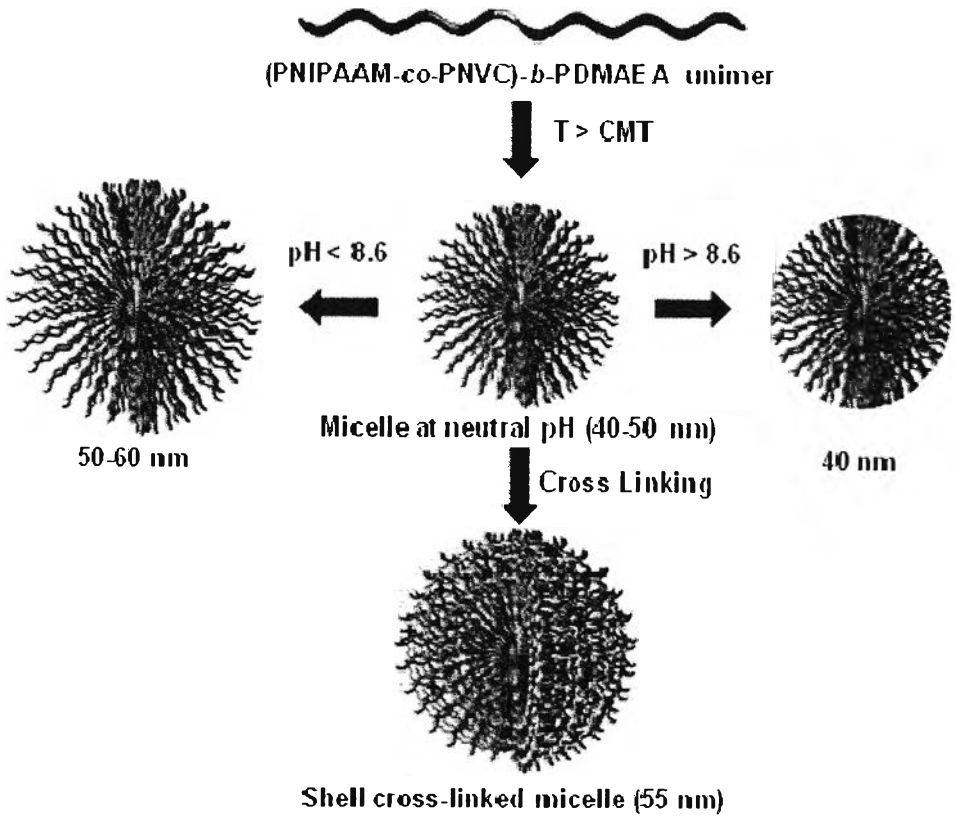
CHAPTER IV

pH AND THERMO- MULTI-RESPONSIVE FLUORESCENT MICELLES FROM BLOCK COPOLYMERS VIA REVERSIBLE ADDITION FRAGMENTATION CHAIN TRANSFER (RAFT) POLYMERIZATION

4.1 Abstract

Well-defined pH- and thermo- multi-responsive fluorescent micelles based on the self-assembly of diblock copolymers poly[(*N*-isopropyl-acrylamide-*co*-*N*-vinylcarbazole)-*b*-*2*-(dimethylamino)ethyl acrylate], (PNIPAAM-*co*-PNVC)-*b*-PDMAEA, are described. The diblock copolymers are prepared via the reversible addition fragmentation chain transfer (RAFT) copolymerization of *N*-isopropyl-acrylamide (NIPAAM) and *N*-vinylcarbazole (NVC) followed by chain extension in presence of *2*-(dimethylamino)ethyl acrylate) (DMAEA). The micelles are formed in aqueous solutions in a wide range of temperature (25 to 60°C), and their sizes increase from 40 to 65 nm when varying pH from basic to acidic. The cross-linking of the PDMAEA-containing shell with *1,2*-bis(*2*-iodoethoxy)ethane (BIEE) results in spherical soft nanoparticles which size is increased by 20-25 % (change from 25-30%) when compared to the micelles. The presence of NVC in concentrations as low as 4% in the core of the micelles allow the nanoparticles to be tagged by fluorescence, making them well suited for therapeutic applications.

4.2 Graphical Abstract



4.3 Introduction

Polymers that can self-assemble in solution and form micelles or vesicles with a hydrophobic core and a hydrophilic corona, or vice versa, have received much attention in recent years.[1-3] Among them, responsive or ''smart'' polymers that undergo significant changes in their physicochemical properties with environmental stimulus, such as pH, temperature, and ionic strength are potential materials for applications in drug delivery, biotechnology, and biomedical fields.[4]

Poly(*N*-isopropylacrylamide), PNIPAAM, possesses a lower critical solution temperature (LCST) in aqueous solution with a sharp phase transition at 32 °C (depending on molecular weight).[5] Upon increase in temperature, PNIPAAM undergoes a coil-to-globule transition, and above LCST it changes its hydrophilic properties to hydrophobic. PNIPAAM is widely used in block copolymers to produce responsive self assemblies; examples of such diblok copolymers include a PNIPAAM segment associated with poly(acrylic acid),[6] poly(*N*-dimethylacrylamide),[7] poly(methacrylic acid)[8] and poly(methyl methacrylate).[9]

Poly(*2*-(dimethylamino)ethyl acrylate), PDMAEA, is a pH-sensitive polymer in aqueous solution, as its tertiary amine can be quaternized at low pH, or in the presence of compounds such as methyl chloride or dimethyl sulfate.[10] PDMAEA and its copolymers have been used in a wide range of applications, including pharmaceuticals, surfactants and in water treatment industries.[11] The controlled polymerization of DMAEA is relatively difficult since this monomer is highly reactive and the polar amino group can deactivate polymerization catalyst.[12] Zeng et. al. were the first to control the living radical polymerization of DMAEA by using atom transfer radical polymerization (ATRP).[10] However, the authors observed that accumulation of chains terminated by quaternization of the amino pendant group during polymerization resulted in increased polydispersity of PDMAEA. By using nitroxide mediated polymerization, Bian and Cunningham avoided the use of a catalyst, and they demonstrated that good control over DMAEA polymerization could be achieved.[12] In addition, the synthesis of block copolymers based on poly(*2*-(dimethylamino)ethyl methacrylate, the methacrylate derivatives of PDMAEA, and the studies of their properties are well documented.[13-15] McCormick and coworkers

demonstrated that well-controlled PDMAEMA and its copolymers with PNIPAAM could be obtained by reversible addition fragmentation chain transfer (RAFT) polymerization. The authors found that the copolymers exist as unimers in aqueous solution, and formed vesicles when the solution temperature is increased above the LCST of the PNIPAAM chain.[16]

Carbazole-based compounds are receiving increasing interest due to their properties of hole-transportation, their charge carrier ability, and electroluminescence.[17] Recent developments in their use have found applications in polymeric light-emitting diodes[18] and organic photo-refractive materials.[19] Although several attempts to prepare poly(*N*-vinyl carbazole) (PNVC) using controlled/ living radical polymerization systems have been reported in the past, it is still not clear whether the homopolymerization of NVC can be controlled. The difficulty in controlling the radical polymerization of NVC may come from the high reactivity of its propagating radicals, which is mainly due to the electron-donating carbazolyl pendant group.[20-22] Mori et al. have demonstrated that PNVC of well defined structures could be produced via controlled radical polymerization of NVC mediated by a xanthate as a chain transfer agent (CTA).[20] The synthesis of four-arms star PNVC [23] and amphiphilic star block copolymers containing PNVC and poly(acrylic acid) including the characterization on the optical properties were also reported.[24] The copolymerization of NVC with vinyl acetate by RAFT polymerization was found to reduce the T_g of PNVC and improve mechanical properties at low temperature.[25]

The combination of a thermosensitive PNIPAAM block containing a fluorescent tag (NVC) with a pH-responsive PDMAEA block can lead to systems that respond to both temperature and pH stimuli, while exhibiting fluorescence properties, thus resulting in multifunctional smart materials. These unique properties would make the (PNIPAAM-*co*-PNVC)-*b*-PDMAEA copolymers potential candidates for applications in biomedical areas, such as in controlled release system.[26] However, the practical applications of the reversible self-assembled aggregates from these double hydrophilic block copolymers might be limited by their structural instability upon dilution, or changes of external conditions. An elegant

route to ensure the structural integrity of these nanostructures is the cross-linking of their shell.[27-31]

The present work focuses on a thermo- and pH-responsive polymer based on the diblock copolymers of NIPAAM and DMAEA combined with the fluorescent tag NVC. This work shows that RAFT polymerization is a good technique to control the block copolymerization of these monomers, so as to obtain stable self-assemblies in aqueous solution. RAFT[32] was chosen, as it is a versatile synthetic tool that gives access to a wide range of polymeric architectures.[33-35] We explore the pH induced micellar self-assemblies and the effect of subsequent shell cross-linking on the size of the resulting soft nanoparticles, as well as their optical properties. This work demonstrates a simple and original preparation of multi-responsive soft nanoparticles via RAFT polymerization.

4.4 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. *1,2*-bis-(*2*-iodoethoxy)ethane (BIEE) (96%) was purchased from Aldrich. RAFT agent, 2-{[(butylsulfanyl)-carbonothioyl]sulfanyl} propanoic acid was prepared as previously described.[36, 37] MilliQ water was used in the preparation of micellar solutions. All other materials were used without further purification. Dialysis experiments were carried out using a Spectra Por dialysis tubing with MWCO 12000-14000.

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 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. For transmission electron microscope (TEM) observation, the samples were prepared by dropping the sample solution onto a carbon coated copper grid followed by adding staining solution (2% phosphotungstic acid). Excess solution was carefully blotted off using filter paper and the samples were air dried before analysis. TEM images were obtained using a H-7650 Hitachi transmission electron microscope at 100kV. The particle diameter and size distribution were measured by using a SemAfore digitizer (JEOL(Skandinaviska)AB) for 100 particles per sample. 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, 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 and then deoxygenated with dry air (20 min). After degassing, the polymerization vial was transferred to a heated oil bath

maintained at 60°C. Polymerization was then stopped at certain times by quenching the reaction in an ice bath followed by determination of the conversion by ^{1}H NMR (92%).

M_n (determined by GPC) = 31 500 g/mol; *PDI* = 1.08; M_n (determined by NMR = 14 700 g/mol.

^{1}H NMR (δ, ppm): 1.1 (6H, s, C**H**$_3$), 1.2-2.1 (C**H**-C**H**$_2$), 4.0 (1H, s, NH-C**H**-(CH$_3$)$_2$), and 8.0 (Ar-**H**).

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. Conversion (99%) was measured by gravimetry, since the ^{1}H NMR signals of the carbazole aromatic protons overlap with the signal of the DMAEA alkene proton.

M_n (determined by GPC) = 52 400 g/mol; *PDI* = 1.20; M_n (determined by NMR = 32 500 g/mol.

^{1}H NMR (δ, ppm): 1.1 (6H, s, C**H**$_3$), 1.2-2.1 (CH-C**H**$_2$-C**H**-C**H**$_2$-C**H**-C**H**$_2$-C**H**), 2.26 (6H, s, C**H**$_3$), 2.6 (2H, t, CH$_2$-C**H**$_2$-N(CH$_3$)$_2$, 4.0 (1H, s, NH-C**H**-(CH$_3$)$_2$), 4.1 (2H, t, O-C**H**$_2$-CH$_2$), and 8.0 (1H, d, Ar-**H**).

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 ranging from pH 2 to 10 using hydrochloric acid and sodium hydroxide. After pH adjustment, the solutions were filtered through a 0.2 micron membrane filters.

Crosslinking of (PNIPAAM-co-PNVC)-b-PDMAEA micelles. A known volume of *1,2*-bis(*2*-iodoethoxy)ethane (BIEE) solution prepared by dissolution of BIEE in water was added to 150 ml aliquots of stirred aqueous micelle solution at a rate of

0.04 ml/min which was then left to stir for 3 days. The shell cross-linked micelles were purified by dialysis against distilled water for 2 days.

4.5 Results and Discussion

Synthesis and characterization of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymers. Block copolymers of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA were synthesized via the RAFT process, following a two-step approach (Scheme 4.1). NIPAAM and NVC were initially copolymerized in dioxane at 60°C to yield a PNIPAAM-*co*-PNVC macro-chain transfer agent (macroCTA, 92% conversion), which was then used to mediate the RAFT polymerization of DMAEA (99% conversion).

Scheme 4.1 Synthesis of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymers.

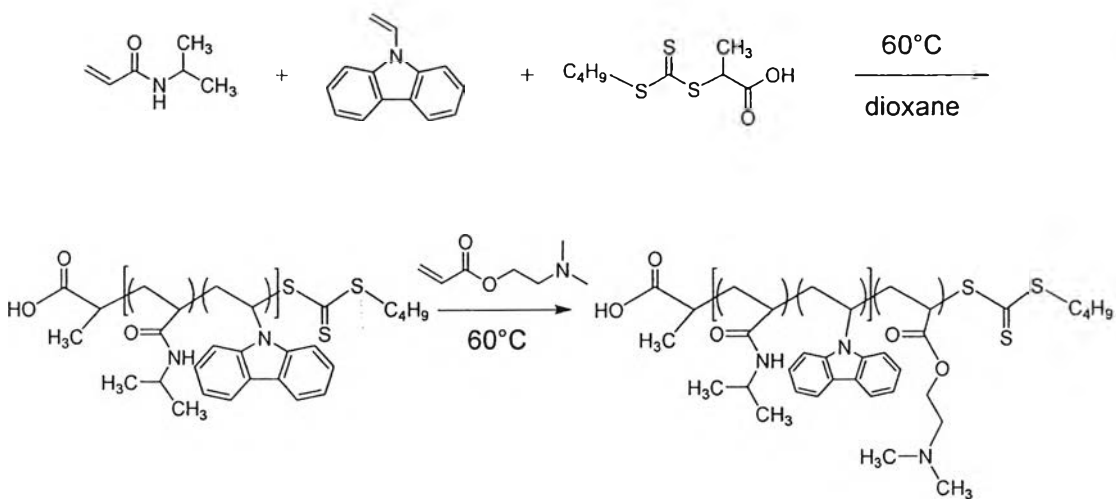


Figure 4.1 shows an example of ^{1}H NMR spectrum of the block copolymer (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$. The peaks corresponding to PNIPAAM, PNVC and PDMAEA are clearly observed in the spectrum.

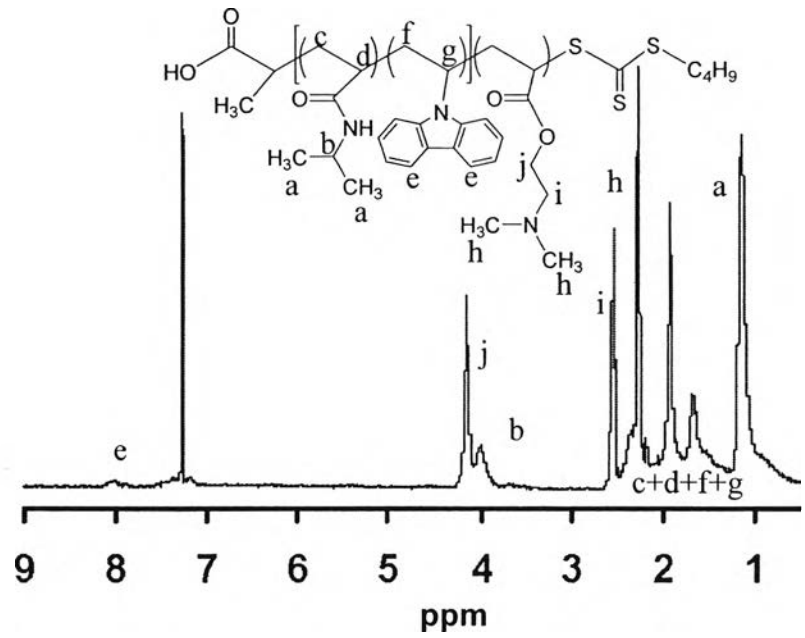


Figure 4.1 ^{1}H NMR spectrum of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ diblock copolymer in $CDCl_3$.

The molecular weight distributions (MWDs) of the PNIPAAM-*co*-PNVC macroCTA (PDI = 1.08) and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymer (PDI = 1.20) were determined by size exclusion chromatography (SEC) and revealed a good control of the polymerization over molecular weight. SEC analyses revealed molecular weights of 31 500 g/mol and 52 400 g/mol for PNIPAAM-*co*-PNVC and (PNIPAAM-*co*-PNVC)-*b*-PDMAEA, respectively. The MWD of the diblock copolymer is clearly shifted toward higher molecular weights by comparison to the first block PNIPAAM-*co*-PNVC macroCTA (Figure 4.2), thus suggesting a good initiator efficiency of the macroCTA. The discrepancy between experimental and theoretical M_n ($M_{n,\,theo}$ (PNIPAAM-*co*-PNVC) = 16 300 g/mol and $M_{n,\,theo}$ (PNIPAAM-*co*-PNVC)-*b*-PDMAEA) = 34 200 g/mol) is due to the inadequacy of the narrow MWD polystyrene standards to approximate the hydrodynamic volume of PNIPAAM and PDMAEA in DMF.[38, 39] Indeed, Müller *et al.*[40] have shown that SEC overestimates the molecular weight of PNIPAAM by a factor of *ca.* 4 by comparing molecular weights determined by SEC and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) techniques. In

order to approximate the molecular weight more accurately, the M_n values of PNIPAAM-*co*-PNVC macroCTA (14 700 g/mol, corresponding to a molecular formula $PNIPAAM_{115}$-*co*-$PNVC_{10}$) and of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymers (32 500 g/mol, corresponding to a molecular formula ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$) were calculated by ^1H NMR spectroscopy. The composition in NIPAAM, DMAEA and NVC for each polymer was determined by integration of the appropriate peaks (1.13, 2.26 and 8.04 ppm, respectively) and their ratios. The obtained values were close to the molecular weights expected from theory.

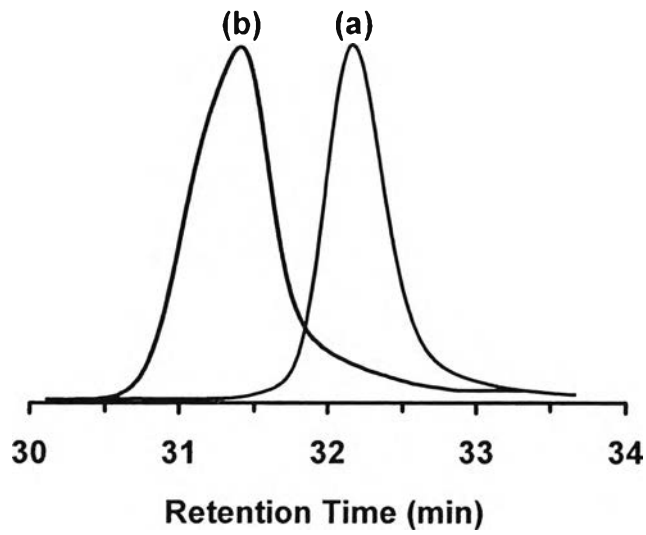


Figure 4.2 SEC chromatograms of (a) PNIPAAM-*co*-PNVC macroCTA and (b) ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$ diblock copolymers.

pH- and thermo-responsive properties. pH- and thermo-responsive amphiphilic copolymers are attracting a growing attention as a new class of materials with versatile properties.[41, 42] Therefore, the synthesis of multi-responsive block copolymers such as (PNIPAAM-*co*-PNVC)-*b*-PDMAEA by combining a polyelectrolyte block (PDMAEA) to a hydrophilic block with an LCST (PNIPAAM), is important so as to obtain pH and thermo-multi-responsive material. In this block copolymer, PDMAEA is a hydrophilic polyelectrolyte at all pH, whose charge depends on the pH of the solution. Indeed, the pendant amine groups are quaternized below the pK_a of the polyelectrolyte, and they are deprotonated at pH > pK_a

(pK_a(PDMAEA) = 8.6).[43] Deprotonation of the tertiary amine pendent groups of the DMAEA unit causes the PDMAEA to change its conformation from highly extended chain to a random coil. The PNIPAAM chain conformation, on the other hand, depends on change in temperature in aqueous solution. When the temperature increases above LCST, the hydrogen bonds between water and the NIPAAM units are weaken, and the PNIPAAM block collapses into hydrophobic chains. Overall, this variation in temperature causes the double hydrophilic block copolymer to change its character to amphiphilic. The attraction between hydrophobic segments drive the formation of micelles by the block copolymer, where the hydrophobic blocks form the core, and the hydrophilic segments spread into the aqueous solution as the corona. The temperature at which this phenomenon occurs is the critical micelle temperature (CMT). The CMT represents the thermo-responsive micelles than the LCST, since the LCST is only representative of the phase transition of one segment of the block copolymers.

The CMT and the consequent micelle sizes of the samples were measured by DLS (Figure 4.3). With an increase in temperature, the mean diameters of the micelles were found to be nearly constant, i.e., 40 to 65 nm depending on pH, within the temperature range of 25°C to 60°C. As the mean diameter values measured by DLS (40 to 65 nm) are much higher than the expected unimers size (5–10 nm), we conclude that the block copolymers form micelles at temperatures as low as 25°C, thus suggesting that the CMT is below room temperature. This observation may come as a surprise when considering that the LCST of PNIPAAM is expected around 32°C. This demonstrates the effect of both the NVC hydrophobic co-monomer on the NIPAAM segment and of the PDMAEA hydrophilic segment on the block copolymer, and similar observations have been made by others. For instance Liu et al. reported that the CMT of a PNIPAAM-*b*-poly(diethylamino)ethyl methacrylate (PNIPAAM-b-PDEAEMA) block copolymer is 25°C, due to the hydrophobic PDEAEMA segment.[44]

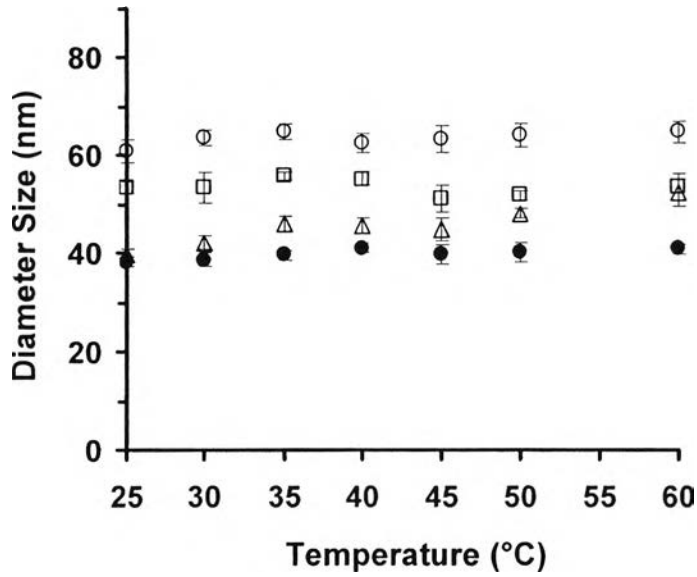


Figure 4.3 Particle sizes observed by DLS under various temperatures for 1 g/L aqueous solutions of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ diblock copolymers at pH 2 (○), 4 (□), 7 (Δ) and 10 (●).

As variation in pH affects the protonation of the PDMAEA block, the effect of pH on the self-assembly of the block copolymers was investigated. Light scattering analyses are usually carried out in presence of an added salt, to suppress Coulombic interactions between particles (an assumption made when using the appropriate algorithm for size and size distribution determination). Unfortunately, introducing a salt in this system may result in the neutralization of the quaternised amine pendant group, thus cancelling the intramolecular elctrostatic repulsion between pendant groups. However, potential Coulombic interactions is only of concern for pH under the pK_a of the hydrophilic block (PDMAEA; pK_a = 8.6)[43], when the tertiary amine is quaternised. Indeed, at pH above pK_a (pH = 10), the particles are neutral, and their size is therefore accurately represented by light scattering measurements without adding salt. Furthermore, as we reach pH = 2 by adding HCl to our aqueous solution of micelles, the solution concentration in [Cl^-] is at least equal to 10mM (In fact, the [Cl^-] is a lot higher, since the Cl^- counterions are not all complexed to a quaternised amine and they remain present at the surface of the particles). Since a concentration in an electrolyte (Cl^- here) of 10mM results in a thickness of the electrical double layer of 3 nm, we argue that the electrolyte

concentration is high enough to prevent any Coulombic interactions.[45] On this ground, we argue that our light scattering measurements at these pHs are representative of the size of the micelles without requiring addition of a salt.

DLS analyses show that (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ diblock copolymers form micelles at all pH, with the hydrophobic (PNIPAAM$_{115}$-*co*-PNVC$_{10}$) block forming the core and the PDMAEA the corona of the micelles. The size of the micelles at pH 2.0 to 4.0 was found to range from 50 to 65 nm, while at pH 7.0 to 10 the micelles become smaller, with sizes ranging from 40 to 50 nm. This variation in size is due to the protonation of the PDMAEA chains at pH below pK_a (PDMAEA) = 8.6, which results in electrostatic repulsion of the positive charges, thus forcing the chains to fully expand. In contrast, at pH above pK_a, the PDMAEA chains are deprotonated and adopt a coil conformation, thus leading to smaller apparent size of the micelles. Interestingly, at pH close to pK_a, the sizes of the micelles are observed to increase with temperature. In the range of temperatures concerned, the constant of self-ionization of water, K_W, decreases with an increase in temperature, leading to more protons in solution.[46] This increase in proton concentration leads to quaternization of the amine pendant group and increased internal elctrostatic repulsion within the PDMAEA chains, which in turns yields to a swollen outer shell and larger micelles. Alternatively, it could be that at pH 4 and 7, the concentration in electrolyte is too low to suppress Coulombic interactions, as discussed above, thus leading to inaccurate measurement of the micelle sizes by light scattering. However, since the micelles size variation is coherent with the variation in pH, we argue that the Coulombic interaction can still be neglected.

Critical micelle concentration (CMC). The concentration at which the amphiphilic block copolymers form a micelle at a certain temperature is defined as the CMC, which can be evaluated by measuring the surface tension.[47, 48] The CMC relies on the reduction of the force at the interface between liquid and gas molecules upon the addition of a surfactant to an aqueous solution. When the concentration in surfactants is increased, the surface tension decreases dramatically, until CMC is reached. To determine the CMC of our system, the surface tension of aqueous solutions of amphiphilic block copolymers was measured in various concentrations. In the case of

($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$, the CMC was found to be independent of the temperature, *ca.* 2×10^{-3} g/L at 25°C and 45°C, with a surface tension of 0.037 - 0.040 N/m. The constant value of the CMC at both temperatures confirms that micelles are readily formed at 25°C.

Shell cross-linked micelles. Despite the high thermodynamic and kinetic stability of the polymeric micelles at equilibrium, further stabilization is needed to avoid disintegration of the aggregate at low polymer concentrations, or upon environmental changes such as decreasing in temperature, alteration of pH or increase in ionic strength of the solution. An easy and promising approach to obtain a robust delivery system is to crosslink the shell of the micelles.[27-31] Shell cross-linked micelles were obtained by reacting the partially protonated ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$ diblock copolymer micelles with a difunctional quaternizing agent, BIEE, in aqueous solution. BIEE reacts with the DMAEA residues on adjacent block copolymer chains to "lock in" the micellar structure.[49-52] BIEE has relatively low water solubility and was initially dispersed as a droplets suspension in water, in a molar ratio of BIEE to DMAEA = 1:2. After 30 min, these droplets disappeared to form a homogeneous solution; the reaction solution was then stirred at 60°C for a further 3 days. The success of the quaternization reaction was estimated by ^{1}H NMR spectroscopy analysis in D_2O as solvent, by comparing the characteristic signals of protons adjacent to the unquaternized and quaternized tertiary amine pendant groups of DMAEA. Signals of the CH_3 and CH_2 groups connected to the tertiary amine group of DMAEA were clearly shifted after cross linking, when compared to signals of the same groups before cross–linking. This is representative of a successful quaternization of the DMAEA tertiary amine (Figure 4.4). The decrease in pH for shell cross-linked micelles from 9.2 to 5.8 further confirms the successful quaternization.[52]

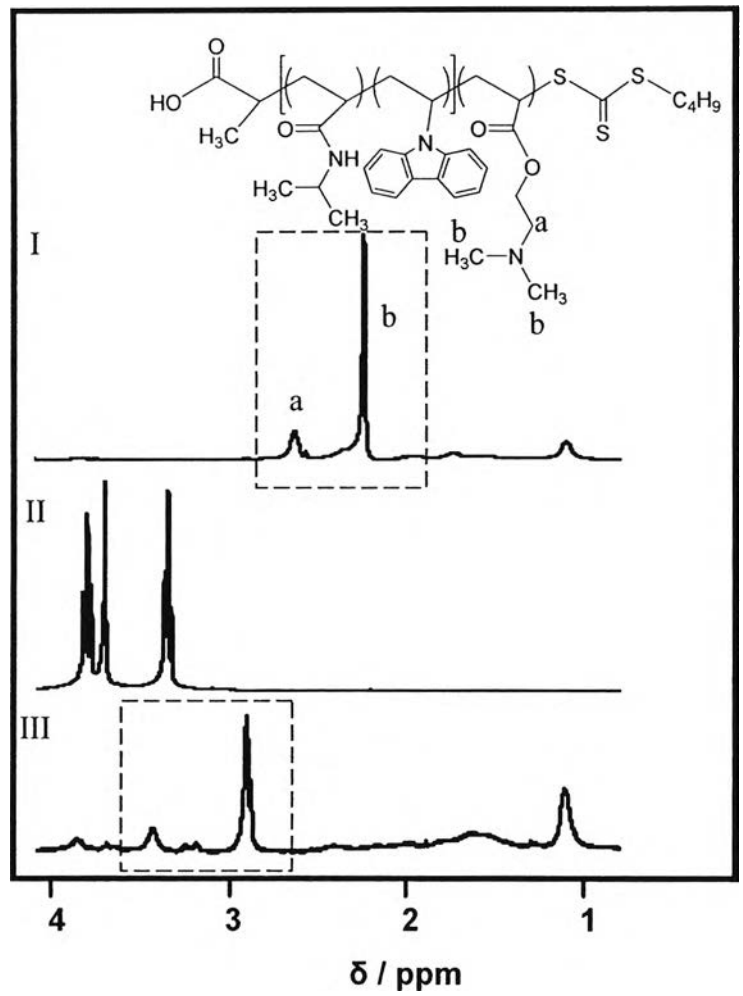


Figure 4.4 ^{1}H NMR spectra of (I) (PNIPAAM-*co*-PNVC)-*b*-PDMAEA micelle before crosslinking in D_2O, (II) BIEE in MeOD, and (III) shell cross-linked micelle of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA in D_2O.

Having demonstrated the quaternization of PDMAEA chains was successful, the cross-linking of the micelles shell was investigated by light scattering and TEM. The particle size measured by DLS of the shell cross-linked micelles (55 nm, PDI = 0.231) was found to be larger than that of the non-cross-linked micelles (40-50 nm, PDI = 0.237), as expected from the electrostatic repulsion of the PDMAEA chains within the corona after quaternization. TEM images of the shell cross-linked micelles (Figure 4.5) confirm the DLS analysis, by showing spherical micelles of average diameter 28 nm and 37 nm, before and after cross linking, respectively. 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.[47]

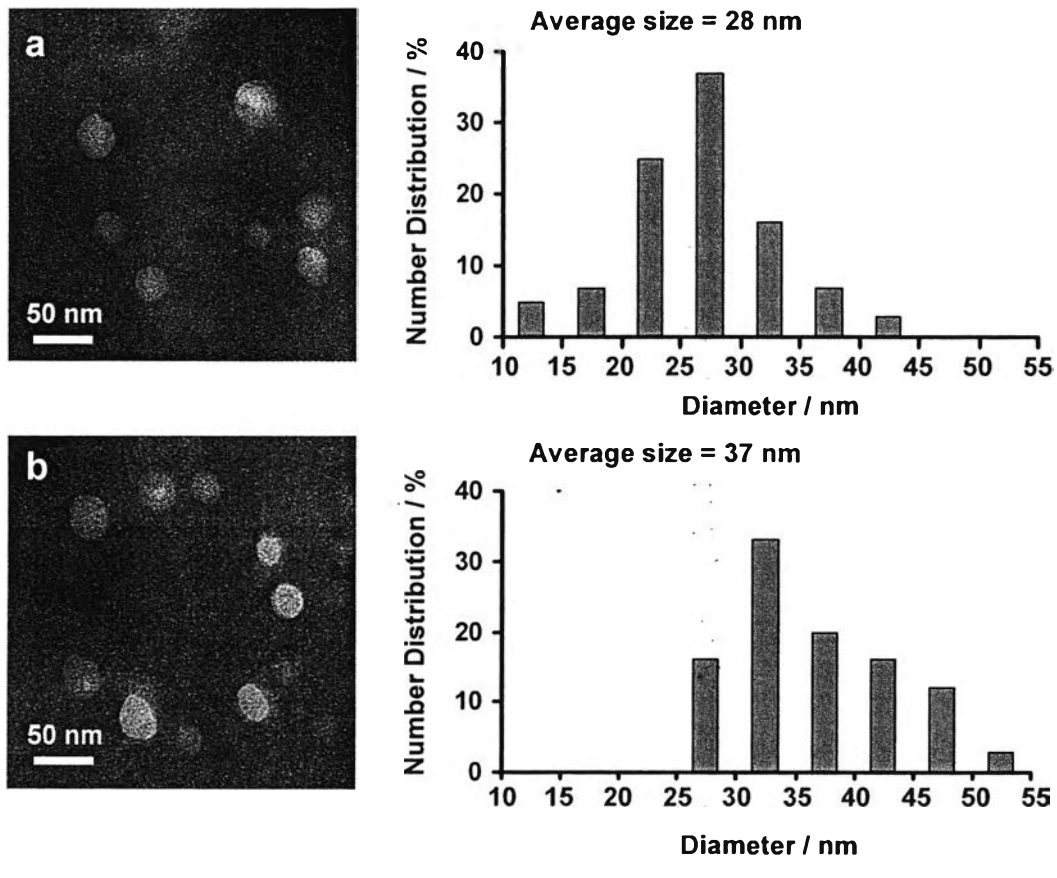


Figure 4.5 TEM images and size distribution of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ micelle (a) before crosslinking, (b) after crosslinking.

Fluorescent properties. The fluorescence properties of the particles were investigated for the cross-linked and non-cross-linked micelles. Solutions of micelles and cross-linked micelles were excited at 300 nm, and two exeimer emissions were observed at 354 and 365 nm (Figure 4.6), close the known partial overlap (second) and a full-overlap (normal) excimer emission at 370 and 420 nm of carbazole.[53, 54] The shift of the peaks position from 370 to 354 nm and from 420 to 365 nm can be explained by the copolymer affecting the transfer of energy of the chromophore in the excited state, and the scattering induced by the micelles in solution. However, the

analyses clearly show that the fluorescence of the carbazole is maintained when incorporated in the particles. It is also noteworthy that the intensity of the excimer emission is reduced when the micelles are cross-linked, although the fluorescence is still strong.

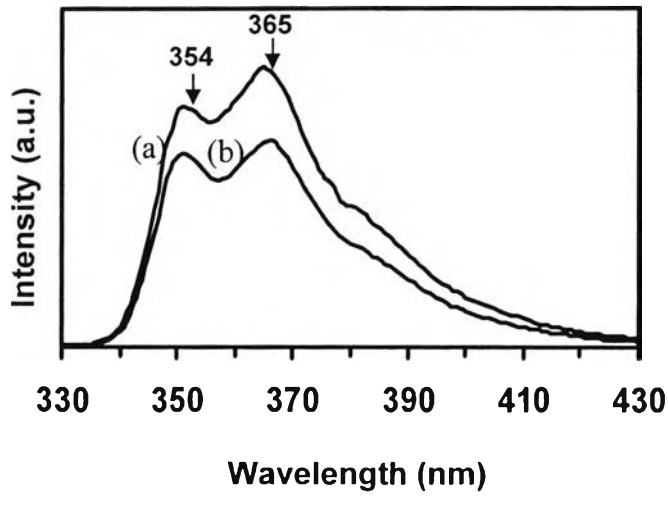


Figure 4.6 Fluorescence spectra for 0.5 g/L aqueous solutions of ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$ (a) before crosslinking, and (b) after crosslinking at room temperature.

Effect of NIPAAM:NVC ratio on CMT. It is likely that the low CMT of the block copolymers with a ratio NIPAAM:NVC of 115:10 arises from the hydrophobicity of the NVC unit. In order to confirm this assumption and produce particles with higher CMT, a copolymer with a lower content of NVC and NIPAAM was prepared (molar ratio NIPAAM : NVC = 115 : 5), while maintaining the degree of polymerization of PDMAEA at 125, and its CMT was assessed by light scattering. Figure 4.7 shows that a signal corresponding to very small particles (*ca.* 5nm, probably unimers) is observed for temperatures below 25 °C, but a dramatic increase in particle size is observed when the temperature is raised from 25 °C to 30 °C, leading to particles of *ca.* 40 nm diameter, similar in size to the particles formed by ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$. From this observation, it is clear that a decrease in concentration of NVC in the NIPAAM segment increases the hydrophilicity of the block and causes an increase of the CMT to *ca.* 27.5 °C.

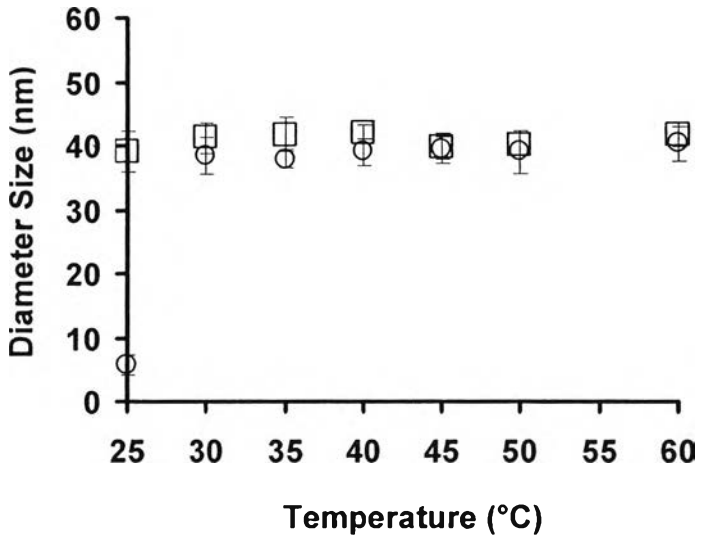


Figure 4.7 Size of micelles under varied temperature for 1 g/L aqueous solutions of (○)(PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ and (□)(PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$.

Micelles of (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$ were also analyzed by fluorescence spectroscopy. Figure 4.8 clearly demonstrates that the fluorescence properties of PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$, are similar to those of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$, although micelles formed from the latter polymer exhibit stronger intensity, due to the higher concentration in NVC in the PNIPAAM segment. It is surprising that the maximum intensity of (PNIPAAM$_{115}$-*co*-PNVC$_{10}$)-*b*-PDMAEA$_{125}$ is lower than twice the maximum intensity of (PNIPAAM$_{115}$-*co*-PNVC$_5$)-*b*-PDMAEA$_{125}$; this could be an effect of the polymeric chains, or scattering from the micelles, as discussed above.

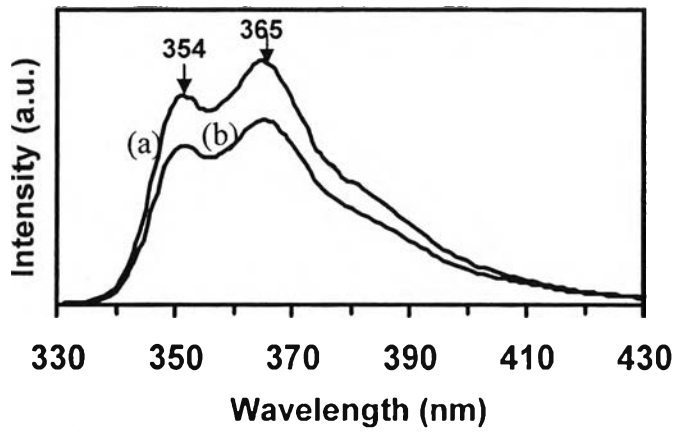
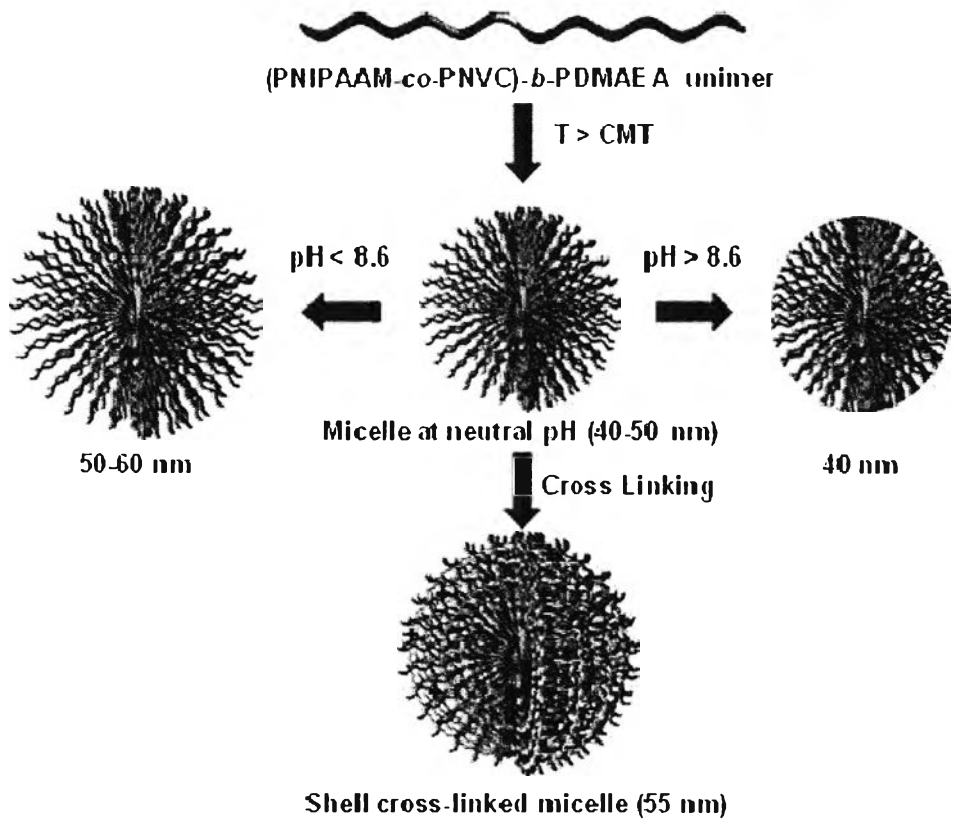


Figure 4.8 Fluorescence spectra for 0.5 g/L aqueous solutions of (a) ($PNIPAAM_{115}$-*co*-$PNVC_{10}$)-*b*-$PDMAEA_{125}$, and (b) ($PNIPAAM_{115}$-*co*-$PNVC_5$)-*b*-$PDMAEA_{125}$ at room temperature.

4.6 Conclusions

pH- and thermo- multi-responsive fluorescent micelles of the block copolymers (PNIPAAM-*co*-PNVC)-*b*-PDMAEA were designed and synthesized for the first time. The block copolymer synthesis was performed via RAFT polymerization of DMAEA mediated by a PNIPAAM-*co*-PNVC macro-chain transfer agent. The resulting (PNIPAAM-*co*-PNVC)-*b*-PDMAEA showed a CMT dependent on the LCST of PNIPAAM chain and the content in the hydrophobic NVC. The protonation ability of the PDMAEA chains permits to control the size of the micelles from 60 to 40 nm, by simply varying the pH from acidic to basic. The cross-linking of the PDMAEA corona with BIEE was demonstrated to be an effective strategy to permanently fix the core/shell nanostructures, and produce positively charged soft nanoparticles, as a consequence of the quaternization of the PDMAEA corona. Scheme 2 summarizes the properties of these nanoparticles. Finally, the introduction of NVC units in concentrations as low as 4% (molar ratio of NVC:NIPAAM = 5:115) allow to tag these particles, by fluorescence. We expect these particles will be of great importance in the field of therapeutics, for instance as tagged positively charged DNA delivery vectors.

Scheme 4.2 Micellization behavior of (PNIPAAM-*co*-PNVC)-*b*-PDMAEA diblock copolymers in aqueous solution and shell crosslinked micelle.



4.7 Acknowledgements

SC and NS acknowledge The Royal Golden Jubilee Program (The Thailand Research Fund) grant number PHD/0087/2549. The authors thank Hitachi High-Technologies Corporation for the TEM measurements, Dr. Hank De Bruyn for assistance with the light scattering measurements and interpretation and Mr. Ben Hornby for designing the micelles schematics.

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