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Schizophrenic copolymer from natural biopolymer by facile grafting

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ABSTRACT

A novel schizophrenic copolymer responsive to pH and temperature change was developed through copolymerization of *N*-isopropylacrylamide (NIPAm) and acrylic acid (AA) on chitosan chains by a bio-friendly initiator. The behavior of purified graft copolymer in solution phase was studied in detail by NMR, Zetasizer, TEM, SEM and ATR-FTIR techniques. In dilute aqueous media, two types of reversible nanoparticles can be obtained by changing temperature and pH: positively charged micelles with chitosan-shell in acidic solutions above 33 °C and negatively charged micelles with P(NIPAm-co-AA)-shell in alkaline solutions. The transform between these two oppositely charged micelles can be easily controlled by changing pH at human body temperature. The effect of graft degree on the solution behavior was also studied. This novel schizophrenic copolymer has potential applications in drug delivery and other controlled releases.

1. Introduction

Smart or stimulus-responsive polymers have attracted great attention due to their interesting properties and potential applications in sensors, therapeutic devices, and optoelectronic switches [1–5]. Responsive properties of these polymers in terms of conformation, hydrophilicity and chain density upon stimuli of temperature, pH, ionic strength, UV light and electric potential have been studied [6–9]. Among the reported smart polymers, one type of copolymers can form micelles in aqueous solution and reverse the core and shell components upon suitable stimuli. Armes et al. have prepared a series of these copolymers, and defined them as 'schizophrenic' copolymers [10–12]. Thereafter, more schizophrenic copolymers were synthesized by several others [13–18]. They generally combined hydrophilic, hydrophobic and charged monomers in a single chain, coupling with their sensitivities to specific stimuli.

The schizophrenic copolymers have been synthesized by group transfer polymerization (GTP), atom-transfer radical polymerization (ATRP), and radical addition fragmentation and transfer polymerization (RAFT) [10,11,13]. Although these living polymerizations can finely control the structure and composition of desired copolymers, they generally require critical conditions or organic solvents, contain catalysts toxic to bio-organisms, and produce non-biodegradable linear copolymers. All of these factors prohibit the applications of the resultant schizophrenic copolymers in biomedical field. Here, we report the development of a new schizophrenic copolymer from a widely available biopolymer-chitosan, by a radical graft polymerization with a bio-friendly initiator.

Chitosan is a versatile biopolymer widely applied in medical and pharmaceutical fields. Many graft copolymers from chitosan have been prepared for targeted deliveries, biomedical scaffolds and biodevices [19–21]. In this work, chitosan was employed to develop a schizophrenic copolymer for the first time, by grafting with *N*-isopropylacrylamide (NIPAm) and acrylic acid (AA), using an initiation system of *tert*-butyl hydroperoxide (TBHP)/amino group ($-NH_2$). Poly(*N*-isopropylacrylamide) (PNIPAm) is the most often employed thermo-responsive polymer that undergoes a sharp coil–globule conformation transition in water at its lower critical solution temperature (LCST) 32 °C, changing from a hydrated coil to a dehydrated globule by forming intramolecular hydrogen bonding between –NH and C═O [6]. It is non-toxic to cell at a concentration $C < 1$ wt% [22]. By copolymerization with other monomers, its LCST has been tuned in a wide range [23]. Biodegradable copolymers containing thermally sensitive PNIPAm block have been prepared by Cheon and Kohori [24–26], and their micelles and nanoparticles have been employed as controlled release carriers. However, these reported nanoparticles showed only gradual release dynamics following a biodegradation process, without the feature of shizophrenic copolymer micelles that release all encapsulated substance once upon a suitable stimuli. AA is the simplest ionizable monomer enabling solubility change dependent on pH [27]. In addition, its low cost and adhesion to biological surfaces as partially protonated attract long-standing interest in its pharmaceutical applications

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[28]. By combining NIPAm with AA, a series of sensitive copolymers and hydrogels have been created and imparted tunable LCST depending on both the monomer composition and the solution pH [29–32]. In this study, NIPAm and AA were grafted onto chitosan together to create a schizophrenic copolymer, which were initiated by an efficient redox pair TBHP/–NH_2. This initiation can generate reactive radical on the nitrogen atom of –NH_2 without any toxic residue [33–36]. Compared to the reported initiators for grafting from chitosan, e.g., ammonium persulfate, ammonium persulfate/sodium bisulfite, ammonium persulfate/maleic anhydride, and maleic anhydride/2,2′-azoisobutyronitrile (which all gave a grafting efficiency less than 30% with random grafting positions) [37–41], this redox pair produces an evidently higher grafting efficiency. It also controls grafting position mainly at the –NH_2 groups. TBHP has a high decomposition temperature ($t_{1/2}$ = 170 h at 100 °C), therefore it is seldom used as an initiator alone. However, with an amine group as a reducer, it can initiate polymerization below 100 °C by forming two radicals RO· and -NH·. Therefore, in this chitosan/TBHP system, the grafting point is controlled mainly at –NH_2 of chitosan. Even if RO· radicals abstract a few H atoms and induce additional radicals on chitosan, –NH_2 is preferred over –OH.

All these carefully chosen components lead to a novel schizophrenic graft copolymer having potential bio-applications in targeted delivery of drugs and imaging reagents. Although these components have been explored for a long time, there is still a growing number of researches employing them to create novel functional reagents, e.g., recently reported multi-membrane hydrogels from pure chitosan, pH responsive and nanostructured hydrogel from grafted chitosan, composite vesicles from PNIPAm, and Janus supraparticles from PNIPAm, poly(acrylic acid) and polyacrylamide [41–44]. As a new responsive unit suitable for reliable biomedical applications usually requires a substantial research investment (similar to a new drug), creatively designing compound systems from the old units becomes an important pathway to meet the growing demand on novel functional reagents. This work is featured by combining bio-friendship and schizophrenic behavior, which offers two types of nanoparticles transforming into each other reversibly upon specific stimuli, supporting potential applications in controlled release.

2. Experimental section

2.1. Materials

Chitosan (low molecular weight M_w = 120,000; 89% deacetylation), acetic acid, *N*-isopropylacrylamide (NIPAm), acrylic acid (AA), *tert*-butyl hydroperoxide (TBHP, 70% solution in water), D_2O and $HAuCl_4$ were all purchased from Aldrich. To remove inhibitor, NIPAm was recrystallized from a mixture of hexane and toluene (65:35 v/v), and acrylic acid was distilled. Freshly deionized water was used as the dispersion medium.

2.2. Synthesis

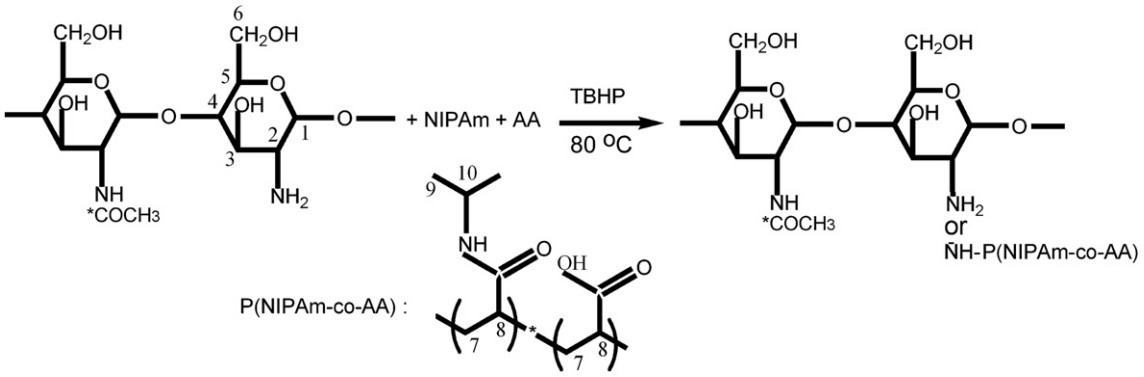
A typical synthesis of chitosan-g-P(NIPAm-co-AA) was described as follows (see Scheme 1 for the schematic illustration). In a flask equipped with a condenser and a nitrogen inlet, chitosan solution (0.5 wt%, 100 mL) containing acetic acid (0.6 wt%, pH 4.5) was stirred, purged with nitrogen, and heated to 80 °C. Then 1.0 g NIPAm and 0.08 mL AA (mole ratio 8:1) were added, followed by addition of 1.0 mL 20 mM TBHP aqueous solution. The reaction was continued for 6 h under nitrogen. Finally white latex was obtained, which subsequently became transparent at room temperature.

The reaction product was purified at room temperature in a dialysis tube with a 50 K molecular weight cutoff (Spectra/Por), against 5 L 0.6 wt% acetic acid solution renewed daily for one week. The copolymer solution was further neutralized to its isoelectric point (IP) around pH 6.5, centrifuged and washed by water. The resultant precipitate was then dried into pure product. In this way, non-grafted monomers (NIPAm and AA) and polymers (PNIPAm, PAA, and P(NIPAm-co-AA)) were removed, and pure copolymer chitosan-g-P(NIPAm-co-AA) (CNA) was obtained in a weight of 0.94 g. The graft degree (the ratio of the increment in copolymer weight to the beginning polymer weight) was calculated as 88%. The purification efficiency was confirmed by a gel permeation chromatography (GPC) assay, in which a single peak of a weight average molecular weight (M_w) around 225,000 was observed for the graft copolymer CNA. From the as prepared latex, an additional peak of M_w around 31,000 was observed, which was attributed to the ungrafted free polymer P(NIPAm-co-AA) (see Table 1).

In order to investigate the effect of graft degree on the copolymer micellization, two more copolymers were prepared by changing only the monomer amount: one using 0.25 g NIPAm and 0.02 mL AA and the other using 2.0 g NIPAm and 0.16 mL AA. Compared to the previous one having a graft degree of 88%, the two additional copolymers (CNA-L and CNA-H) gave graft degrees of 26% and 156% after purification with the weight of 0.63 g and 1.28 g respectively. Their M_w′s were determined by GPC as 156,000 and 307,000 respectively (see Table 1).

2.3. Characterization

The molecular weight distributions of as prepared latex and purified graft copolymers were analyzed by a GPC/LS/RI method. The assay system consisted of a Waters 515 HPLC pump, a Razel syringe pump, a Shodex Protein KW-804 column (Showa Denko Co.), a miniDAWN tri-angle laser light scattering detector (Wyatt Technology Co.), and an Optilab rEX refractive index detector (690 nm, Wyatt Technology Co.). An eluent of acetic acid (0.6 wt%, pH 4.5) was employed at a flow rate of 0.5 mL/min, injection volume 200 μL, temperature 25.0 °C. Chromatograms were collected by Astra V software, provided by Wyatt Inc. The aqueous solution behavior of the purified graft copolymer was studied by changing pH and temperature. ^1H NMR measurements were recorded on a Varian Inova AS 500 spectrometer in deuterium oxide (D_2O), at 25 °C and 40 °C for pH 4.2 and at 25 °C for pH 9.2. The pH of D_2O medium was adjusted by adding DCl or NaOD. FTIR spectra were recorded on a Bio-Rad FTS 6000 Spectrometer (Cambridge, MA). The sampling station was equipped with an overhead attenuated total reflectance (ATR) accessory (Pike Technologies, Madison, WI), comprising of transfer optics within the chamber, with a detachable ATR crystal – zinc selenide crystal with 45° parallelogram geometry and 10 internal reflections – mounted into a plate with a shallow trough for sample containment. The depth of penetration, which gives a measure of the intensity of the resulting spectrum, is 1.46 μm. Acetic acid solution (0.6%) was used as the control solution, which spectrum was subtracted from those of samples. The size distribution and ζ-potential of obtained micelles in solution were measured by a Malvern Zetasizer 3000HSA equipped with a 10 mW He–Ne laser (633 nm), operating at an angle of 90° and a constant temperature. D_n and D_v are the number and volume average particle diameters, respectively. D_v/D_n is the polydispersity index of the micelle size distribution. The micelles were stained by $HAuCl_4$ in solution and observed under a transmission electron microscope (TEM) (JEOL 2010, 200 kV) after dropping on a carbon coated Cu grid; the corresponding nanoparticles of dried micelles were observed under a scanning electron microscope (SEM) (JEOL, JSM-6335F, 3 kV) after spin-drying on a glass wafer.



Scheme 1. Synthesis of copolymer chitosan-g-P(NIPAm-co-AA) (CNA) by a radical graft polymerization.

3. Results and discussion

The response of purified copolymer CNA to the changes in solution temperature and pH was studied. In order to avoid the chain entanglement and serious gelation upon phase separation, we employed a dilute solution of 0.08 w/v%. Its behavior was proved to be complex and fascinating. This copolymer dissolved molecularly in the solution of pH = 4.2 and $T = 25$ °C, since both the chitosan backbone and the P(NIPAm-co-AA) graft were hydrophilic and hydrated (a clear solution in Fig. 1). At this concentration, the adjacent polymer chains may contact to each other. This short distance allows the existence of hydrogen bonding and electrostatic interaction between molecules. Despite the coexistence of protonated amine groups on chitosan and dissociated carboxylic groups on acetic acid and P(NIPAm-co-AA), the inter-chain electrostatic association was weak and did not prohibit the dissolution of copolymer. GPC/LS/RI measurement revealed that copolymer CNA had a radius of gyration $R_g = 24$ nm and $M_w = 225{,}000$. When heated above 33 °C, the P(NIPAm-co-AA) graft became dehydrated and hydrophobic, and several grafts assembled into a micellar core. The clear solution changed into a light-blue phase and was stable in storage, indicating micellization occurred. On the other hand, as the medium pH was increased from 4.2 to its isoelectric point (IP) pH 6.5 by slowly dropping 0.1 N NaOH under stirring, the clear solution changed into a gel-like fluid. This gelation is due to the strong (large quantity of) electrostatic association between protonated amine groups and dissociated carboxylic groups, analogous to the case of a protein at its IP. While the pH of gel-like fluid was further increased to 9.2, it became a bluish solution and was stable in weeks. On the basis of chemical intuition, the inter-chain electrostatic association should become weaker and the deprotonated chitosan chains should form the hydrophobic cores with the solvated P(NIPAm-co-AA) chains forming the micellar coronas (Fig. 1). The ^{1}H NMR studies confirmed this proposition to be the case. In the micellization process, inter-chain hydrogen bonding between –OH and $–NH_2$ may result in complex micelles of large size.

The NMR spectrum of the copolymer dissolved in D_2O is shown in Fig. 2 a and is used to calculate the block composition. The unit mole ratio of chitosan to P(NIPAm-co-AA) was calculated as 1:1.3 based on peak areas assigned to "2" and "7" (referenced to Scheme 1). Thus the grafting efficiency (the mole ratio of grafted monomers to beginning monomers) of NIPAm and AA is nearly 40%. This value was also supported by the calculation from the weight of purified product.

When the solution was heated to 40 °C, the P(NIPAm-co-AA) graft became progressively dehydrated and self-assembled into hydrophobic core, as judged by ^{1}H NMR spectroscopy (Fig. 2c) that is similar to that of pure chitosan (Fig. 2d). The chitosan main chain remained solvated under these conditions (note the prominent signals at $\delta = 2.9$–3.6), whereas the signals due to the P(NIPAm-co-AA) graft at $\delta < 1.6$ disappeared. Zetasizer measurements indicated a zeta potential $\zeta = 62$ mV (proving protonated chitosan as the shell), a number-average hydrodynamic diameter $D_n = 180$ nm and a polydispersity $D_w/D_n = 1.10$. This micelle diameter was much larger than those reported for flexible linear copolymers (e.g. 80 nm from ref.[45]). The stretching conformation of chitosan chain should contribute to this large micelle size. As reported by Wu [46], in a solution of pH 5.1 buffered with 0.2 M CH_3COOH and 0.1 M CH_3COONa, the hydrodynamic radius $R_h = 20$ nm of a dissolved chitosan chain ($M_w = 120{,}000$, 91% deacetylation) was much larger than that (9 nm) of well dissolved flexible polystyrene with a same M_w, indicating that the protonated chitosan chain was rather stretched. However, even if the chitosan chains were fully stretched, one or two chains could not possibly give such a large compact micelle of 180 nm. This unusually large micelle size was not consistent with a simple core-shell structure. At this stage, we propose that these P(NIPAm-co-AA)-core aggregates are actually compound micelles with a complex core structure of partially swollen intercalate or interpenetrate networks, leading to rather loosely packed aggregates. In previous reports by Yuan and Morris [47,48], casein micelle with $R_h = 80$ nm and self-assembling micelles of poly(styrene-co-methacrylic acid) and poly(-vinylpyrrolidone) with $R_h = 100$ nm were studied in an aqueous medium, both of which were compound micelles resulted from complex interactions between H-bonding groups. Therefore, the large micelle size of copolymer CNA can be attributed to the inter-chain hydrogen bonding interaction between –OH and $–NH_2$

Table 1
The characterization data of all the synthesized copolymers from different recipe.

Reactions	Beginning chitosan/NIPAm/AA (g/g/mL)	M_w of graft copolymer	M_w of free P(NIPAm-co-AA)	Monomer composition in graft (NIPAm/AA)	Graft degree
1#	0.5/1.0/0.08	225,000	31,000	8/1	88%
2#	0.5/2.0/0.16	307,000	59,000		156%
3#	0.5/0.25/0.02	156,000	8000		26%

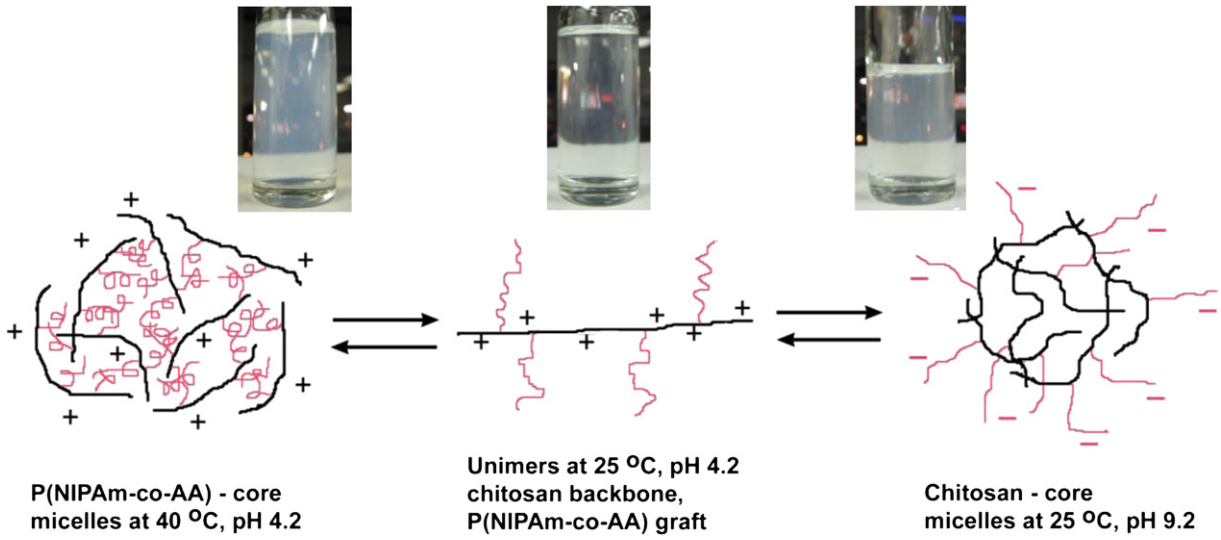


Fig. 1. Schematic representation of two types of reversible micelles transformed from copolymer chitosan-g-P(NIPAm-co-AA) in aqueous solution.

groups of chitosan. When these micelles were stained by $HAuCl_4$ in solution, many Au nanoparticles of ~10 nm size were immobilized on the micelle surface, due to the reduction effect of chitosan $-NH_2$ (Fig. 3 a). The micelle size under TEM agrees with the result from zetasizer measurement. Its non-shrinkage upon drying and non-void appearance indicates a dense distribution of Au nanoparticles within the micelles. While the original micelles were dried, SEM observation (Fig. 3c) showed nanoparticles with a mean diameter ~80 nm, which was much lower than the $D_n = 180$ nm in solution. These results support our assumption of swollen compound micelles in solution. This complex problem may be resolved by forming single-molecule micelles upon the extreme dilution (with a concentration below 5.0×10^{-6} g/mL). In another report by Wu [49], a combination of static and dynamic laser light scattering revealed that at temperatures higher than 33 °C, a linear poly(*N*-isopropylacrylamide) chain grafted with poly(ethylene oxide) (PNIPAm-g-PEO) in water undergoes a "coil-to-globule" transition to form a stable single chain core-shell nanostructure of $R_h = 30$ nm

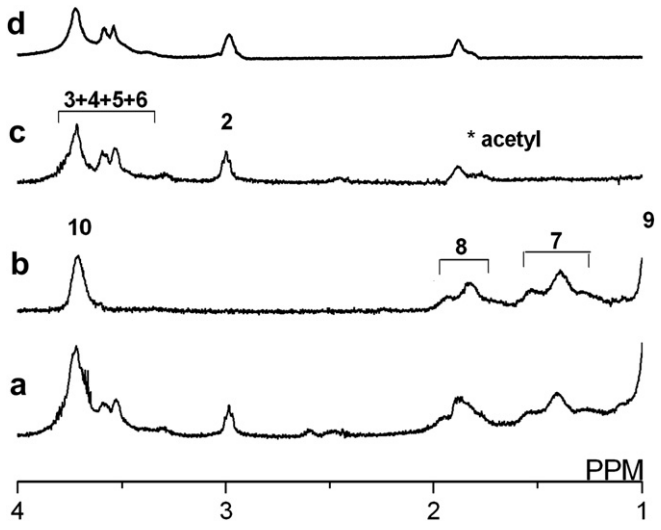


Fig. 2. ^{1}H NMR spectra recorded for the pure chitosan in D_2O/DCl (d) and the graft copolymer chitosan-g-P(NIPAm-co-AA) (CNA) in D_2O/DCl and D_2O/NaOD: a) at pH 4.2 and 25 °C (molecularly dissolved copolymers); b) at pH 9.2 and 25 °C (chitosan-core micelles); c) at pH 4.2 and 40 °C (P(NIPAm-co-AA)-core micelles).

(concentration = 6.7×10^{-7} g/mL), with the collapsed PNIPAm chain as the hydrophobic core and the grafted PEO branches as the hydrophilic shell. Regardless of the precise morphology of these aggregates, the temperature-induced micellization process was fully reversible: molecularly dissolved copolymer solution was obtained upon being cooled to 25 °C.

Reverse micelles with chitosan cores were obtained by adjusting the solution pH from 4.2 to 9.2. The signals due to chitosan residues at $\delta = 2.9$–3.6 had almost disappeared (Fig. 2b), indicating much lower mobility and decreased solvate for this main chain. On the other hand, the signals due to the P(NIPAm-co-AA) graft at $\delta < 2.0$ and $\delta = 3.7$ were prominent, indicating that this graft formed the solvated micellar corona. From Fig. 2b, the mole ratio of NIPAm to AA in graft can be calculated as around 8:1 (see Table 1), based on peak areas assigned to "10" and "7". This value is the same as their mole ratio in starting materials. Zetasizer measurements indicated a zeta potential $\zeta = -32$ mV, proving ionized P(NIPAm-co-AA) graft as the corona, since AA's pKa is around 4.5. The number-average hydrodynamic diameter D_n and the polydispersity D_v/D_n of micelles were measured as 140 nm and 1.06. This micellization was completely reversible: as the solution pH was reduced from 9.2 to 4.2 by adding acid, the micelles dissolved and the characteristic bluish color disappeared. When the chitosan-core micelles were stained by $HAuCl_4$ in solution, a typical large and complex core-shell structure was observed under TEM (Fig. 3b). A few Au nanoparticles (~10 nm) were immobilized in the chitosan-core area and isolated by entangled P(NIPAm-co-AA) chains, which clearly presented the compound micelle structure. In the corona area, thin Au dots (~2 nm) were deposited due to the weak reducibility of NH group of NIPAM. The micelle size under TEM (~140 nm) agrees with the result from zetasizer measurement. SEM image of the dried chitosan-core micelles (Fig. 3d) showed nanoparticles of a mean diameter ~ 60 nm, which was much lower than that in solution, mainly due to dehydration upon drying of the swollen micelles.

In order to thoroughly clarify the CNA transition mechanism in aqueous medium, its micelle size was further monitored in a wide range of pH and temperature. The obtained phase diagram against temperature was shown in Fig. 4. When the solution pH was lower than 6.0, it remained as a clear solution below its LCST (lower critical solution temperature) while changed into positively charged micelles above its LCST. This LCST increased with the solution pH: it was near 33 °C at pH 4.2, 35 °C at pH 5.2 and 40 °C at

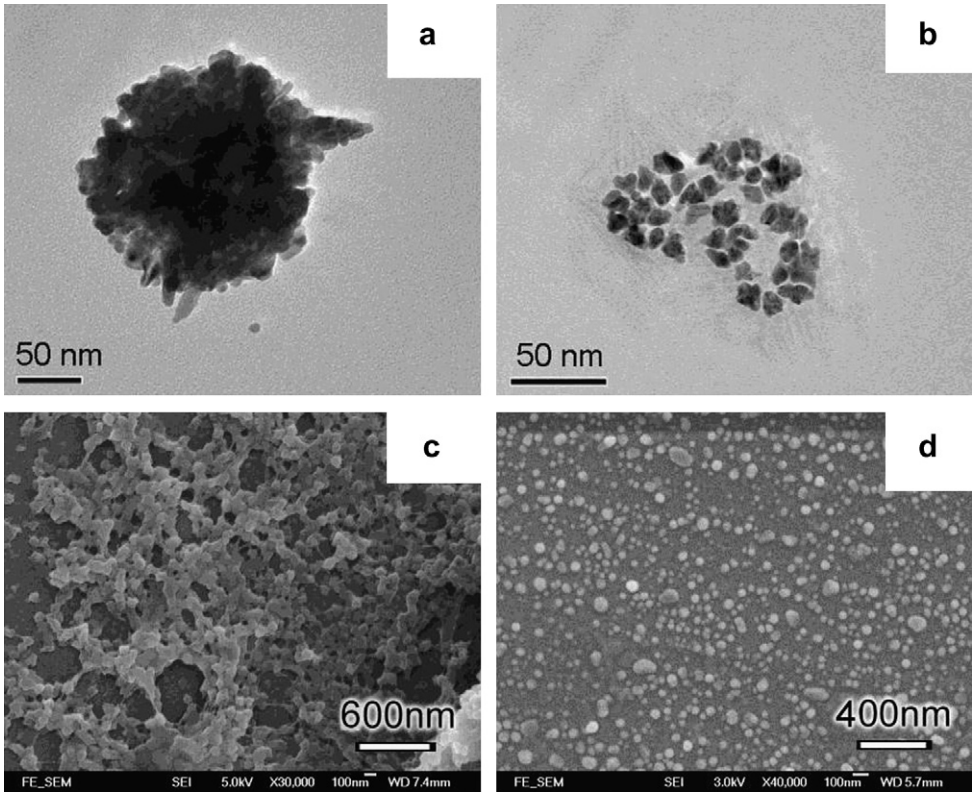


Fig. 3. TEM images of compound micelles from chitosan-g-P(NIPAm-co-AA) stained by $HAuCl_4$ solution: P(NIPAm-co-AA)-core micelles (a) and chitosan-core micelles (b); SEM images of dried P(NIPAm-co-AA)-core micelles (c) and chitosan-core micelles (d).

pH 5.8. Above their LCST, the micelles gradually shrunk from ~300 nm to ~180 nm within 15 °C increment and reached a stable size finally. This final stable size also increased with the solution pH, e.g. 172 nm at pH 4.2, 181 nm at pH 5.2, and 193 nm at pH 5.8. The increase of LCST and micelle size with solution pH is due to the dissociated carboxylic groups imparting electrostatic repulsion between AA units. Between pH 6.0 and 7.5, a gel-like fluid was noticed due to its isoelectric point around pH 6.5. When the solution pH was increased above 7.5, CNA changed into negatively charged micelles that might further shrink above its LCST (~50 °C). At pH 7.5, the micelle size was 310 nm at temperatures below 45 °C and shrunk into 251 nm at 50 °C. At pH 8.2 and 9.2, the micelle had smaller size of 170 and 140 nm respectively, which shrunk to 120 and 105 nm at 50 °C. This decrease of micelle size with the increase in pH is due to the denser contracting of chitosan-core at a higher pH, where chitosan $-NH_2$ is increasingly deprotonated and the hydrogen bonding between –OH and $-NH_2$ become stronger. These micelles at temperatures above 50 °C were rather condensed particles and remained dispersed in water, mainly depending on the electrostatic repulsion between negative shells. In this phase diagram, it is interesting that between 33 and 45 °C the two types of micelles can transform freely into each other by only pH change, benefiting from the dependence of its LCST on solution pH [30]. As shown in Fig. 5, at a constant temperature, the copolymer behaves much differently at the pH values below or above the gel-like region. At 25 °C, the copolymer does not form detectable micelles below pH 5.8 and aggregates into negative micelles above pH 7.5; at 40 °C, the copolymer aggregates into positive micelles below pH 5.8 and negative micelles above pH 7.5. This property allows the free conversion of two types of micelles in human body, suggesting a potential application in controlled release [41].

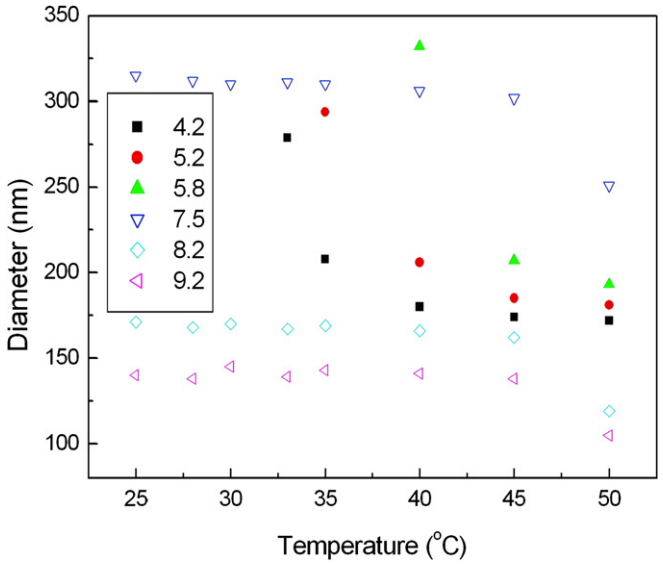


Fig. 4. Micelle size of CNA in various pH solutions changes with temperature solid symbols represent positively charged micelles with chitosan-shell; void symbols represent negatively charged micelles with hydrolyzed P(NIPAm-co-AA) shell.

The above discussion of CNA transition was also supported by ATR-FTIR measurement (Fig. 6). When the CNA solution was heated to 40 °C, the characteristic absorption of P(NIPAm-co-AA) at around 1631 cm^{-1} (ascribed to the carbonyl stretching vibration in amide groups) increased evidently, due to its transition into dyhydrated mode and resultant absorption onto the ATR crystal surface (resembling the protein absorption on hydrophobic surface in the ATR measurement) [50]; while the pH of CNA solution was adjusted from 4.2 to 9.2 by dropping 0.1 N NaOH, the characteristic

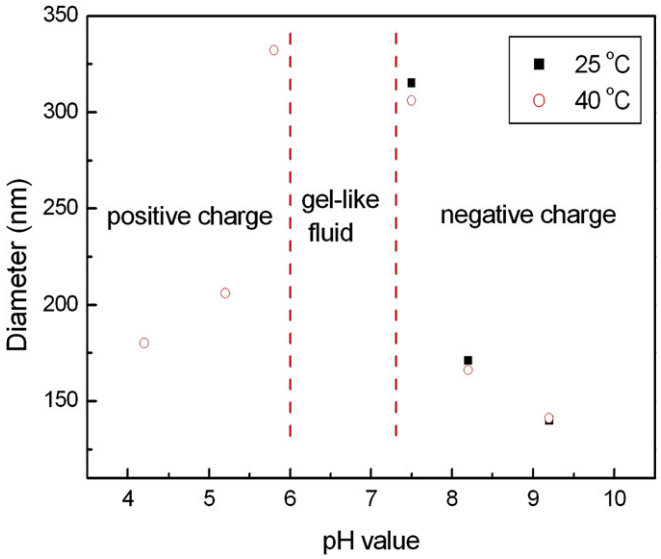


Fig. 5. Micelle size of CNA changes with pH adjustment at 25 and 40 °C.

absorptions at 1554, 1414, and 1080 cm^{-1} (which are ascribed to N–H bending vibration, O–H bending vibration, and C–O stretching vibration in chitosan chain, in well agreement with those of pure chitosan.) increased, due to the dehydration and resultant absorption of chitosan chains onto the ATR crystal surface.

The solution behaviors of another two copolymers with different graft degrees (CNA-L and CNA-H) were also studied by changing temperature and pH. Zetasizer measurement was performed on CNA-H (at a concentration of 0.08 w/v% too), which gave a phase diagram similar to that of CNA: they are different only by the micelle sizes, e.g., $D_n = 193$ nm and $\zeta = 59$ mV at pH 4.2 and 40 °C; $D_n = 148$ nm and $\zeta = -41$ mV at pH 9.2 and 25 °C. However, the CNA-L cannot transform into stable micelles at all conditions. When the CNA-L solution was heated up to 40 °C at pH 4.2, it remained clear without micelle formation. This result is due to its low graft degree and difficulty in the graft aggregation. When the CNA-L solution was changed into alkaline phase, it appeared turbid and produced a gel-like precipitate. This non-stability of CNA-L at alkaline condition is due to the low quantity of hydrophilic grafts and the weakness of charge repulsion from AA units.

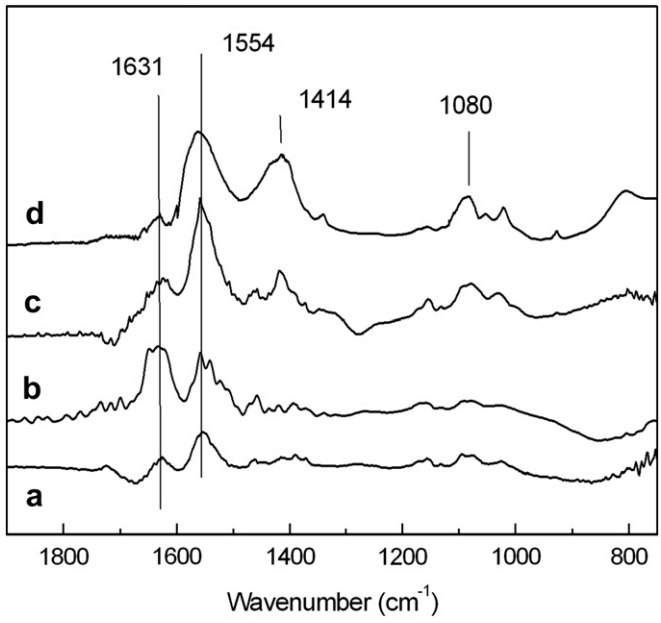


Fig. 6. ATR-FTIR spectra of chitosan solution (d) and the graft copolymer chitosan-g-P(NIPAm-co-AA) (CNA) solutions: a) at pH 4.2 and 25 °C (molecularly dissolved copolymers); b) at pH 4.2 and 40 °C (P(NIPAm-co-AA)-core micelles); c) at pH 9.2 and 25 °C (chitosan-core micelles).

In summary, this copolymer CNA has a more complex phase behavior than the recently reported copolymer poly(*N*-isopropylacrylamide-co-propylacrylic acid). The latter only showed adjustable LCST depending on composition and pH, without formation of any stable micelles or nanoparticles [31]. Our schizophrenic copolymer can form two types of freely reversible micelles at body temperature, supporting potential applications in controlled release and sensor.

4. Conclusions

A schizophrenic graft copolymer chitosan-g-P(NIPAm-co-AA) (CNA) was synthesized using a bio-friendly initiator TBHP. The CNA solution showed complex and fascinating responses to pH and temperature change. From its clear dilute solution, two types of reversible nanoparticles were obtained, which were P(NIPAm-co-AA)-core micelles in acidic solution above 33 °C, and chitosan-core micelles in alkaline solution. These charged micelles can be transformed into each other by simply tuning pH at temperatures close to human body temperature. However, when the graft degree of copolymer was lower than 26%, difficulty in micellization at all conditions was noticed. A large number of natural biopolymers could be the candidates to be coupled with NIPAm and AA to prepare schizophrenic copolymers in the same way. Thus synthesized schizophrenic copolymers would have high potential in wide applications such as are hopefully applied in controlled release, sensoring, and other intelligent processes.

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