Colloid Polym Sci (2009) 287:919–926
DOI 10.1007/s00396-009-2046-8

ORIGINAL CONTRIBUTION

Tunable phase transition behaviors of pH-sensitive polyaspartamides having various cationic pendant groups

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Received: 9 February 2009 / Revised: 27 April 2009 / Accepted: 30 April 2009 / Published online: 21 May 2009
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Abstract New pH-sensitive graft copolymers based on poly(2-hydroxyethyl aspartamide) (PHEA) were prepared by attaching various cationic monomers, such as 4-(aminomethyl)pyridine (PY), 1-(3-aminopropyl)imidazole (IM), and *N*-(3-aminopropyl)dibuthylamine (BU), as pH-sensitive units and octadecylamine (C_{18}) as a hydrophobic segment on poly (succinimide). Phase transition of each copolymer solution occurred at a vicinity of the pK_a value of the cationic groups, and their insoluble pH ranges were broadened as the feed amount of pH-sensitive moieties was increased. Depending on the cationic grafts having different pK_a values, the pH ranges where the copolymer became insoluble could be tuned. Copolymers PHEA-*g*-C_{18}-PY, PHEA-*g*-C_{18}-IM, and PHEA-*g*-C_{18}-BU exhibited phase separations in solutions at pH ranges of 4~6, 6~8, and 9~12, respectively. These polymers have the unique feature of their pH sensitivity profiles being identified to three regimes. Under low pH conditions (below pK_a), the polymer solution is transparent. At medium pH (around pK_a), polymer precipitation occurred in solution. At pH>pK_a, the polymer solution is gradually dissolved again.

Keywords Polyaspartamide · pH-sensitive · Phase transition

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Introduction

In recent years, there has been rapid development of stimuli-sensitive polymers that change their molecular conformations and, hence, their functions in response to external stimuli such as pH, temperature, electric field, and magnetic field [1–4]. In particular, pH-sensitive polymers are being extensively studied owing to their potential use in targeted drug delivery systems in both scientific and industrial fields [5–10].

Ionizable groups such as bases and acids play an important role in pH-sensitive behavior. A basic group such as pyridine or imidazole is substantially charged below its pK_a value, and conversely, an acidic group such as carboxylic acid is ionized at pH above pK_a. The resulting ionic repulsion overcomes hydrophobic interactions of polymer chains and leads to uncoiling of the polymer chain. Meanwhile, as the pH is changed such that a less ionizing environment is created, the proportion of charged groups decreases, and hydrophobic interactions become the predominant factor for both backbone and side chain polymers [11]. This causes the polymer chain to form a compact, insoluble, globular molecule, which precipitates from the aqueous solution. Representative pH-sensitive materials are sulfonamide [5, 8] and L-histidine [12, 13].

Poly(amino acids) have received considerable attention for their biomedical applications as potential drug carriers, owing to their protein-like nature and biodegradable and non-toxic properties. Poly(2-hydroxyethyl aspartamide) (PHEA), a poly(amino acid) derivative, is composed of an amino acid derivative based on aspartic acid and is easily synthesized by simple aminolysis of poly(succinimide) (PSI). Since the succinimide ring in PSI readily reacts with primary or secondary amines by nucleophilic attack,

diverse molecules can be grafted to PHEA to provide various functions [14–17].

The aim of this study is to prepare a new class of pH-sensitive polymers containing several basic molecules of different pK_a values, resulting in phase transitions at various pH ranges. To this end, we selected three kinds of weak basic monomers containing pyridine, imidazole, and tertiary amine groups, whose pK_a values are around 5, 7, and 9, respectively. We then synthesized graft copolymers with varying composition by attaching the aforementioned weak basic monomers as pH-sensitive units and octadecylamine (C_{18}) as a hydrophobic segment. Their pH-induced phase transition behavior was investigated by light transmittance measurements.

The characteristic feature of pH-sensitive polymers in this study is that when copolymers having a certain ratio of hydrophobic and hydrophilic cationic units are formed, the pH-induced turbidity profiles are distinctly identified to three regimes instead of two regions. The first regime is a transparent region where pH<pK_a, and the second regime is around pK_a, where the polymer precipitates. When the pH is higher than the pK_a, the polymer is gradually dissolved again to generate a homogeneous solution. Each of these pH regimes was characterized by dynamic light scattering (DLS) and zeta-potential measurements.

A pH-induced phase transition is a unique property that has been observed with polymers having ionizable units and a hydrophobic component within the polymer chain [18–20]. The competitive interactions between electrostatic repulsion and hydrophobic interactions in the polymer chain could play the main role in the pH-dependent behaviors when amphiphilic polyelectrolytes dissolve in aqueous media. The polymers in the present study have a hydrophobic C_{18} unit and ionizable amine derivatives as the pendant groups. By changing the ratio between these two distinctive units of poly(aspartamide)s, we could control the critical phase transition pHs. The phase transition behaviors of these polyaspartamide derivatives having pH-sensitive pendant groups are expected to facilitate various applications including controlled drug delivery.

Experimental section

Materials

L-aspartic acid and ethanolamine were purchased from Sigma. *N*,*N*-dimethylformamide (DMF), C_{18}, phosphoric acid (85%), mesitylene, sulforane, 4-(aminomethyl)pyridine (PY), 1-(3-aminopropyl)imidazole (IM), *N*-(3-aminopropyl) dimethylamine (ME), *N*-(3-aminopropyl)diethylamine (ET), and *N*-(3-aminopropyl)dibuthylamine (BU) were purchased from Aldrich. All chemicals were used as received except DMF. DMF was dried over a 4Å molecular sieve before use. The dimethyl sulfoxide-d_6 (DMSO-d_6) used for proton nuclear magnetic resonance (^{1}H-NMR) experiments was obtained from Aldrich.

Methods

Synthesis of pH-sensitive polyaspartamides

PSI was synthesized by acid-catalyzed thermal polycondensation of L-aspartic acid, as previously reported [14]. In brief, a mixture of L-aspartic acid and phosphoric acid in a mesitylene/sulforane ratio of 7/3 was stirred at 160 °C under an N_2 atmosphere. The water formed in the reaction mixture was removed using a Dean–Stark trap with a reflux condenser. After 24 h, the reaction mixture was precipitated in excess methanol and washed with distilled water several times until the pH of the suspension became neutral. The molecular weight (M_n) of PSI was determined by gel permeation chromatography in H_2O as 13,500 (PDI=1.32) [21].

The series of pH-sensitive polyaspartamides was obtained by successive aminolysis of C_{18} and each pH-sensitive monomer among PY, IM, ME, ET, and BU. All polymers and the compositions of the monomers are listed in Table 1. The general synthetic procedure of pH-sensitive polyaspartamide was as follows: Purified PSI (0.97 g, 0.01 mole equivalent as succinimide units) was dissolved in 8 mL of dried DMF, and a selected amount of C_{18} was added to the mixture rapidly. The reaction mixture was stirred at 70 °C for 24 h. A pH-sensitive molecule was then added to the mixture, and the solution was stirred for another 12 h and cooled to 30 °C. A slightly excess amount of ethanolamine was subsequently added to the reaction mixture. After stirring for 6 h, the insoluble products were filtered out, and the clear solution was extensively dialyzed (MWCO 6~8 kDa) by distilled water and then freeze-dried.

^{1}H-NMR characterization

^{1}H-NMR spectra (AL 400, JEOL) was used to characterize the conjugation of C_{18} and pH-sensitive units and to determine the degree of substitution (DS). DMSO-d_6 was used as a solvent.

The light transmittance measurement

The light transmittance of polymer solutions with varying the pH was determined at 500 nm using a UV–vis spectrophotometer (Cary 1 Bio, Varian). In each case, 1 mg/ml of the polymer was dissolved in distilled water and titrated to pH 3.0 with 0.1 N HCl, and the pH was gradually

Table 1 DS of polymers

Sample name	M_n[a]	Feed mole ratio (C_{18})	DS_{C18} (mol%)	Feed mole ratio (pH-sensitive unit)	DS_{amine} (mol%)
PHEA-*g*-C_{18}0-PY70	24,600	0	–	70	55.0
PHEA-*g*-C_{18}10-PY25	24,100	10	8.1	25	20.1
PHEA-*g*-C_{18}10-PY50	26,800	10	9.0	50	47.7
PHEA-*g*-C_{18}10-PY70	28,000	10	9.6	70	59.3
PHEA-*g*-C_{18}10-PY90	28,900	10	9.6	90	70.0
PHEA-*g*-C_{18}20-PY70	29,100	20	18.2	70	41.5
PHEA-*g*-C_{18}30-PY70	30,800	30	24.6	70	37.5
PHEA-*g*-C_{18}10-IM25	25,400	10	9.5	25	23.7
PHEA-*g*-C_{18}10-IM50	28,500	10	9.7	50	50.9
PHEA-*g*-C_{18}10-IM90	31,000	10	9.5	90	74.1
PHEA-*g*-C_{18}10-ME70	27,900	10	9.7	70	63.4
PHEA-*g*-C_{18}10-ET70	31,000	10	9.8	70	68.8
PHEA-*g*-C_{18}10-BU70	33,700	10	9.3	70	56.0

[a] Number average molecular weight calculated from M_n of PSI taking into the account amount of grafts

raised by adding 0.1 N NaOH solution given in various volume increments. Before the experiments, all samples were filtered through a filter membrane (pore size 0.4 μm).

Dynamic light scattering and zeta-potential measurement

The particle size of the pH-sensitive PHEA sample was measured by the DLS method based on the particle size option in ELS-8000 (Otsuka Electronics Co., He-Ne laser). The scattered intensity was registered at the scattering angle of 90° and 25 °C. Zeta potentials were measured by an ELS flat board cell coated with platinum. Each data point for the zeta potentials is an average of at least five measurements.

The concentration of all solutions was prepared as 3 mg/mL. Before the experiments, all samples were filtered through a filter membrane (pore size 0.4 μm), and the pH of the polymer solution for these measurements was precisely adjusted by using 0.1 N HCl or 0.1 N NaOH solution.

Results and discussion

Synthesis and characterization of polymers

pH-sensitive polymers were successfully synthesized by performing the reaction of PSI with C_{18} as a hydrophobic group and a cationic monomer as a pH-sensitive group. The five pH-sensitive molecules used in this study were chosen because they had distinctly different pK_a values. The pyridine side chain of PY has a pK_a of around 5, and the imidazole side chain of IM has a pK_a of 6.9. Three different tertiary amines (ME, ET, BU) having similar pK_a values between 9 and 10 were chosen as the study of a polymer system that undergoes a phase transition immediately above pH 9.

All synthetic routes and the molecular structures are shown in Fig. 1. The structures of the polymers synthesized and the DS were determined by ^{1}H-NMR. The characteristic ^{1}H-NMR peaks assigned for polymer structure verification are listed below:

<PHEA> -NH-CO-**CH_2**: 4.6 ppm, -CO-CH2-**CH**-: 2.6 ppm.
<C_{18}> -NH-(**CH_2**)$_{17}$-CH_3: 1.1 ppm, -NH-(CH_2)$_{17}$-**CH_3**: 0.8 ppm.
<PY> =N-**CH**-CH-: 8.4 ppm, =N-CH-**CH**-: 7.2 ppm, -NH-**CH_2**-C≡: 4.3 ppm.
<IM> -N-**CH**=N-: 7.6 ppm, =N-**CH_2**-CH-: 7.1 ppm, -CH_2-**CH**=N-: 6.9 ppm, =N-**CH_2**-CH_2-: 3.9 ppm, -NH-**CH_2**-CH_2-: 3.0 ppm, -CH_2-**CH_2**-CH_2-: 1.8 ppm.
<ME> -N^+-(**CH_3**)$_2$: 2.3 ppm, -CH_2-**CH_2**-CH_2-: 1.6 ppm.
<ET> -N^+-(**CH_2**)$_3$-: 2.6 ppm, -CH_2-**CH_2**-CH_2-: 1.5 ppm, -N^+-(CH_2-**CH_3**)$_2$: 1.0 ppm.
<BU> -N^+-(**CH_2**)$_3$-: 3.0 ppm, -CH_2-**CH_2**-CH_2-: 1.9 ppm, -N^+-(CH_2-**CH_2**-CH_2-CH_3)$_2$: 1.6 ppm, -N^+-(CH_2-CH_2-**CH_2**-CH_3)$_2$: 1.3 ppm, -N^+-(CH_2-CH_2-CH_2-**CH_3**)$_2$: 0.9 ppm.

Table 1 shows the grafted mole percents of C_{18} and pH-sensitive groups as the feed mole percents. The first number is the feed mole percent of the hydrophobic amine (DS_{C18}) and the last is the feed mole percent of pH-sensitive amine

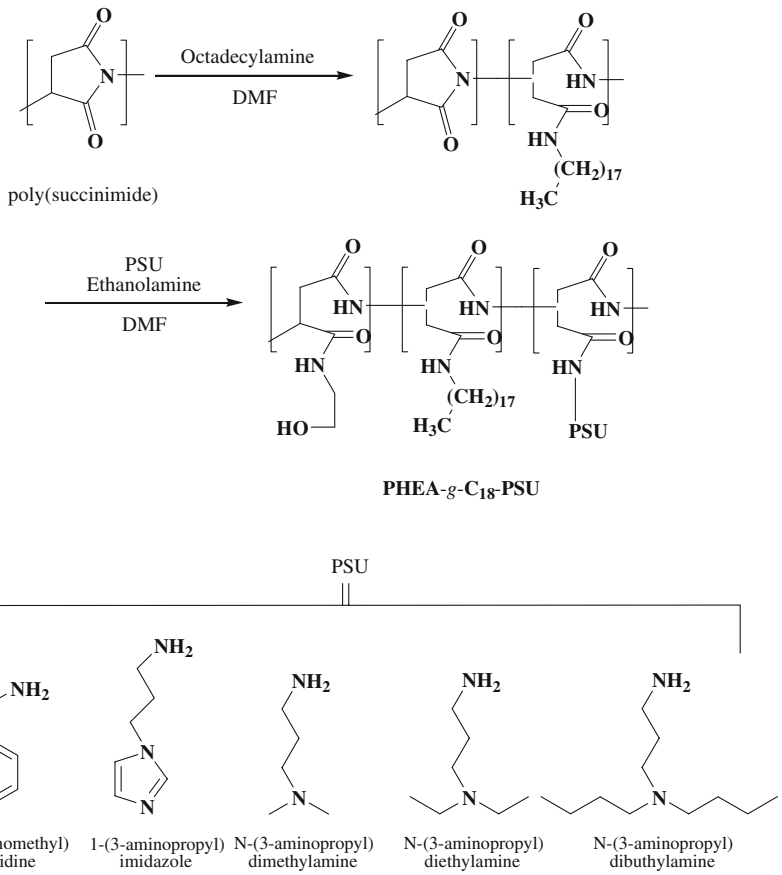

Fig. 1 Synthetic scheme of pH-sensitive polymers

(DS_{amine}). In this paper, the DS of C_{18} and pH-sensitive groups were determined as the mole ratios of C_{18} and of pH-sensitive molecules to the mole ratio of all the repeating units in the polymer. These were calculated by comparing the integral of characteristic peaks assigned to C_{18} and pH-sensitive groups respectively, with the integral peak assigned to the methine proton of the main chain of the backbone polymers. Equation 1 and the characteristic peaks of C_{18} and pH-sensitive groups are used for determining each DS,

$$\mathrm{DS}(\%) = \frac{\mathrm{Area}_{\text{Characteristic peaks}}}{\mathrm{Area}_{4.5\sim4.7\mathrm{ppm}} \times n} \times 100 \qquad (1)$$

where n is the number of hydrogen molecules assigned for characteristic peaks. The chemical shift and n for characteristic peaks from each monomer are as follows: <C_{18}>: sum of 1.1 and 0.8 ppm, n=37; <PY>: 7.2 ppm, n=1; <IM>: 6.9 ppm, n=1, <ME>: 1.6 ppm, n=2; <ET>: 1.5 ppm, n=2; and <BU>: 1.6 ppm, n=4.

The grafted mole percent of the C_{18} unit linearly increased according to the increase of the feed amount, with a grafting reaction efficiency of 80~95%. However, the yield of grafted pH-sensitive moieties decreases with an increase of the feed amount of C_{18} and bulkiness of pH-sensitive molecules. In the case of PHEA-*g*-C_{18}10-PY70, which was grafted with 10 mol% of C_{18} in advance, 85% of the pyridine units were attached to the backbone polymer. In the case of PHEA-*g*-C_{18}20-PY70 and PHEA-*g*-C_{18}30-PY70, the yields of grafted pyridine groups were 59% and 53%, respectively. Furthermore, ME and ET can be attached to PHEA with a conjugation efficiency of more than 90%; however, the bulkier molecule BU showed lower conjugation efficiency of about 80%.

Phase transition

The transmittance of aqueous solution of pH-sensitive polymers with different pH-sensitive groups was observed in a range of pH 3.0~10. The initial polymer solution was prepared at pH 3.0 with 0.1 N HCl, and the solution pH values were adjusted by adding 0.1 N NaOH solution. In Figs. 2, 3, 4, and 5, the transmittances of polymer solutions with different pendant groups are shown with the pH values. Figure 2 presents the percent transmittance of aqueous solutions of PHEA-*g*-C_{18}*n*-PY70 (n=0, 10, 20, and 30) according to the pH change. The transmittances of the solutions reversibly changed without hysteresis for all polymers studied. For the polymer without C_{18}, the transparency of aqueous solutions was maintained over

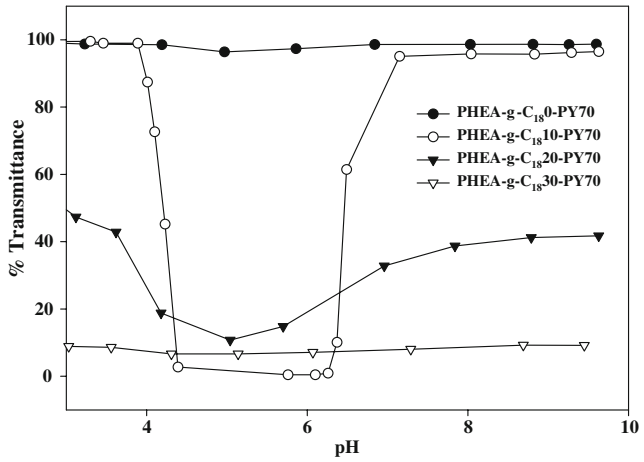

Fig. 2 Turbidity of PHEA-*g*-C_{18}*n*-PY70 (*n*=0, 10, 20, and 30) solution as pH change

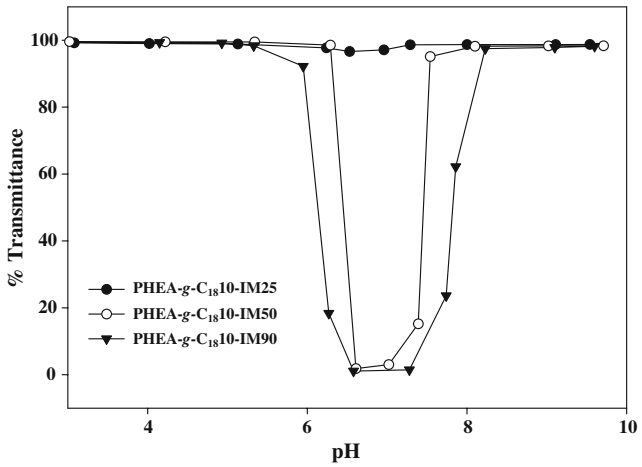

Fig. 4 Turbidity of PHEA-*g*-C_{18}10-IM*n* (*n*=25, 50, and 90) series solutions as pH change

the complete range of pH, indicating that no large scatters, i.e., greater than about λ/20, were formed regardless of the pK_a values of the graft groups. However, the percent transmittance significantly decreases with an increase in the feed molar ratio of C_{18}. The polymers with a high DS of C_{18} over 30% showed the significant drop of transmittance to less than 5% in all range of pH. At the neutral condition, it is known that the C_{18} grafted PHEA forms micelle, wormlike micelle, and lamellar phase as the DS of C_{18} increases [14, 22]. At very low DS, the aqueous solution of alkyl-grafted polymer forms relatively large micelles of multicore [23] but is transparent, apparently forming hydrogel-type loose aggregates. As the DS increases, the micelles become smaller and more tightly packed; while, at high DS=30, it becomes turbid and highly organized with a structural transition. In Fig. 2, a sharp transmittance change was clearly observed for polymers with DS 10%. The feed amount of C_{18} was thus fixed at 10 mol% for the preparation of pH-sensitive polymers.

Figure 3 shows the transmittance change of the PHEA-*g*-C_{18}10-PY series according to pH. In the UV–vis spectroscopic measurements, the aqueous solution of pyridine-grafted PHEA with a DS value exceeding 50% gave high transmittance at pH below 4.0. However, it drastically decreased when the pH was between 4 and 6. This was attributed to the hydrophobic nature of the pendant groups arising from deprotonation of pyridine moieties whose pK_a value is 5.2. The pK_a values of copolymers tend to slightly increase as DS of pyridine group gets larger (PHEA-*g*-C_{18}10-PY50=5.25, PHEA-*g*-C_{18}10-PY70=5.35, and PHEA-*g*-C_{18}10-PY90=5.38), but all the values are around 5.2. As the pH of the solution was raised over 6, the transmittance

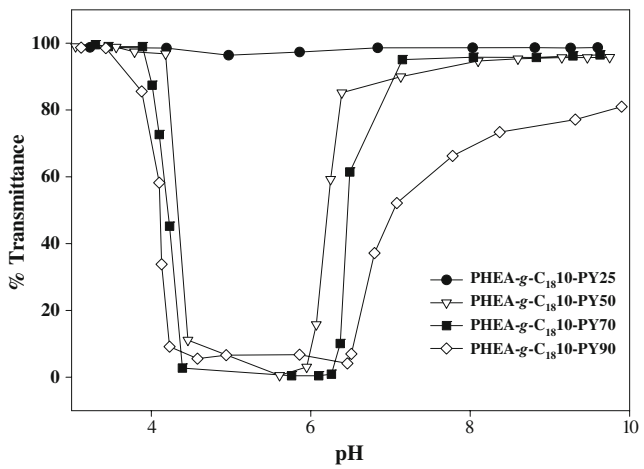

Fig. 3 Turbidity of PHEA-*g*-C_{18}10-PY*n* (*n*=25, 50, 70, and 90) series solutions as pH change

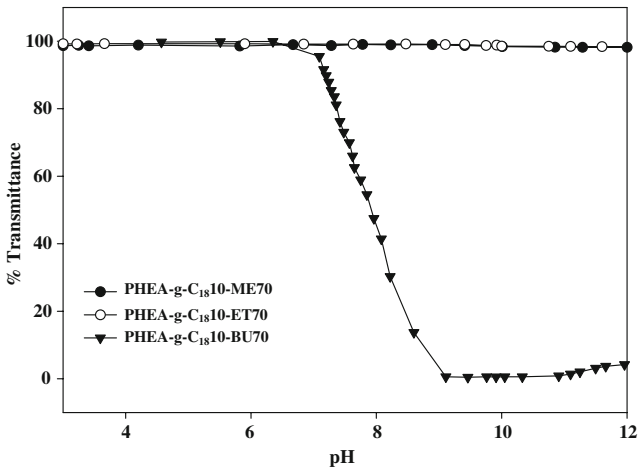

Fig. 5 Turbidity of tertiary amine grafted PHEA series solutions as pH change

gradually increased again. The insoluble pH range of pyridine-grafted polymer broadened with increasing grafting density of the pyridine moiety.

The pH-dependant transmittance changes of imidazole-grafted polymers are shown in Fig. 4. The insoluble turbid range appeared between pH 6 and 8, around pK_a=6.9 of imidazole moieties, and the insoluble range broadened with an increase of the amount of grafted imidazole groups, as was the case for the pyridine groups. Both polymers of grafted pyridine and imidazole groups with DS below 25% showed no phase transition, and the transmittance value was maintained at a high level over the complete pH range. This may be due to the weakness of the role of pH-sensitive groups in the polymer as the hydrophilic aspartamide backbone plays the main role in determining the polymer solubility.

Figure 5 shows the pH-induced transmittance profiles of polymer solutions with three different tertiary amines. For the case of PHEA-*g*-C_{18}10-BU70, the transmittance gradually decreased beyond pH 7, and finally, there was no transmittance at pH 9. A slight increase in transmittance above pH 11 was also observed. For PHEA-*g*-C_{18}10-ME70 and PHEA-*g*-C_{18}10-ET70, the solution remained transparent in the range of pH variation, because of their lack of hydrophobicity. Deionized pyridine or imidazole units tended to engage in hydrophobic interactions such as π–π interaction and hydrogen bonding among aromatic heterocycles, resulting in polymer aggregation and precipitation with a sharp phase transition [8]. On the contrary, aliphatic pH-sensitive units did not interact with each other, and thus, the aqueous solution failed to precipitate throughout the pH range (PHEA-*g*-C_{18}10-ME70 and PHEA-*g*-C_{18}10-ET70) or showed a gradual decrease of transmittance with precipitation in a wide range of pH (PHEA-*g*-C_{18}10-BU70).

Phase transition behavior

The characteristic feature of pH-sensitive polymers in this study is that the pH-induced turbidity profiles can be distinctly classified into three regions: (1) pH<pK_a soluble range, (2) pH≈pK_a insoluble range, and (3) pH>pK_a soluble range.

As demonstrated with the phase transition behavior of the polymers evaluated here, the transition point shifts to lower pH with an increase in the feed ratio of pH-sensitive groups. For the case of the PHEA-*g*-C_{18}10-PY series, the insoluble precipitation pH shifted from 4.33 to 4.11 with an increase of the feed mole percent of pyridine moiety from 50% to 90% (Table 2). As more pyridine molecules are conjugated, less ethanolamine, a part of the polymer involved in hydrogen bonding, is grafted to the backbone polymer. Thus, the chain becomes more hydrophobic than that of ethanolamine-grafted polymers.

The negative logarithm of an acid dissociation constant (pK_a) of pH-sensitive units can be expressed as a function of the degree of ionization (α), known as the Henderson–Hasselbalch equation [24].

$$\mathrm{p}K_a = \mathrm{pH} + \log\frac{1-\alpha}{\alpha} \tag{2}$$

At the precipitation pHs, the pH in which the percent transmittance of polymer solution is at 50%, the composition of ionized and unionized fraction of pyridine units can be calculated using Eq. 2. As shown in Table 2, the fractions of unionized units of ionizable molecules in PHEA-*g*-C_{18}10-PY series were 8.0~8.4% at their transition pHs, indicating that the precipitation occurs at similar hydrophobic fractions in the polymer chains. However, the variation of ionized fractions at the same point of pHs was considerably larger than that of unionized fractions. Therefore, it may be proposed that the polymers in solution would be changed from a soluble state to an insoluble state by rearranging the alignment of hydrophobic units and hydrophilic charged moieties and by breaking the balance of intermolecular forces. Thus, the phase transition occurs at almost constant content of the unionized form. Supporting this hypothesis, the polymer phase transition pH could be precisely controlled by changing the amount of pH-sensitive units.

The pH-dependant solution behaviors for the selected polymers were further investigated by DLS and zeta-potential

Table 2 Ionization and phase transition of PHEA-*g*-C_{18}10-PY series

Sample name	PHEA-*g*-C_{18}10-PY50	PHEA-*g*-C_{18}10-PY70	PHEA-*g*-C_{18}10-PY90
pH (percent T=50)	4.333	4.207	4.110
α[a]	0.823	0.861	0.886
Ionized fraction (percent)[b]	39.26	51.10	62.01
Unionized fraction (percent)[c]	8.45	8.23	7.99

[a] α=degree of ionization at pH (percent T=50)

[b] Ionized fraction (percent)=$DS_{PY}\times\alpha$

[c] Unionized fraction (percent)=$DS_{PY}\times(1-\alpha)$

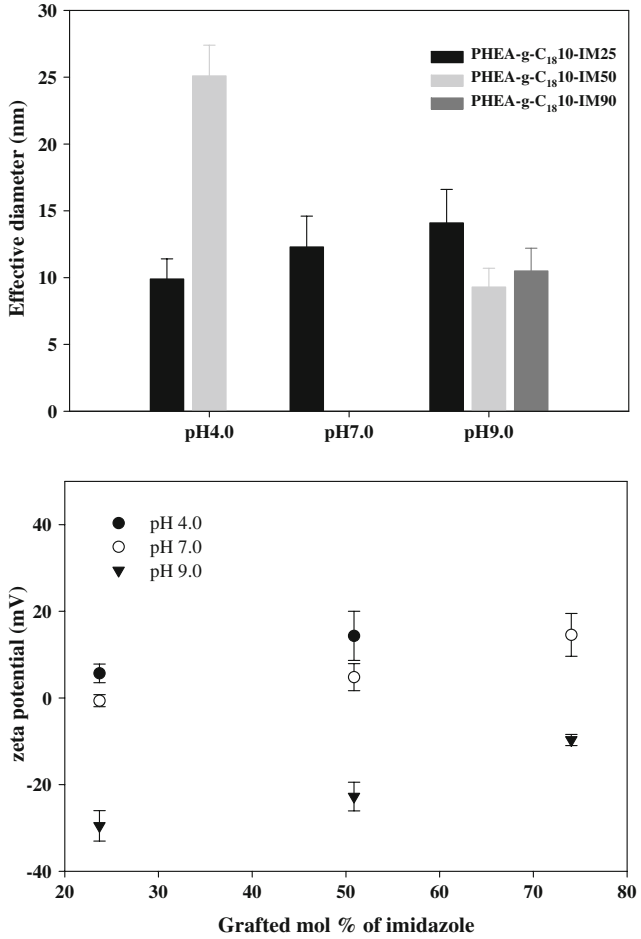

Fig. 6 Effective diameter and zeta potential of PHEA-*g*-C_{18}10-IM aggregates at pH 4.0, 7.0, and 9.0

measurements at pHs 4.0, 7.0, and 9.0 to understand the polymeric microstructures in aqueous solution. The results are shown in Fig. 6. The DLS and zeta-potential data of PHEA-*g*-C_{18}10-IM90 at pH 4.0 were not obtained because this polymer was completely dissolved at pH 4.0, and thus, there was no formation of self-assembled microstructure measurable with light scattering, and also, the sizes of turbid samples of PHEA-*g*-C_{18}10-IM50 and PHEA-*g*-C_{18}10-IM90 at pH 7.0 could not be determined by DLS because of the precipitation.

All of the polymer samples are transparent at pH 4.0. For the pK_a of the imidazole moiety, 99.9% of imidazole units were in an ionized form at this pH. This indicated the formation of a self-assembly consisting of a hydrophobic C_{18} core is surrounded by a protonated imidazole shell. The effective diameter of aggregates increased from 9.9 to 25.1 nm as the amount of conjugated imidazole was increased from 25 to 50 mol%. The reason for this is that the polymer backbone bending required for the formation of hydrophobic domains was affected by the imidazole conjugation. The increased charge density induced the PHEA to form hydrophobic domains, and electrostatic repulsion between imidazole moieties of the PHEA in the aggregate cause the aggregate to swell at pH 4.0. The zeta-potential measurements at pH 4.0 showed a similar trend, validating the trend observed with DLS measurement. Zeta potentials of PHEA-*g*-C_{18}10-IM series increased with an increase of the feed amount of imidazole moiety due to the cationic amine groups of imidazole in the side chain. The zeta potentials of the PHEA-*g*-C_{18}10-IM series increased from +5.7 to +14.3 mV when the feed molar ratio of the imidazole group was increased from 25% to 50%. This strongly indicates that the conjugated imidazole moieties were located in the shell surface of the aggregate. For the PHEA-*g*-C_{18}10-IM series, a small change of pH of about 0.1 is sufficient to alter the curvature of the alkyl chain layer and the surface charge. Therefore, it may be concluded that the internal structure of the polymer solution is significantly curved, leading to the formation of a small micelle and/or dissolution at low pH. However, as the surface charge of the micelle is reduced such that the repulsive force is reduced, the curvature of the micelle is reduced to form a large-sized micelle or lamellar structure [8].

At pH 7, the particle size of PHEA-*g*-C_{18}10 was about 25 nm [14] but that of the PHEA-*g*-C_{18}10-IM25 was about 12 nm. Therefore, the addition of imidazole moieties and its repulsive charge might contribute to increased curvature of the micelle. Meanwhile, at pH 9.0, where the polymer solutions are dissolved, the deprotonation of imidazole renders the micelles charged negatively. As the amount of imidazole is increased, the zeta potential becomes neutralized and, further, a significant amount of imidazole remains uncharged and intercalated in the hydrophobic layer, to causing curvature of the micelle. Thus, ethanolamine and imidazole may exchange the proton interaction at high pH but essentially are in a soluble form in water. The micelles are stabilized for PHEA-*g*-C_{18}10-IM series, which contains a negatively charged surface, similar with PHEA-*g*-C_{18}10. At pH 9.0, the measured particle sizes of PHEA-*g*-C_{18}10-IM series remained almost the same, irrespective of the amount of conjugated imidazole, because most of the imidazole moieties are presented in a unionized form at this pH. Therefore, the phenomenon that the PHEA-*g*-C_{18}10-IM solutions are dissolved at high pH might occur irrespective of the content of pH-sensitive units in the polymer. The possible cause of this phenomenon could be as follows: as pH rises, hydroxide ions are adsorbed on the uncharged aggregates by hydrogen bonding between nonbonding electrons (oxygen of ethanolamine and nitrogen of unionized imidazole) of the polymer and hydroxide ions, resulting in dissolution [25, 26].

These pH-sensitive polyaspartamide derivatives have many possible applications over a broad range of both scientific and industrial fields, such as intracellular drug

delivery systems for endosomal escape and cosmetic systems employing triggered release of active materials by skin pH.

Conclusion

Various weak basic monomers such as PY, IM, and BU were successfully conjugated to PHEA with varying degrees of substitution. The phase transition of each copolymer occurred in the vicinity of the pK_a value of the attached pH-sensitive unit, and their insoluble pH ranges were broadened as the feed amount of pH-sensitive units was increased. By simple tuning of the attached pH-sensitive units with different pK_a values, we could obtain pH-sensitive polymer series that are insoluble at pH ranges of 4~6, 6~8, and 9~12, respectively.

Acknowledgements This work was part of the project of the Human Resource Development for Industrial Demand, and Basic R&D Research Program, financially supported by the Ministry of Education, Science, and Technology (MEST) and a Korea Science and Engineering Foundation (KOSEF) grant (R11-2007-050-03003-0).

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