Journal of Applied Polymer Science

pH and Thermo-Responsive Poly(*N*-isopropylacrylamide) Copolymer Grafted to Poly(ethylene glycol)

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ABSTRACT: pH and thermo-responsive graft copolymers are reported where thermo-responsive poly(*N*-isopropylacrylamide) [poly (NIPAAm), **poly A**], poly(*N*-isopropylacrylamide-*co*-2-(diethylamino) ethyl methacrylate) [poly(NIPAAm-*co*-DEA), **poly B**], and poly(*N*-isopropylacrylamide-*co*-methacrylic acid) [poly(NIPAAm-*co*-MAA), **poly C**] have been installed to benzaldehyde grafted poly-ethylene glycol (PEG) back bone following introducing a pH responsive benzoic-imine bond. All the prepared graft copolymers for PEG-*g*-poly(NIPAAm) [**P-N1**], PEG-*g*-poly(NIPAAm-*co*-DEA) [**P-N2**], and PEG-*g*-poly(NIPAAm-*co*-MAA) [**P-N3**] were characterized by ^{1}H-NMR to assure the successful synthesis of the expected polymers. Molecular weight of all synthesized polymers was evaluated following gel permeation chromatography. The lower critical solution temperature of graft copolymers varied significantly when grafted to benzaldehyde containing PEG and after further functionalization of copolymer based poly(NIPAAm). The contact angle experiment showed the changes in hydrophilic/hydrophobic behavior when the polymers were exposed to different pH and temperature. Particle size measurement investigation by dynamic light scattering was performed to rectify thermo and pH responsiveness of all prepared polymers. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. 130: 168–174, 2013

KEYWORDS: grafting; thermal properties; stimuli-sensitive polymers

Received 21 November 2012; accepted 7 February 2013; published online 8 March 2013
DOI: 10.1002/app.39150

INTRODUCTION

In the last few years, attentions have been devoted to the materials responsive to various physical and chemical stimuli like temperature, pH, ionic strength, or light.[1–4] Among them, poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is one of the most widely investigated environmentally responsive polymer which exhibits a hydrated expanded state at lower temperature while at high temperature it undergoes to collapsed state following dehydration.[5] Poly(NIPAAm) shows a lower critical solution temperature (LCST) around 32°C below which it is dehydrated and form random coiled architecture in water where water is served as a good solvent. But above the LCST the coiled conformation converts into globular design.[6,7] It is particularly advantageous that the LCST value of poly (NIPAAm) solutions and gels falls between human body temperature and room temperature, this being the main reason why this polymer has been widely investigated in the field of biotechnology, bioengineering, and medicine.[8] Literatures report that thermo-responsive polymers based on poly(NIPAAm) homopolymer and its copolymers have gained a potential attraction because of their attractive application in different scientific arena like biomedical application, tissue engineering, drug delivery system, and so on.[9–11]

On introducing a monomer during particle synthesis with NIPAAm, the functionality of the NIPAAm can be expected to increase. For example, the product of copolymerization of NIPAAm with ionizable acrylic acid responds to external stimuli.[12] Efforts are under investigation to perk up the switch ability of poly(NIPAAm) following copolymerization using other monomers, grafting, modification of monomers, and installation of surface roughness.[13–17] However, there is a critical copolymer content in NIPAAm above which thermosensitivity of NIPAAM is reduced or eliminated. This can be attributed to the influence of these monomer units on the subtle balance of hydrogen bonding in poly(NIPAAm).[18]

Much attention has been paid recently to poly(NIPAAm) based polymers for the development of temperature-stimulus sensitive polymer. For biomedical application, the LCST of poly (NIPAAm) is not favorable enough regarding physiological

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temperature. Modification of poly(NIPAAm) can be a proper way for their application by increasing the LCST. As a corollary, in this present work, we demonstrate the synthesis and characterization of a dual pH and thermo-responsive graft copolymer between different amine end-capped poly(NIPAAm) copolymer and oligo(benzaldehyde) grafted polyethylene glycol (PEG). In the polymer structural design, we have introduced a pH sensitive benzoic imine bond as linker between PEG and poly(NIPAAm) copolymers having carboxyl or amine groups for pH/temperature LCST behavior into synthesized graft copolymers. Dual-responsive behaviors of the polymers against the mentioned stimuli with pH changing and temperature conditions were investigated through LCST.

EXPERIMENTAL

Materials

PEG (Mw =1000), hydroxyethyl methacrylate (HEMA), *tert*-butyl peroxybenzoate, *tert*-butyl methacrylate (*t*-BMA), 4-formylbenzoic acid, anhydrous toluene, hexane, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino)pyridine (DMAP), *N*-isopropylacrylamide (NIPAAm), 2-(diethylamino)ethyl methacrylate (DEA), 2,2′-azobis(isobutyronitrile) (AIBN), cysteamine, trifluoroacetic acid, anhydrous tetrahydrofuran (THF), anhydrous dimethyl sulfoxide (DMSO), 1,4-dioxane, dichloromethane (DCM), dimethylformamide (DMF), sodium hydroxide (NaOH), lithium bromide (LiBr), and diethyl ether were purchased from Sigma Aldrich (St. Louis, MO).

Synthesis of Oligo(benzaldehyde) Grafted PEG

Benzaldehyde grafted PEG was synthesized following two steps. At first, PEG-*g*-oligo(HEMA-*co*-*t*-BMA) [**Pre-polymer 1**] was synthesized in a similar method described by Park and coworkers.[1] Briefly, PEG (4 g), *t*-BMA (0.34 g), and HEMA (0.65 g) dissolved in 80 mL of anhydrous toluene and added with *t*-butylperoxy benzoate (0.8 g) as initiator in anhydrous toluene to produce grafted PEG copolymers. The reaction was allowed to proceed for 24 h at 120°C under a dry nitrogen flow and magnetic stirring. The product was purified by precipitation in hexane, dried in vacuum. The polymer was purified by dialysis (molecular weight cut-off: 3000; Spectrum Laboratories, Rancho Dominguez, CA) for 2 days.

Finally to prepare the benzaldehyde grafted PEG [PEG-*g*-oligo (benzaldehyde), **Pre-polymer 2**], **Pre-polymer 1** (3.5 g), 4-formyl benzoic acid (0.42 g), DCC (0.86 g), and DMAP (0.09g) was dissolved in 70 mL of THF in a 250-mL two-neck flask.[2] The reaction was allowed to proceed for 24 h at room temperature under a dry nitrogen flow and magnetic stirring. The formed precipitate (urea form) was removed by filtration, and the filtrate was added to 30 mL of same solvent mixture. After mixing, the solution was evaporated with a rotary evaporator and precipitated in hexane. The resulting polymer was dried in vacuum and analyzed. Purification was done by dialysis (molecular weight cut-off: 1000; Spectrum Laboratories, Rancho Dominguez, CA) for 2 days. Yield: 77%.

^{1}H-NMR of benzaldehyde grafted PEG (400 MHz, $CDCl_3$): δ (ppm) 10.0 (—C*H*O of benzaldehyde), 8.00–8.20 (Ar *proton* of benzaldehyde), 4.15 (—CH_2— of HEMA), 3.81 (—CH_2OH of HEMA), 1.9–2.2 (—CH_2 of *t*-BMA), 1.2–1.4 (—C(CH_3)$_3$ of *t*-BMA), 3.8–3.6 (—CH_2—O— of PEG).

Synthesis of Amine End-Capped Poly(NIPAAm), Poly(NIPAAm-*co*-DEA), and Poly(NIPAAm-*co*-*t*-BMA)

The poly(NIPAAm) [**poly A**], poly(NIPAAm-*co*-DEA) [**poly B**], and poly(NIPAAm-*co*-MAA) [**poly C**] were synthesized following a similar method reported elsewhere.[19] NIPAAm (5 g), cysteamine (0.34 g), and AIBN (0.73 g) were dissolved in 40 mL of DMSO for the preparation of poly(NIPAAm). Predetermined amount of DEA (3.70 g) and *t*-BMA (2.84 g) was added to the solutions containing NIPAAm (2.5 g), cysteamine (0.17 g), and AIBN (0.36 g) at a feed molar ratio of NIPAAm/DEA (1 : 1 ratio) and NIPAAm/*t*-BMA (1 : 1 ratio) for the preparation of **poly B** and poly(NIPAAm-*co*-*t*-BMA), respectively. Polymerization was carried out at 60°C for 24 h. After stirring, the solvent was evaporated with a rotary evaporator after the polymer was collected by precipitation in 500 mL of diethyl ether. To prepare the amine end-capped poly(NIPAAm-*co*-MAA) [**poly C**], poly (NIPAAm-*co*-*t*-BMA) (5 g) was added into a 250-mL round-bottom flask and dissolved with DCM (50 mL). The solution was stirred at room temperature for 2 h to ensure complete dissolution. Trifluoroacetic acid (3.2 g) was then added into the flask. After the reaction mixture was stirred at ambient temperature for 24 h, the solvent was evaporated with a rotary evaporator after the solution was precipitated by precipitation in 400 mL of diethyl ether. The polymer was precipitated in 400 mL of diethyl ether and was purified by dialysis (molecular weight cut-off: 1000; Spectrum Laboratories, Rancho Dominguez, CA) for 2 days. The degree of polymerization and molecular weight of each copolymer was determined by ^{1}H-NMR spectroscopy (Bruker Avance 400 spectrometer operating at 400 MHz) and gel permeation chromatography (GPC, YL9112 Isocratic pump, Younglin instrument, Korea) with a KD-804, KD-803 column (Shodex, Japan) using DMF in 1 wt % LiBr), respectively. Yield: **poly A** (85%), **poly B** (87%), and **poly C** (83%).

Synthesis of pH and Thermo-Responsive Graft Copolymers Using Benzaldehyde Grafted PEG With Amine End-Capped Poly(NIPAAm)

The process of temperature and pH sensitive graft copolymers was synthesized in a similar method.[2,20] Amine end-capped copolymer based poly(NIPAAm) and **Pre-polymer 2** were dissolved in 30 mL of DMSO in a 150-mL flask. For PEG-*g*-poly (NIPAAm) [**P-N1**], the molar ratio of **Pre-polymer 2**/poly (NIPAAm) in feed was 1/7. For PEG-*g*-poly(NIPAAm-*co*-DEA) [**P-N2**] and PEG-*g*-poly(NIPAAm-*co*-MAA) [**P-N3**], the ratio of poly(NIPAAm-*co*-DEA) or poly(NIPAAm-*co*-MAA)/**Pre-polymer 2** was 1/9 ratio. The mixture was stirred for 4 h at 40°C and after completing the reaction, polymer solution was purified by dialysis (molecular weight cut-off: 3000) of 0.1*M* NaOH aqueous solution (pH 8.5–9) respectively for 2 days.

- PEG-*g*-poly(NIPAAm) [**P-N1**]; yield (75%), GPC (DMF in 1 wt % LiBr, PEG standard): M_n = 11,700 [Poly Dispersity Index (PDI): 2.3].
  ^{1}H-NMR of **P-N1** (40 0MHz, $CDCl_3$): δ (ppm)7.90 (Ar proton of benzaldehyde), 4.15 (—CH_2— of HEMA), 3.81 (—CH_2OH of HEMA), 2.6 (—CH_2CHCH_3 of HEMA), 1.0

(—CH_2CHCH_3 of HEMA), 1.9–2.2 (—CH_2— of *t*-BMA), 1.2–1.4 (—$C(CH_3)_3$ of *t*-BMA), 3.8–3.6 (—CH_2—O— of PEG), 2.6 (—$CHCH_2$— of NIPAAm), 4.0 (—$CH(CH_3)_2$ of NIPAAm), 1.0 (—$CH(CH_3)_2$ of NIPAAm), 2.85 (—CH_2— of cysteamine).

- PEG-*g*-poly(NIPAAm-*co*-DEA) [**P-N2**]; yield (78%), GPC (DMF in 1 wt % LiBr, PEG standard): M_n = 12,600 (PDI: 2.1). ^{1}H-NMR of **P-N2** (400 MHz, $CDCl_3$): δ (ppm) 7.90 (Ar proton of benzaldehyde), 4.15 (—CH_2— of HEMA), 3.81 (—CH_2OH of HEMA), 2.6 (—CH_2CHCH_3 of HEMA), 11.0 (—CH_2CHCH_3 of HEMA) 11.9–2.2 (—CH_2— of *t*-BMA), 1.2–1.4 (—$C(CH_3)_3$ of *t*-BMA), 3.8–3.6 (—CH_2—O— of PEG), 2.6 (—$CHCH_2$— of NIPAAm), 4.0 (—$CH(CH_3)_2$ of NIPAAm and —$CH(CH_2CH_3)_2$ of DEA), 1.0 (—$CH(CH_3)_2$ of NIPAAm), 2.7 ($NH(CH_2CH_3)_2$ of DEA), 1.1 (—$N(CH_2CH_3)_2$ of DEA), 2.85 (—CH_2— of cysteamine).
- PEG-*g*-poly(NIPAAm-*co*-MAA); yield (73%), GPC (DMF in 1 wt % LiBr, PEG standard): M_n = 15,000 (PDI: 2.2). ^{1}H-NMR of **P-N3** (400MHz, $CDCl_3$) [**P-N3**]: δ (ppm) 7.90 (Ar proton of benzaldehyde), 4.15 (—CH_2— of HEMA), 3.81 (—CH_2OH of HEMA), 2.6 (—CH_2CHCH_3 of HEMA), 11.0 (—CH_2CHCH_3 of HEMA) 11.9–2.2 (—CH_2— of *t*-BMA), 1.2–1.4 (—$C(CH_3)_3$ of *t*-BMA), 3.8–3.6 (—CH_2—O— of PEG), 2.6 (—$CHCH_2$— of NIPAAm), 4.0 (—$CH(CH_3)_2$ of NIPAAm), 1.0 (—$CH(CH_3)_2$ of NIPAAm), 2.85 (—CH_2— of cysteamine).

LCST Measurements

The LCST of the polymer solution was determined using a UV/vis spectrophotometer (Optizen 2020UV, Mecasys) connected to a temperature controller (RW-0525G, Jeio Tech). Each polymer concentration was always maintained at 1 wt % at different pH conditions (pH = 3.0, 6.0, 7.4, and 12.0), and the absorbance at 500 nm was measured. The pH was adjusted by adding 0.1*M* HCL or NaOH solution. The cloud point was analyzed as increasing temperature of solutions from 10 to 80°C under heating rate of 1 °C/min.

Dynamic Light Scattering and Dynamic Contact Angle

Glass slides were cleaned with acetone, followed by rinsing successively with distilled water and then drying in a vacuum oven. The each copolymer was dissolved in solution at pH 3.0 and 7.4 at a concentration of 10 mg/mL and placed at predetermined temperatures (25 and 37°C). Dynamic contact angles were measured using the static sessile drop method on a data drop shape analyzer (DSA 100, KRUSS GmbH). Dynamic light scattering (DLS) investigation was performed to evaluate the particle size using a Zetasizer Nano S90 (Malvern Instruments, UK) at 37°C.

RESULTS AND DISCUSSION

Characterization

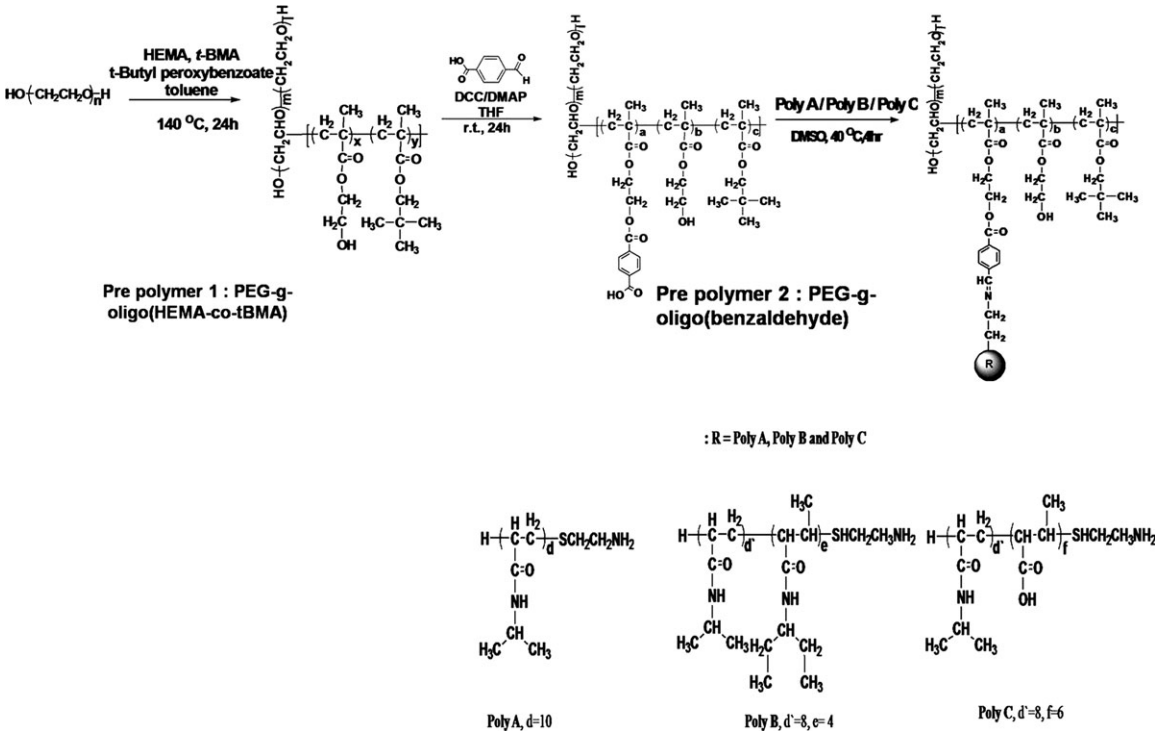
Poly(NIPAAm) is a temperature sensitive polymer which is characterized by an LCST, below which the side chains form hydrogen bonds with water molecules, wherein, above the LCST, the hydrogen bonds between water molecules and the hydrophilic (—NHC=O)groups of polymer (PNIPAM) are broken, and then the hydrophobic interactions between the hydrophobic isopropyl groups of polymer (PNIPAM) becomes dominant. For that reason, the linear PNIPAM precipitates at the temperatures higher than LCST, and the crosslinked one releases water molecules and it collapses at these (higher) temperatures.[21] The polymers become more hydrophobic and show reversible phase transition between hydrophilic to hydrophobic states around the LCST of the polymer. Changes of the LCST have been found due to the modification or copolymerization of poly(NIPAAm) to the responsive characteristics for temperature, pH stimuli within polymer chains. In our study, we have applied PEG to enhance the LCST of the NIPAAm. To prepare a pH responsive polymer, we prepared PEG grafted benzaldehyde and amine end-capped PNIPAAm containing copolymer.

The synthesis procedure of grafted PEG coplymers having different pH senstive copolymers based on poly(NIPAAm) is illustrated in Scheme 1. Firstly, grafted PEG copolymer (**Pre-polymer 1**) containing hydroxyl groups onto the backbone was obtained by radical polymerization using HEMA and *t*-BMA in the presence of *t*-butylperoxybenzoate as initiator at 140°C. The degree of substitution (DS) of HEMA and *t*-BMA was calculated from the integration ratio between the oxirane protons (4H, CH_2CH_2O) of the PEG at 3.6 ppm, the methyl protons [2H, CH_2CH_2OH] of HEMA at 4.1, and *t*-butyl protons [9H, $C(CH_3)_3$] of *t*-BMA at 1.4 ppm, as shown in Figure 1(a). GPC analysis indicated that the resultant grafted PEG copolymer with a M_n of 3400 was successfully synthesized and purified; the results were in close agreement with the ^{1}H-NMR data. Additionally, the DS of HEMA and *t*-BMA onto PEG was determined to be 10 and 6, respectively.

PEG-*g*-oligo(benzaldehyde) (**Pre-polymer 2**, containing 10 grafted benzaldehyde groups onto the backbone) with a molecular weight 4900 was synthesized by reacting PEG-*g*-oligo (HEMA-*co*-*t*-BMA) and *p*-formyl benzoic acid, where peak for aldehyde group was assigned at 10.0 and 7.9–8.2 ppm in the ^{1}H-NMR spectrum [Figure 1(b)].[2] Then PEG grafted copolymers (**P-N1**, **P-N2**, and **P-N3**) were synthesized by controlling the molar ratio of **Pre-polymer 2** and the amine end-capped **poly A** [poly(NIPAAm)], **poly B** [poly(NIPAAm-*co*-DEA)], and **poly C** [poly(NIPAAm-*co*-MAA)] at 40°C for 4 h. The poly (NIPAAm) based copolymer grafted PEG copolymers with benzaldehyde groups of **Pre-polymer 2** can effectively react with **poly A, poly B**, and **poly C** containing amine groups forming a benzoic imine bond.[2] The aldehyde peak at 10.0 ppm of **poly A, poly B,** and **poly C** significantly disappeared, while the imine proton at 8.1 ppm was clearly seen in ^{1}H-NMR spectrum in Figure 1. GPC investigation was applied to determine the molecular weight of the synthesized crosslinked polymer indicating the molecular weight of 32,700 for **P-N1,** 26,800 for **P-N2**, and 30,500 for **P-N3,** respectively (Table I).

LCST Behavior of the Prepared Polymers

Note should be recalled that copolymerization of poly (NIPAAm) in graft copolymers with a pH sensitive polymers typically changes the LCST behaviors. The influence of pH on the LCSTs of different copolymer compositions based poly (NIPAAm) was systematically investigated under different pH conditions. According to Figure 2(a), the LCST of pure poly



Scheme 1. Synthesis of dual pH and thermo-responsive graft copolymers.

(NIPAAm) is around 32°C at pH 7.4. It has been found that the LCST increased significantly, almost 36°C, when the poly (NIPAAm) was grafted onto PEG and it remains constant under neutral at pH 7.4 and slightly acidic at pH 6.0. Change in LCST was resulted when the poly(NIPAAm) grafted PEG was present in mild acidic environment. The LCST decreased from 36°C to 32°C, when polymer was treated at pH 3.0. The significant changes of LCST under different conditions are the results of grafted poly(NIPAAm) copolymer to PEG via pH responsive linker. The presence of PEG aids the hydrophilicity of poly (NIPAAm) and subsequently increased the LCST compared to only poly(NIPAAm). On the other hand, the pH responsive benzoic imine bond was broken down when the polymer was subjected to acidic environment. Due to cleavage of imine bond, the poly(NIPAAm) copolymer disassociate from the PEG and gained its individual hydrophobic features.[2] Next, we evaluated the influence of functionalization of NIPAAm regarding LCST within a broad range of pH. According to Figure 2(b), the transition state of **P-N2** was broader and extends at neutral and acidic environment compared with when it was treated at highly basic condition. A high LCST has been resulted at lower pH. The LCST values decreased with increasing pH. It was found that at pH 12.0 the LCST value was 18°C. The high protonation and solvation effects of DEA under acidic environment show higher LCST. On the other hand, at basic environment, the methacrylic block becomes hydrophobic due to deprotonation forcing to show lower LCST.[21] It is seen that at pH 3 there is no LCST for poly(NIPAAm-*co*-DEA) while it is found for **P-N2**. Figure 2(c) shows that LCST of **P-N3** was surprisingly changed under a wide range of pH. The copolymer does not exhibit any LCST within the experimental temperature range, not only at pH 7.4 but also at higher pH 12. The effect of breaking down of imine bond was significantly found, when it was treated in highly acidic environment (pH 3.0). LCST of **P-N3** dropped dramatically to 30°C. Accompanied with dissociation from the PEG, hydrophobic nature of MAA at lower pH played a vital role to show lower LCST value under the same condition. It should be noted that the LCST of thermo-responsive polymers depends on the hydrophilic/hydrophobic balance of the polymers regarding the hydrophobic and hydrogen bond interactions of water molecules with polymer chains.[22] Below LCST, water is a good solvent to solubilize the polymer due to the strong hydrogen bonding interaction between polar groups and water which is opposed by the hydration of the apolar groups. The water surrounding the apolar groups is in a low entropy state relative to free water, leading to an entropic penalty.[12] pH responsive property of the synthesized polymer was also confirmed via ^{1}H-NMR spectroscopy (Supporting Information Figure S2). The result shows the characteristics absorbance peak of benzaldehyde at 10.0 ppm for **Pre-polymer 2** which disappeared when poly(NIPAAm) was grafted via imine bond formation at pH 7.4. But, when the solution of **P-N1** was treated at pH 3.0 the peak at 10.0 ppm reappeared. The re-appearance of aldehyde peak clearly demonstrated the degradation of **P-N1** via cleavage of benzoic imine bond.

Contact Angle Measurement

The contact angles measured at 25 and 37°C are plotted and presented in Figure 3. The results indicate that there was significant increase in contact angle at 37°C compared with at 25°C. Figure 3(a) indicates that for **P-N1** contact angle significantly increased at higher temperature. The reason is that at lower

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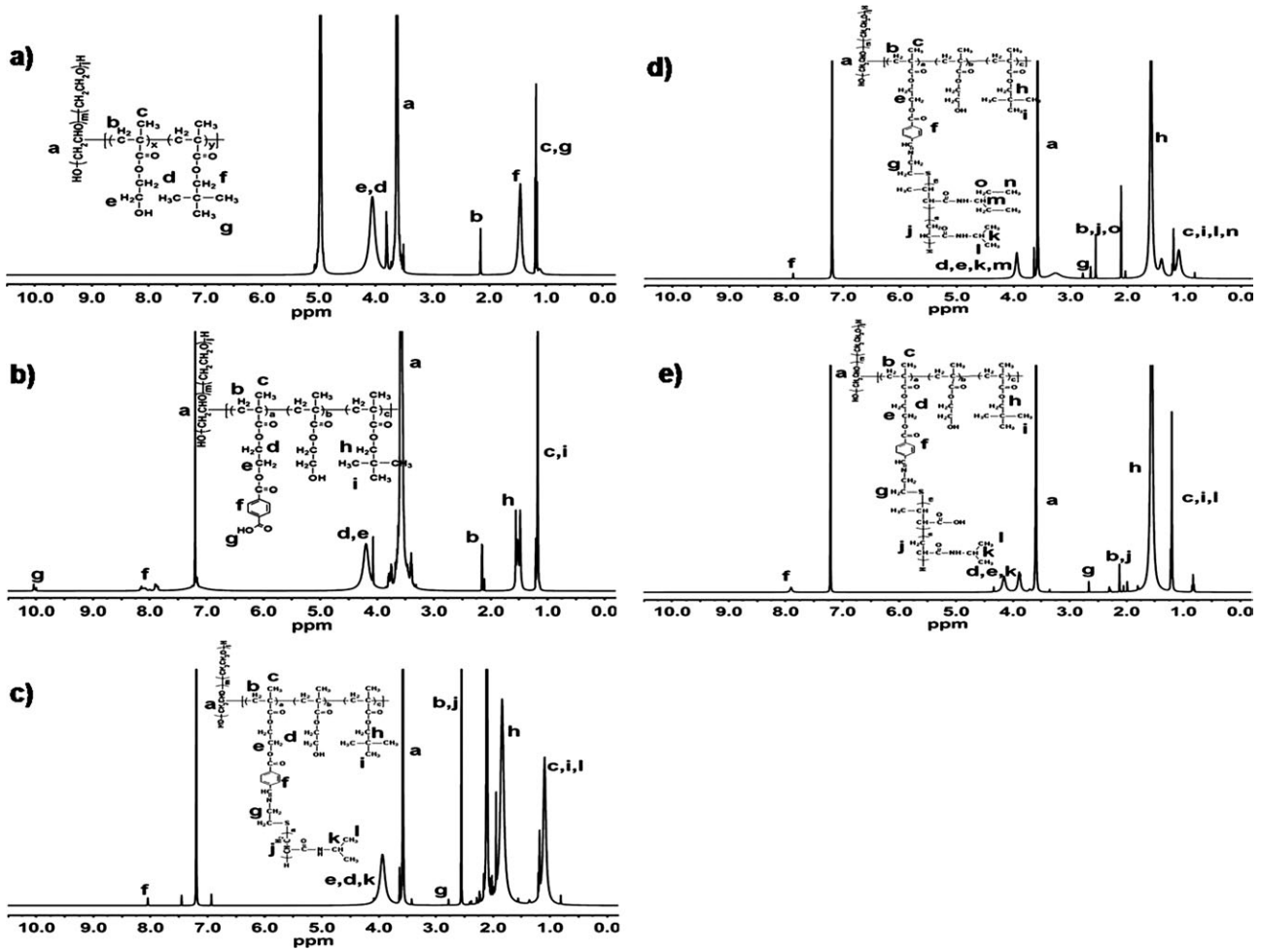

Figure 1. [1]H-NMR spectra of (a) PEG-*g*-oligo(HEMA-*co*-*t*-BMA), (b) PEG-*g*-oligo(benzaldehyde), (c) PEG-*g*-poly(NIPAAm)[**P-N1**], (d) PEG-*g*-poly[(-NIPAAm)-*co*-(DEA)][**P-N2**], and (e) PEG-*g*-poly[(NIPAAm)-*co*-(MAA)][**P-N3**].

temperature NIPAAm swells in water through extensive hydrogen bond formation with its available surrounding water molecules while at higher temperature, by forming intra- and intermolecular hydrogen bonds through entropic forces, the polymer shrinks and expels water molecules.[23] At pH 7.4 for polymer **P-N2**, contact angles were 13.1° and 38° measured at 25 and 37°C, respectively. At lower pH, the dimethyl amino groups are positively charged and exhibit enhanced hydrophilicity. So here, contact angle should be increased for **P-N2**. But the benzoic imine bond played a vital role to increase the contact angle slightly withdrawing hydrophilicity through disassociation from the polymer due to cleavage of benzoic-imine bond. In Figure 3(b), the contact angle was higher at 37°C than at 25°C for all synthesized polymer due to the thermo-responsive behavior of the NIPAAm. Grafted polymer (**P-N3**) showed slightly lower contact angles with 25.3° and 39.2° at pH 7.4. At pH 7.4 the contact angle of **P-N3** was lower at 25°C compared to lower pH condition [Figure 3(c)]. This is due to the hydrophilic effect of the PEG and MAA. At lower pH, due to cleavage of benzoic-imine bond from synthesized copolymer was disassociated and increased the hydrophobicity.[2] The carboxyl group of MAA formed more hydrogen bonds with CONH group of NIPAAm

Table I. Molecular Weight of All Synthesized Polymers Determined by [1]H-NMR and GPC

Polymer	M_n[a]	M_n[b]	PDI[2)]
PEG-*g*-oligo(HEMA-*co*-*t*BMA) [**Pre-polymer 1**]	3100	3400	1.7
PEG-*g*-oligo(benzaldehyde) [**Pre-polymer 2**]	4900	5100	1.8
Poly(NIPAAm) [**poly A**]	1900	2000	2.1
Poly(NIPAAm-*co*-DEA) [**poly B**]	1400	1700	1.8
Poly(NIPAAm-*co*-MAA) [**poly C**]	1500	1000	1.7
PEG-*g*-poly(NIPAAm) [**P-N1**]	32,700	11,700	2.3
PEG-*g*-poly[(NIPAAm)-*co*-(DEA)] [**P-N2**]	26,800	12,600	2.1
PEG-*g*-poly[(NIPAAm)-*co*-(MAA)] [**P-N3**]	30,500	15,000	2.2

[a]Estimated by [1]H-NMR using $CDCl_3$ solvent.
[b]Estimated by GPC (DMF) using PEG standard.

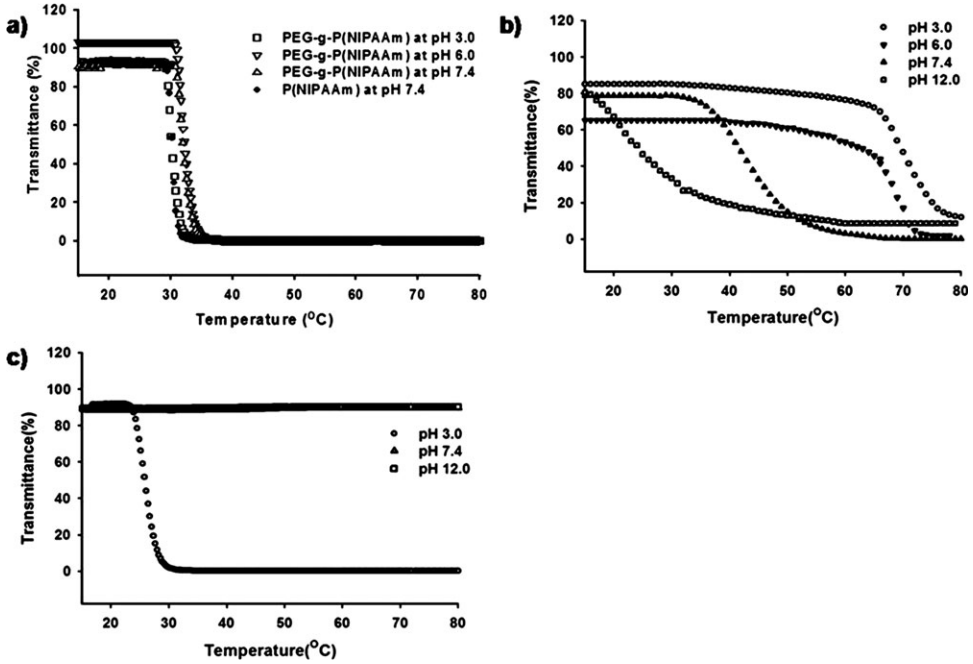

Figure 2. Temperature dependent transmittance changes of (a) **P-N1**, (b) **P-N2**, and (c) **P-N3.**

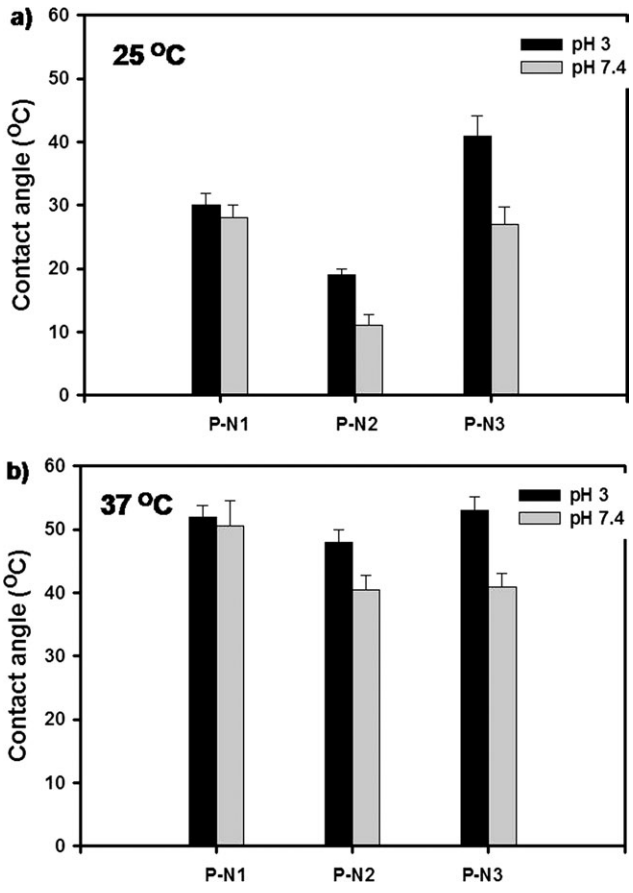

Figure 3. Water contact angle of **P-N1, P-N2**, and **P-N3** (**a**) at 25°C and (b) at 37°C.

which added more hydrophobic properties to **P-N3** polymer at lower pH. The increase of contact angle may be due to the expanded conformation of **P-N3** where the copolymer offered enough free volume to accommodate water molecules below the LCST and an optimum copolymer composition for intra- and intermolecular NIPAAm hydrogen bonding which subsequently enhanced hydrophobicity above LCST. As our synthesized polymers are responsive to pH we can compare the data under different pH condition at different temperature.[24]

pH Induced Size Change of the Polymer

According to Figure 4(a), the hydrodynamic diameter of **P-N1** was not significantly different during their presence in acidic and neutral accommodation. For, **P-N2**, the particle size

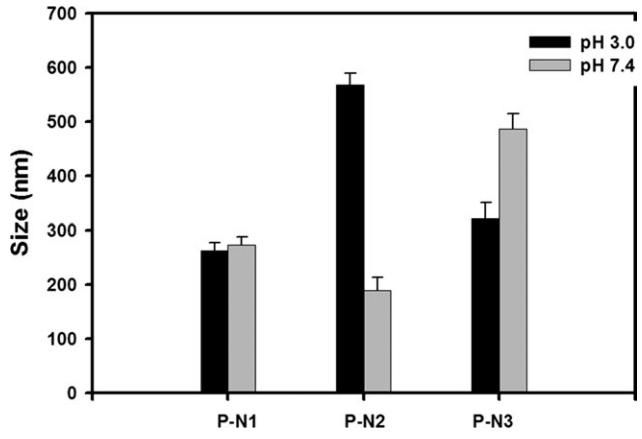

Figure 4. Particle size investigation by dynamic laser light scattering (DLS) of **P-N1**, **P-N2**, and **P-N3**.

increased at pH 3.0 [Figure 4(b)]. Under acidic environment, DEA segments become hydrophilic and leave the core of the formed micelles by **P-N2** wherein a highly compact core is the reason of lower particle size at pH 7.4. Same phenomenon has been observed during investigation of the micelle size assembled by **P-N3**. The hydrophobic nature of MAA at lower pH showed a highly compact core and at higher pH, the protonation effect tended to withdraw MAA from the micelles which subsequently increased the hydrodynamic diameter [Figure 4(c)].

CONCLUSIONS

In conclusion, we have successfully synthesized dual pH and temperature responsive poly(NIPAAm) based copolymer grafted to PEG backbone via pH responsive benzoic-imine bond where NIPAAm shows thermo-responsive properties. The LCST investigation clearly showed the degradation behavior of pH responsive bond present in PEG-*g*-poly(NIPAAm) [**P-N1**] which was further rectified following contact angle as well as particle size investigation. Functionalization of poly(NIPAAm) copolymers by DEA and MAA segments indicates a significant influence on LCST behavior of grafted PEG copolymers.

ACKNOWLEDGMENTS

This work was supported by Fusion Research R&D Program from the Ministry of Knowledge Economy (MEK, 2012) and also by a grant bearing the Grant No of R0001435 of the Ministry of Knowledge Economy (MEK).

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