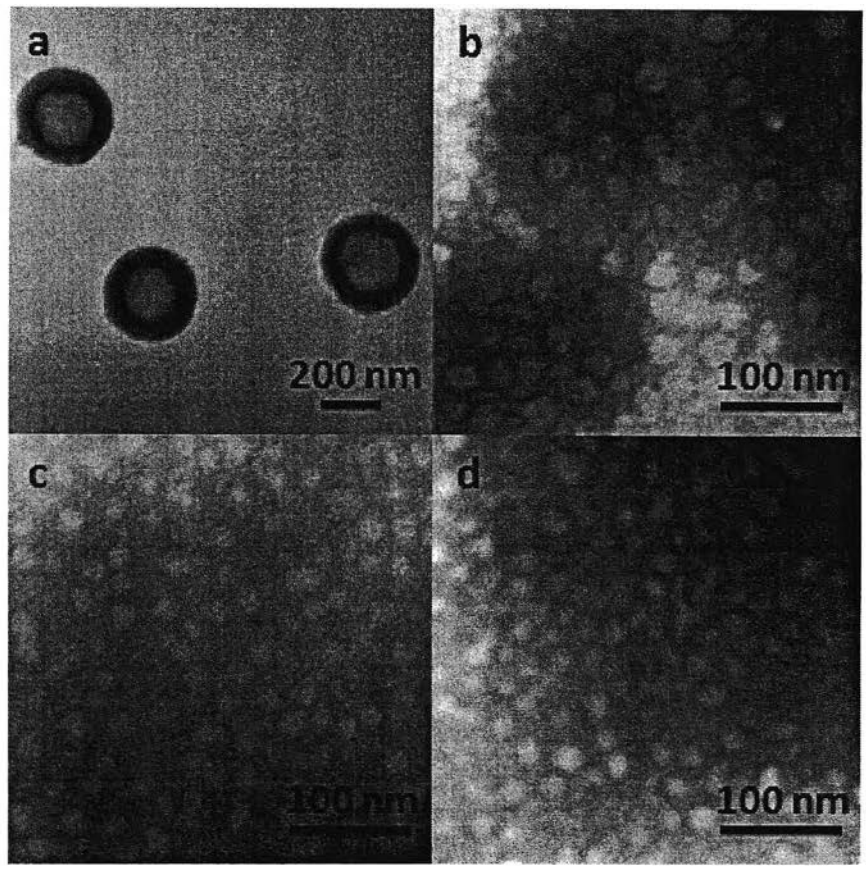


Figure 5.2 TEM images of $PNIPAAM_{115}$-*b*-$PDMAEA_{106}$ at pH 2 prepared by slow (a) and fast heating process (b) and at pH10 prepared by slow (c) and fast heating process (d) after negative staining.

The aggregate formation was also found to depend on the heating rate. The four solutions obtained at pHs 2, 4, 7 and 10 were submitted to two different heating profiles: (1) a slow increase in temperature from 25° to 60°C (slow heating process) and (2) a rapid increase in temperature by setting the samples in an oil bath which was maintained at 60° C (fast heating process). Figure 2 confirms the effects of slow and fast heating processes which induced the micelle and vesicle formation at low pH. The particle morphology evolution was followed by DLS. As shown in Figure 3, the variation in heating rate does not affect the particles formed at pH 7 and 10, and sizes remain around

50nm. However for solutions at pHs 2 and 4, a slow heating process triggers the formation of particles of size around 200 nm, whereas a fast heating rate yields particles of size as small as 50 nm. This difference in size implies that the formation of micelles and vesicles are related to the heating process, as the rate of heating induces a change in morphology, which is based on the switching from hydrophilic to hydrophobic of the PNIPAAM chains. When subjected to a temperature above LCST, the PNIPAAM block undergoes a coil-to-globule transition and the block copolymer self assemble in micelles. However, a slow increase in temperature triggers a slow transition from coil to globule of the PNIPAAM chains, which affects directly the morphology and lead to vesicle formation. These conclusions are further confirmed as the micelle structures obtained from fast heating evolve into vesicles when leaving the solution at high temperatures for more than 1 hour. This observation illustrates the kinetic dependence of the micelles formation, while the vesicle formation is thermodynamically driven at these temperatures.

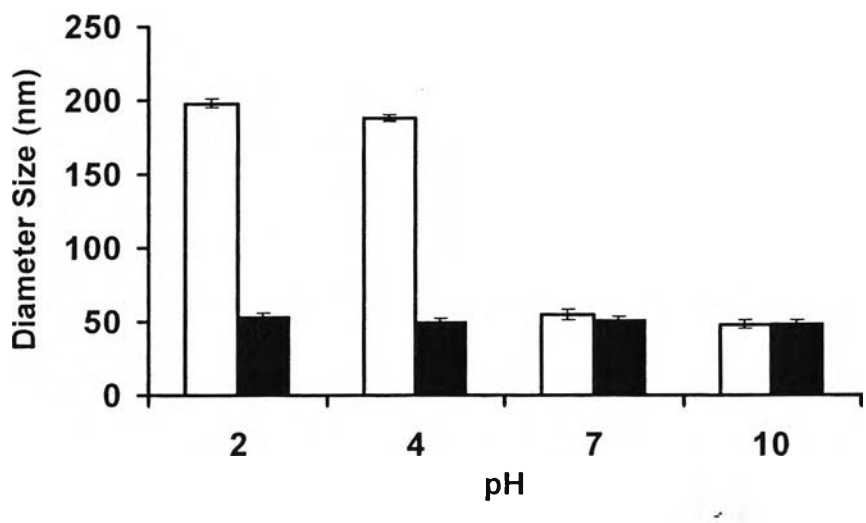


Figure 5.3 Variation of particle size at pH 2, 4, 7 and 10 for 1 g/L aqueous solutions of $PNIPAAM_{115}$-*b*-$PDMAEA_{106}$ diblock copolymers prepared by slow (□) and fast heating (■) at 60°C.

5.6 Conclusions

The pH and temperature showed a synergetic effect to control the morphologies of ($PNIPAAM_{115}$-*b*-$PDMAEA_{106}$). The PDMAEA block allowed the initial aggregation of the bock copolymer to be either micelles or vesicles. In a subsequent step, the PNIPAAM block triggered the formation of vesicular structures, independently of the pH when the temperature was raised above the CMT of the copolymer. This work is a good example to show that a single type of block copolymer can perform differently to be micelles or vesicles if we allow it in the favorable assemble condition.

5.7 Acknowledgements

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5.8 References

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