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Preparation and aqueous solution behavior of a pH-responsive branched copolymer based on 2-(diethylamino)ethyl methacrylate

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ABSTRACT: pH-Responsive amphiphilic branched copolymers were prepared from poly(ethylene glycol) methyl ether methacrylate (PEGMA), 2-(diethylamino)ethyl methacrylate (DEAEMA), 2-(*tert*-butylamino)ethyl methacrylate (*t*BAEMA), and ethylene glycol dimethacrylate (EGDMA) utilizing a thiol-modified free radical polymerization. The molecular structures of copolymers were confirmed by proton nuclear magnetic resonance spectroscopy (^{1}H NMR) and triple-detection gel permeation chromatography (tri-GPC). The aqueous solution behaviors of the obtained copolymers were investigated by dynamic light scattering (DLS). The DLS data showed that about 16 nm polymer particles comprising of hydrophobic poly(*tert*-butylamino)ethyl methacrylate (P*t*BAEMA) and poly(diethylaminoethyl methacrylate (PDEAEMA) core, hydrophilic PEGMA corona were formed above pH 8. With the decrease of pH from 8 to 6, a dramatic increase in the hydrodynamic radius of polymer particles from 16 nm to 130 nm was observed resulting from the protonation of the PDEAEMA segment. Moreover, *in vitro* drug release behaviors of the resulting polymer assemblies at different pH values were also investigated to evaluate their potential as sustained release drug carriers. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2015**, *132*, 42183.

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INTRODUCTION

Over recent decades, the pH-responsive polymers have attracted much attention due to their unique ability to sense small changes in environment pH that trigger a corresponding change in the polymer's physical properties such as size, shape, hydrophobicity, and/or degradation rate.[1] Taking advantage of these distinctive virtues, many of them have been developed to fabricate pH-responsive polymer micelles or nanoparticles. Various polymerization methods have been applied to synthesize pH-responsive polymers with different size, shape, and chemical functionality, such as emulsion polymerization, anionic polymerization, group transfer polymerization (GTP), atom transfer radical polymerization (ATRP), and reversible addition-fragmentation chain transfer (RAFT).[2]

Practically, because of its well-controlled particle size distribution and structure, emulsion polymerization is among the most useful synthetic routes for preparing vinyl-based pH-responsive polymers. Both semi-batch and seeded semi-batch emulsion polymerization techniques have been used to produce colloidal particles or core-shell nano-particles.[3–8] However, well-defined pH-responsive polymers are more commonly prepared by living or controlled polymerization techniques. Eisenberg and coworkers[9] have produced a series of polyacrylic acid (PAA)-containing pH-responsive block copolymers via living anionic polymerization together with group-protection chemistry, where polystyrene-*b*-poly(*tert*-butyl acrylate) (PS-*b*-P*t*BA) were prepared by sequential anionic polymerization of styrene and *tert*-butyl acrylate. The P*t*BA block in the copolymers was selectively hydrolyzed using *p*-toluenesulfonic acid to form PAA block.[9] In addition, AB diblock copolymers of poly(dimethylaminoethyl methacrylate) (PDMA) and poly(diethylaminoethyl methacrylate) (PDEAEMA) were synthesized by Armes and coworkers[10] using GTP technique. Although anionic polymerization and GTP have been successfully used to synthesize pH-responsive polymers, strict reaction conditions are always required, and only a limited number of monomers are suitable for these techniques, both of which limited the application of these techniques.

Additional Supporting Information may be found in the online version of this article.

ATRP is currently one of the most versatile controlled radical polymerization (CRP) techniques for the design and synthesis of stimuli-responsive polymers. Since 2001, Armes and coworkers[11–19] have reported a series of well-defined AB diblock or ABC triblock pH-responsive copolymers synthesized via ATRP. These copolymers consist of either weakly acidic or weakly basic polyelectrolyte segments that can induce the formation of core-shell or shell cross-linked micelles in aqueous solution. RAFT polymerization is also a powerful technique for the preparation of well-defined copolymer architectures. In many cases, RAFT polymerization could be carried out directly in water at ambient temperature without the need for protecting group chemistry. For example, McCormick and coworkers[20–25] fabricated a range of pH-responsive or dual responsive block copolymers by aqueous RAFT polymerization using 4-cyanopentanoic acid dithiobenzoate (CTP), 4-cyano-4-(ethylsulfanylthiocarbonyl)sulfanylpentanoic acid (CEP), 4-cyano-4-(propylsulfanylthiocarbonyl)sulfanylpentanoic acid (CPP) or 2-ethylsulfanylthiocarbonylsulfanyl-2-methyl propionic acid (EMP) as chain transfer agent (CTA). The reversible micellization of these block copolymers in aqueous media at different pH was investigated by ^{1}H NMR spectroscopy, DLS, and fluorescence spectroscopy. While both ATRP and RAFT polymerization are good choices for the synthesis of well-defined block copolymers, toxic catalysts, expensive ligands, or smelly CTA are essential for these techniques, which restrict their industrial application in many fields such as cosmetic, food, and drug.

Compared with linear homopolymers and copolymers with analogous molecular weight, branched copolymers have many advantages such as lower viscosities, more globular morphologies, higher solubilities, higher concentrations of end groups, and great surface adhesion.[26] The most commonly used way to synthesize branched copolymers is the step growth polymerization of AB_2 monomers with extension to addition polymerizations via routes such as self-condensing vinyl polymerization. Sherrington and coworkers[27] reported a so-called "Strathclyde route" for producing branched copolymers using divinyl monomers and CTAs in a conventional free-radical polymerization. The degree of branching can be adjusted by changing the molar ratio of the vinyl monomers to divinyl monomers, and the possible gelation due to the addition of divinyl monomers could be avoided by controlling the concentration of CTAs. More recently, Weaver *et al.*[28] prepared a series of DEAEMA-based pH-responsive branched copolymers using the "Strathclyde route". These copolymers could form micellar structures with hydrodynamic diameters varying in the range of 16 to 46 nm in basic conditions. However, the reliable size of most copolymers (9 out of 10 entries) in aqueous solution at acidic pH has not been determined by DLS.

Herein, we report a series of novel pH-responsive branched copolymers based on the pH-responsive monomer DEAEMA, hydrophilic macromonomer PEGMA, and hydrophobic comonomer *t*BAEMA. EGDMA and 1-dodecanethiol (DDT) with given molar ratio are used as branching monomer and CTA, respectively. The relative molar ratios of different monomers in obtained copolymers were calculated by ^{1}H NMR. Tri-GPC was used to confirm the formation of branching structure. The hydrodynamic diameter of some obtained copolymers in aqueous solution at both acidic and basic pH was determined by DLS. The results showed that up to 130∼145 nm microgel-like polymer particles with P*t*BAEMA and DDT groups as hydrophobic domains, PEGMA, and PDEAEMA residues as hydrophilic corona can be formed at acidic pH. With the increase of pH from 6 to 8, the polymer particles collapsed to form more compact core-shell structure due to the deprotonation of tertiary amine groups.

EXPERIMENTAL

Materials

DEAEMA, *t*BAEMA, and EGDMA were obtained from Aldrich and passed through an alumina column to remove inhibitor prior to use. PEGMA (M_n=950 g·mol^{-1}) and DDT were purchased from Aldrich and used as received. Azobis(isobutyronitrile) (AIBN) recrystallized from methanol prior to use. Ethanol, methanol, 1M standard hydrochloric acid (HCl) solution, and 1M standard sodium hydroxide (NaOH) solution were purchased from Aladdin and used without further purification.

Solution Copolymerization Procedure

Using sample 4 (Table I) as an example PEGMA (2.2 g, 2.0×10^{-3} mol), DEAEMA (5.2 g, 5.6 mL, 2.8×10^{-2} mol), *t*BAEMA (1.9 g, 2.0 mL, 1.0×10^{-3} mol), EGDMA (1.2 g, 1.1 mL, 6.0×10^{-3} mol), and DDT (1.2 g, 1.4 mL, 6.0×10^{-3} mol) were added to a three-necked flask equipped with a stirrer bar and degassed by nitrogen purge for 30 min. About 88 mL of ethanol was degassed separately and added to the monomer mixture, and the solution was heated to 70°C under an inert atmosphere. The polymerization was started by addition of AIBN (0.16 g, 9.7×10^{-4} mol, 1.5% of the amount of monomers) and left stirring for around 48 h. After that, ethanol was removed by evaporation at reduced pressure and unreacted monomers were removed by precipitation of the polymer into cold *n*-hexane. The resulting viscous liquid was dried in a vacuum oven overnight (10.6 g, 91%).

Characterization

Molecular weights, molecular weight distributions, and Mark-Houwink α-values were measured using a Viscotek TDA-305 tri-GPC equipped with two Viscotek T6000M columns and a guard column. Tetrahydrofuran (THF) was used as the mobile phase, the column oven temperature was set to 35°C, and the flow rate was 1 mL/min. The samples were prepared for injection by dissolving 10 mg of copolymer in 1 mL of HPLC grade THF and filtered with a 0.2 μm PTFE membrane. 100 μL of this mixture was then injected, and data were collector for 40 min. The wavelength used was 670 nm. The d*n*/d*c* value used was 0.07. OmniSEC was used to collect and process the signals transmitted from the detectors to the computer and to produce the molar mass distribution and molar mass versus elution volume plots.

^{1}H NMR spectra were recorded on 1.0 w/v % copolymer solutions in either D_2O (pH adjusted with DCl) or $CDCl_3$ using a Bruker AVANCE II400 spectrometer operating at 400 MHz.

Table I. The Recipes for the Synthesis of Linear or Branched Copolymers[a]

Samples	DEAEMA /mmol	tBAEMA /mmol	EGDMA /mmol	DDT /mmol	Polymer composition[b]	M_n[c] /g·mol^{-1}	M_w[c] /g·mol^{-1}	M_w/M_n[c]	α[c]
0	0	31	0	0	-	82 900	152 536	1.84	0.68
1	28	1.0	0	2.0	5/69/26	154 316	214 500	1.39	0.70
2	28	1.0	2.0	2.0	5/71/24	50 637	77 687	1.52	0.38
3	28	1.0	6.0	6.0	6/70/24	64 675	133 230	2.06	0.41
4	28	1.0	6.0	6.0	7/70/23	117 595	151 442	1.28	0.43
5	24	1.4	6.0	6.0	7/62/31	81 492	199 655	2.45	0.31
6	20	1.8	6.0	6.0	7/57/35	61 326	163 740	2.67	0.33

[a] DDT modified free radical solution copolymerization of PEGMA, DEAEMA, *t*BAEMA, and EGDMA performed in ethanol at 70°C under nitrogen, using 2.0×10^{-3} moles of PEGMA and 9.7×10^{-4} moles of AIBN.
[b] Polymer composition (PEGMA/DEAEMA/tBAEMA) were calculated by ^{1}H NMR excluding DDT due to overlapping proton resonances.
[c] Obtained via triple detector GPC.

DLS measurements were made on 0.5 wt % aqueous copolymer solutions at 20°C using a Brookhaven BI-200SM instrument equipped with a 200 mW green laser (λ= 532 nm) with variable intensity at a fixed scattering angle of 90°. The average hydrodynamic diameters and polydispersity index were calculated from the intensity autocorrelation data with the cumulants method. The intensity-intensity time correlation functions were analyzed by the CONTIN method.

Surface tension of branched copolymer solution was determined using the ring single-measurement method on a Krüss K-100 tensiometer. The temperature was controlled at 25±0.1°C with a HAAKE DC 30 thermostatic bath (Karlsruhe, Germany). A series of aqueous solutions with different pH values (2 and 10) and different copolymer concentration (ranging from 1.0×10^{-4} wt % to 1.0 wt %) were prepared and kept for 15 min to equilibrate.

The pK_a values of copolymers were measured by acid titration. Initially, 0.5 wt % of aqueous copolymer solution was prepared and adjusted to pH 2 with 1M HCl solution. Then the copolymer solution was titrated with 1M NaOH solution under magnetic stirring. The pH value after each addition of NaOH solution was measured with a PHS-2C pH meter (Dapu, Shanghai), and then plots of pH value vs. volume of NaOH solution (titration curve) could be obtained.

In Vitro Loaded and Release of Drugs from Copolymer Particles

About 5 mg of indomethacin and 10 mg of the obtained copolymer were added into 10 mL of methanol, and stirred for 1 h. After that, 20 mL of phosphate buffered solution (PBS) was added into the solution and stirred for 12 h to allow indomethacin to diffuse into the assemblies formed from the copolymer.[29] Finally, methanol and free indomethacin was removed by dialysis. The entrapment efficiency (EE) can be calculated by the eq. (1):

$$EE=\frac{m_0-m_f}{m_0}\times100\% \quad (1)$$

where, m_0 stands for the original amount of indomethacin, m_f is the amount of free indomethacin, which was calculated from the absorbance value of indomethacin solution at 204 nm, measured by UV–vis spectroscopy (UV-7504C, Xinmao, Shanghai), using a pre-established calibration curve (Figure S1 in Supporting Information).<<?Please provide the Supporting Materials.>>

The release profiles of indomethacin from assemblies were studied using a dialysis bag (MWCO 3500 Da). Briefly, 20 mL (V_e) of PBS solution with 5 mg of indomethacin-loaded copolymer assemblies was placed in a dialysis bag. The dialysis bag was then immersed in 500 mL of PBS solution at different pH, and stirred at a speed of approximately 120 rpm. The temperature of the solution was kept at 37°C by an oil bath. The samples were taken at desired time intervals and drug concentration was measured using UV–vis spectroscopy at 204 nm. The cumulative drug release percent (E_r) was calculated based on the eq. (2). The *in vitro* release experiments were carried out in triplicate at each pH value.

$$E_r=\frac{V_e\sum_1^{n-1}C_i+V_0C_n}{m_{In}}\times100\% \quad (2)$$

where, m_{In} represents the amount of indomethacin in the copolymer assemblies, V_0 is the whole volume of the release media (V_0=520 mL), and C_i and C_n stand for the concentrations of indomethacin in the i^{th} and n^{th} sample, respectively.

RESULTS AND DISCUSSION

The pH-responsive branched copolymers in this work were prepared by the free-radical statistical copolymerization of PEGMA, DEAEMA, and *t*BAEMA. PEGMA with fascinating water solubility and biocompatibility was selected as the hydrophilic macromonomer to provide steric stabilization for the hydrophobic core. DEAEMA homopolymer is a well-known pH responsive polybase with a pK_a of approximately 7.3.[30,31] It is molecularly soluble in water below its pK_a due to protonation of its tertiary amine groups. When the solution pH exceeds 7.3, DEAEMA homopolymer will precipitate from aqueous solution, because the average degree of protonation drops below a critical value and the chains become hydrophobic. *t*BAEMA, as one of the structural isomers of DEAEMA, was used as a hydrophobic comonomer. The turbidimetric titration curves (Figure 2S in Supporting Information) confirmed that the pure linear

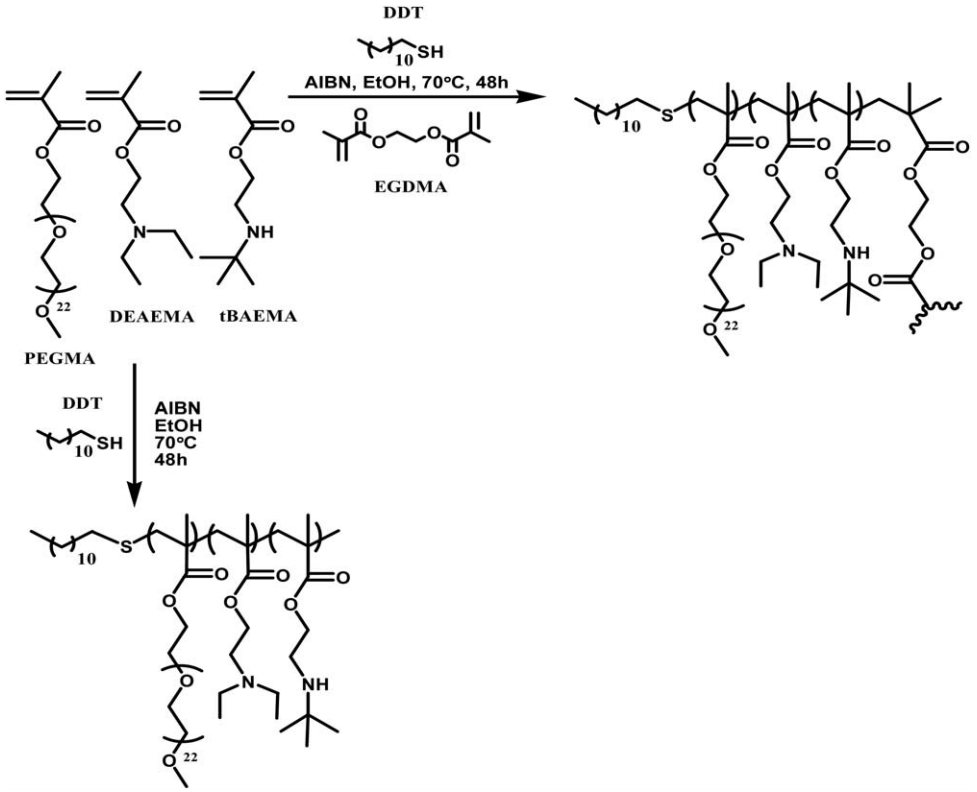


Figure 1. Schematic representation of the synthesis of linear and branched copolymers

P*t*BAEMA homopolymer (see Table I, Sample 0) was hydrophobic in the range of pH 2 to 12. The addition of the bifunctional branching agent EGDMA and the chain transfer agent DDT with stoichiometric ratio resulted in the formation of branched architecture.

Synthesis and Characterization of the Copolymer

Typically, the monomers, EGDMA and DDT were dissolved in ethanol (10.0 w/v % based on total monomer), and the polymerization was initiated by AIBN. The reaction procedure is shown in Figure 1.

In the absence of divinyl monomer EGDMA (see Table I, sample 1), linear copolymer (M_w=214,500 g/mol) was synthesized by the conventional free-radical copolymerization of monofunctional monomers PEGMA, DEAEMA, and *t*BAEMA. The addition of DDT (see Table I, sample 2) only resulted in the reduction in the molecular weight of the copolymer (M_w=77,687 g/mol). In the presence of EGDMA and DDT (see Table I, sample 3∼6), a series of branched copolymers with different degree of branching were synthesized.

In these cases, DDT was used to prevent the possible macroscopic gelation resulting from the addition of EGDMA. DDT could effectively control the number of polymer chains formed during the copolymerization and reduce the molar mass of the primary chains, which promoted the formation of the branched structures.[32] Compared to the linear copolymers, the Mark-Houwink α-values are lower for the branched copolymers and show a general decrease with increasing degrees of branching, indicating more compact structures for the increasingly branched systems.[26]

The molecular composition of PEGMA, DEAEMA, and *t*BAEMA in the purified copolymers were determined by ^{1}H NMR in $CDCl_3$-a good solvent for all monomers. Figure 2 shows a typical ^{1}H NMR spectrum of the copolymer. The protons of –O–C***H***$_2$–C***H***$_2$–O and –O–C***H***$_3$ on PEGMA chain give rise to signals at δ 3.64 (c) and 3.38 (k), respectively. The peaks at δ 2.70 (g), 2.57 (i), and 1.04 (j) can be assigned to the protons of –O–CH$_2$C***H***$_2$–N–, –N–C***H***$_2$–CH$_3$, and –N–CH$_2$–C***H***$_3$ on DEAEMA segment, respectively. Moreover, the –O–CH$_2$C***H***$_2$–NH– peak assigned to the *t*BAEMA appears at δ 2.82 (h). The relative molar ratios of the different monomer unit (n_{PEGMA}/n_{DEAEMA}/n_{tBAEMA}) (as listed in Table I) could be calculated by the eq. (3) based on their integrals:

$$n_{PEGMA}/n_{DEAEMA}/n_{tBAEMA} = \frac{I_k}{3}/\frac{I_i}{4}/\frac{I_h}{9} \quad (3)$$

where, I_k, I_i, and I_h stand for the integral of the peaks at δ 3.38 (k), 2.57 (i), and 2.82 (h), respectively. In all cases, the calculated polymer compositions were found to be very similar to those in the feed solutions (as shown in Table I). This is a result of obtaining high conversions of monomer to polymer in batch polymerizations.

Aqueous Solution Behavior

The aqueous solution behaviors of the linear and branched copolymers were investigated by DLS. Variation of average hydrodynamic diameter and polydispersity of polymer particles

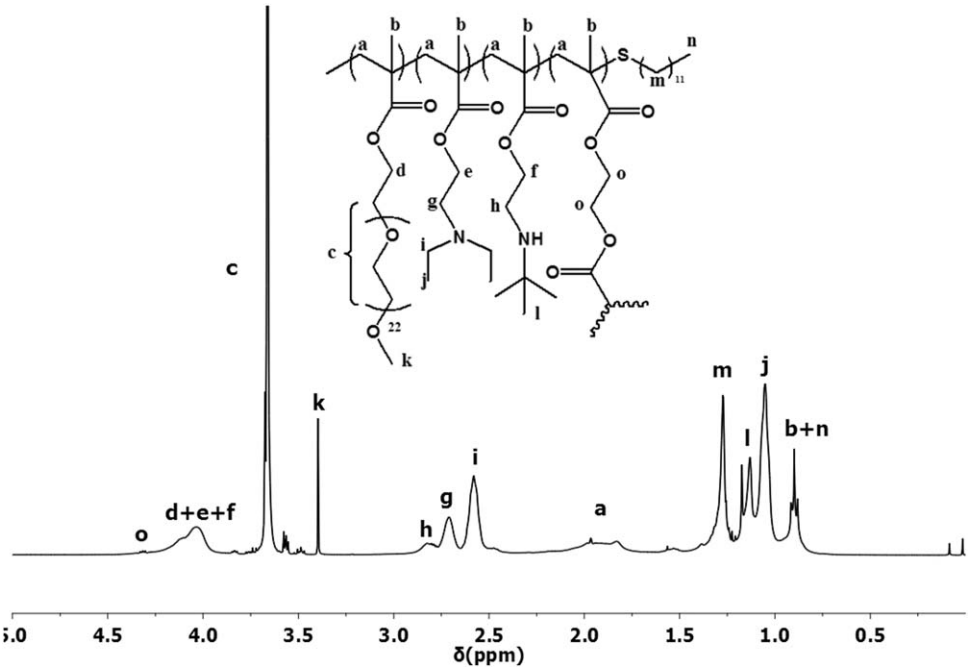


Figure 2. ^{1}H NMR spectrum of sample 4 in $CDCl_3$

vs. solution pH value (Figure 3) were plotted based on the original DLS traces (Figure 3S in Supporting Information). The copolymers were initially dissolved in water as 0.5 wt % solutions water at pH=2. At this pH, all of the copolymers could be dissolved and the obtained solution appeared colorless and transparent. On increasing the solution pH from 2 to 10, the colorless solutions of sample 4–6 turned to be light blue and the transparency reduced as well. However, samples 1–3 kept almost unchanged.

At pH 2, the PDEAEMA fragments of either linear or branched copolymers were completely protonated and became hydrophilic. For linear (sample 1 and 2) or branched copolymer with high content of hydrophilic PEGMA and PDEAEMA but lower amount of hydrophobic DDT (sample 3), the solutions were not capable of scattering sufficient light to allow reliable size determination.[33] This can explain why the hydrodynamic diameters of samples 1–3 were small [see Figure 3(a)] while the polydispersity of the particles were pretty broad [see Figure 3(b)].

However, confident size determination could be obtained for samples 4–6 (as shown in Figure 3), presumably due to the high degree of branching and the high levels of hydrophobic DDT present.[26] These highly branched copolymers may form swollen microgel-like particles with P*t*BAEMA residues and DDT chain-ends as hydrophobic domain stabilized by the hydrophilic PEGMA and extensively protonated PDEAEMA groups. In the ^{1}H NMR spectrum of sample 6 in D_2O at pH 2 (see Figure 4), signals from the hydrophilic segments were clearly visible at δ 1.26 (j, N–CH_2–C$\boldsymbol{H}_3$), 3.12 (i, N–C$\boldsymbol{H}_2$–CH_3), 3.36 (g, O–CH_2C$\boldsymbol{H}_2$–N), 4.22 (e, O–C$\boldsymbol{H}_2$$CH_2$–N), and 3.27(k, O–C$\boldsymbol{H}_3$), 3.59 (c, –[C$\boldsymbol{H}_2C\boldsymbol{H}_2$O]$_{22}$–). However, signals from the hydrophobic P*t*BAEMA and DDT residues were absent in the same spectrum, which could also be attributed to the formation of hydrophobic core by hydrophobic interaction between P*t*BAEMA and DDT.

With the increase of the solution pH from 2 to 12, the average degree of protonation of tertiary amine groups gradually reduced to a critical value (the apparent pK_a of the copolymer) and the PDEAEMA residues became hydrophobic, which resulted in a dramatic increase in the scattering intensity. For samples 1–3, the copolymers gained the ability to scatter sufficient light for reliable size determination by DLS, although the scattering intensity was still insufficient for visual inspection. Take sample 3 as an example, the polydispersity of the particles significantly reduced from 0.932 at pH 4 to 0.056 at pH 8 [as shown in Figure 3(b)], which indicated that the hydrodynamic diameter of 9.4 nm measured by DLS at pH 8 was much more reliable than the diameter of 6.0 nm at pH 4 [see Figure 3(a)]. In cases of samples 4–6, the copolymer particles formed at low pH collapsed from around 145 nm to around 16 nm as the PDEAEMA residues became deprotonated and gained enough hydrophobicity to form more compact hydrophobic core together with P*t*BAEMA and DDT. The hydrophilic PEGMA grafts remained solvated irrespective of the solution pH, and stabilized the hydrophobic core by steric stabilization mechanism. The ^{1}H NMR spectrum of sample 6 recorded at pH 10 (see Figure 4) showed that the signals originally assigned to the tertiary amine groups disappeared, indicating that the PDEAEMA fragments were dehydrated and immersed in the hydrophobic phase. The proton resonances of the PEGMA grafts were still visible at δ 3.27 and 3.59, as expected.

Figure 3 also showed that both the hydrodynamic diameter and the polydispersity of the particles greatly changed at a certain pH—the apparent pK_a of the copolymers. The pK_a values of the copolymers synthesized in this work were determined by

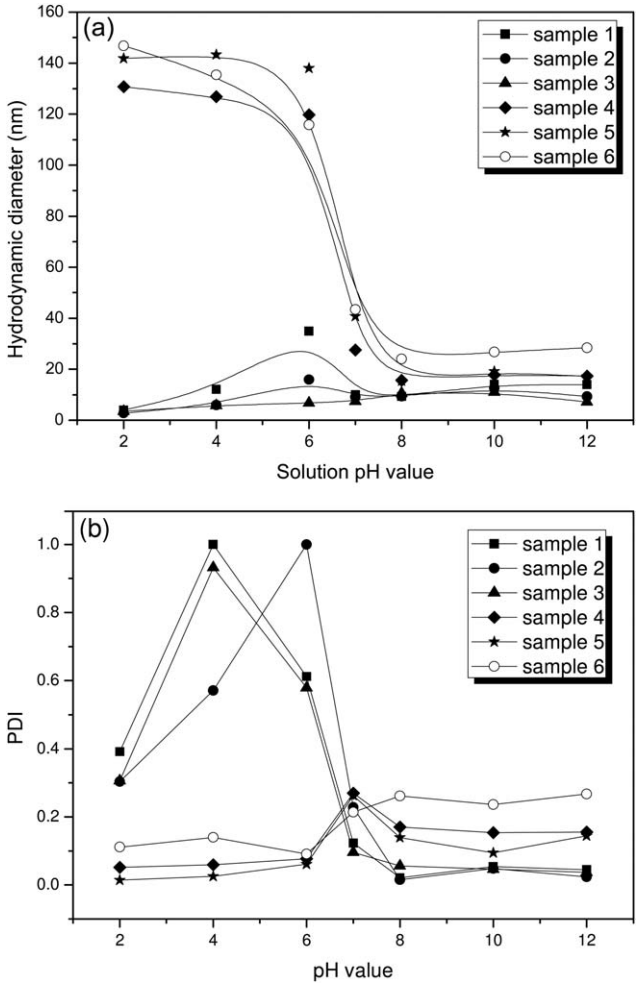


Figure 3. Variation of (a) average hydrodynamic diameter and (b) polydispersity of particles with solution pH value for samples 1∼6 in 0.5 wt % aqueous solution.

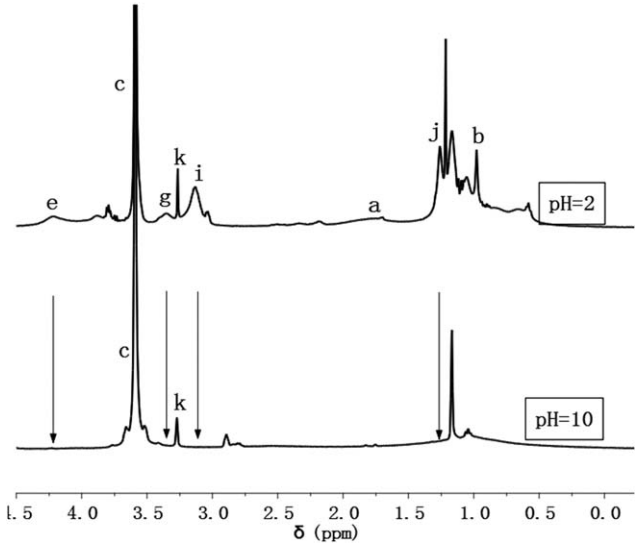


Figure 4. ^{1}H NMR spectra recorded for sample 6 at 0.1 g/L in D_2O at pH 2 and 10.

titrating dilute acidified aqueous solutions of the copolymers against NaOH solution. The obtained plots of the pH value versus the volume of NaOH solution and the corresponding pK_a values were shown in Figure 5.

For linear copolymers (sample 1 and 2), no big change was observed in their pK_a values. However, on increasing the degree of branching (from sample 2 to sample 3, then sample 4), the pK_a values slightly but systematically decreased due to steric constraints of the branched polymers. This allowed us to adjust the apparent pK_a values of pH responsive polymers by varying the degree of branching.

Formation of the Microgel-Like Particles

The variation of particle diameter and polydispersity with solution pH for highly branched copolymers (samples 4–6) is extremely similar to that of weakly basic polyelectrolyte based pH-sensitive microgels with basic groups located near the surface of the microgel.[34] These microgels will form compact structures at high pH, but swell and develop a "hairy" morphology at low pH.[35] For our branched copolymers, at pH 10, the PDEAEMA residues became hydrophobic due to the deprotonation of tertiary amine groups. The primary polymer chain was dominated by continuous hydrophobic PDEAEMA, P*t*BAEMA, and DDT groups. In this case, the branched copolymers were likely to form unimolecular polymer particles with compact core-shell structure by intramolecular hydrophobic interaction. The hydrophilic PEGMA side chain didn't only serve to stabilize the hydrophobic core by steric stabilization, but prevent the formation of multimolecular assemblies. At pH 10, diluting the solution of the branched copolymer from 0.5 wt % to 0.05 wt % caused no obvious change in its hydrodynamic diameter (see Figure 6). This could partly verify the formation of unimolecular polymer particles.

With the decrease of solution pH, the copolymer particles were gradually swelled by water due to the protonation of PDEAEMA residues, and formed particles of around 135 nm with hydrophobic P*t*BAEMA residues and DDT end groups core stabilized by hydrated PEGMA and protonated PDEAEMA corona (as

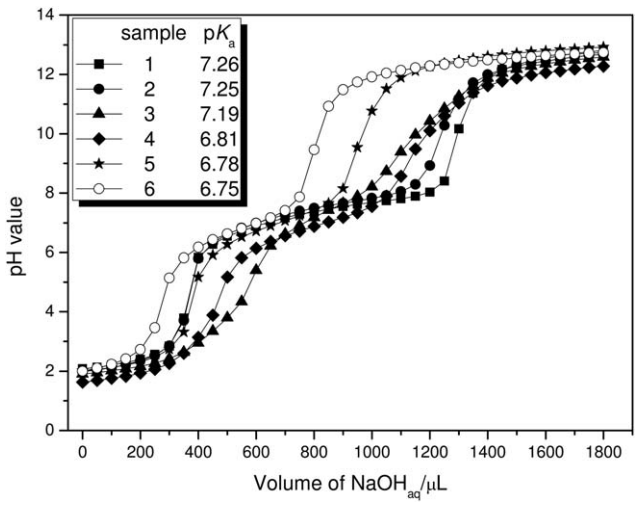


Figure 5. Plots of the pH value versus the volume of NaOH solution as well as the corresponding pK_a values obtained by titration at 25°C.

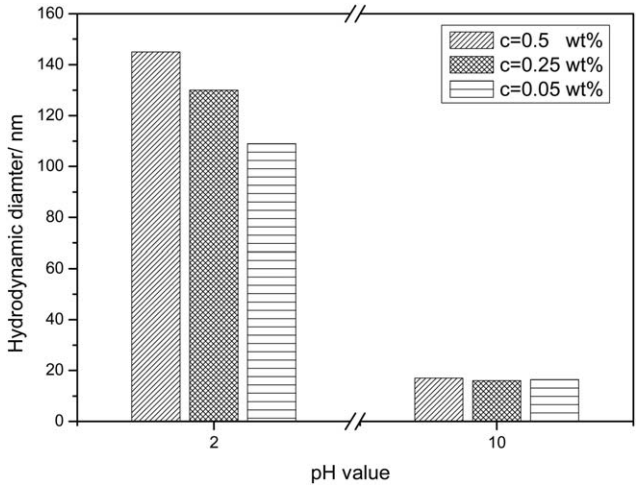


Figure 6. The hydrodynamic diameter of copolymer assemblies of sample 6 at pH 2 and 10 measured by DLS.

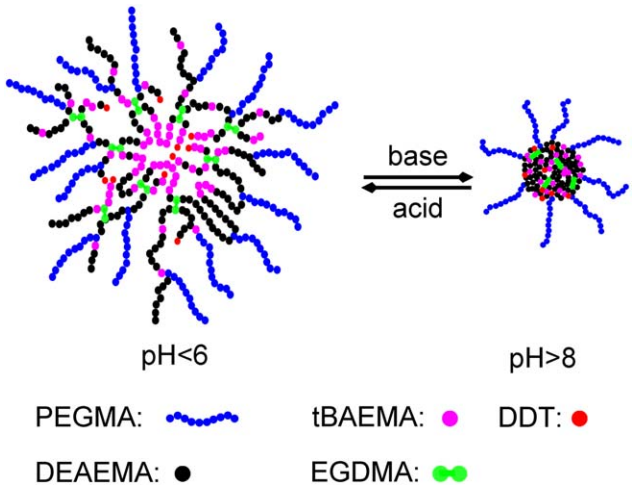
shown in Scheme 1). Although the hydrodynamic diameters of these particles were much larger than those of typical micelles, the solution was still colorless and transparent. This indicated that the particles were more like microgels than typical multimolecular micelles, in case of which the scattering/turbidity would be higher. However, diluting the solution to from 0.5 wt % to 0.05 wt % did cause an obvious reduction in the hydrodynamic diameter from 144 nm to 109 nm (as shown in Figure 6), which could be attributed to the intermolecular interactions between microgel-like particles. When the particles were strongly swelled by water, their surfaces became fuzzy, which allowed for deformation as well as for interpenetration between particles.[35]

pH-Dependent Drug Load and Release

Indomethacin, a nonsteroidal, anti-inflammatory agent, was used as the model drug to evaluate the pH-dependent drug load and release behavior of branched copolymer particles. Originally, 20 mL of pH 8.04 PBS was added into the methanol solution with 5 mg of indomethacin and 10 mg of branched copolymer (sample 6). After the free drug and methanol was removed by dialysis, the final EE of indomethacin was only 10%. However, when pH 5.28 PBS was used as disperse phase instead of pH 8.04 PBS, the EE increased to 84%. This phenomenon could be explained as follows. The water solubility of indomethacin is pH-dependent amounting to only 25 μg/mL at pH 5.1 and 1600 μg/mL at pH 7.2.[36] At pH 8.04, almost all of the indomethacin was dissolved in PBS, which directly led to a low EE. However, the addition of pH 5.28 PBS followed by removal of methanol resulted in the precipitation of indomethacin from methanol. Meanwhile, the branched copolymer was swelled by water and formed soft and porous particles with fuzzy surface. Because of the hydrophobic interaction between indomethacin and the hydrophobic domains of particles, indomethacin was successfully entrapped within the porous particles. In the case of linear copolymer (sample 2), the EE was only 43% in pH 5.28 PBS, which indicated that the formation of highly swollen microgel-like structures played a more important role in the increase of EE than the solubility-rise of indomethacin.

The drug release performances of the branched copolymer assemblies were investigated at pH 5.91, 6.81, and 8.04, as shown in Figure 7. It could be observed that the release rates of indomethacin from the branched polymer assemblies were markedly influenced by pH values. At pH 5.91, only 10% of indomethacin was released in the first 12 h because of the poor solubility of indomethacin. In the case of pH 6.81 PBS, the average degree of protonation of tertiary amine groups decreased and the polymer particles gradually collapsed to compact core-shell particles, which enhanced the release rate of indomethacin and about 22% of indomethacin was released in the first 12 h. However, at pH 8.04, the release rate of indomethacin increased and the cumulative release was up to 40% after 12 h.

Such a profile is different from the previous study on the pH-responsive drug release behavior of doxorubicin (DOX) from



Scheme 1. Schematic illustration of the formation of the branched copolymer assembly. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

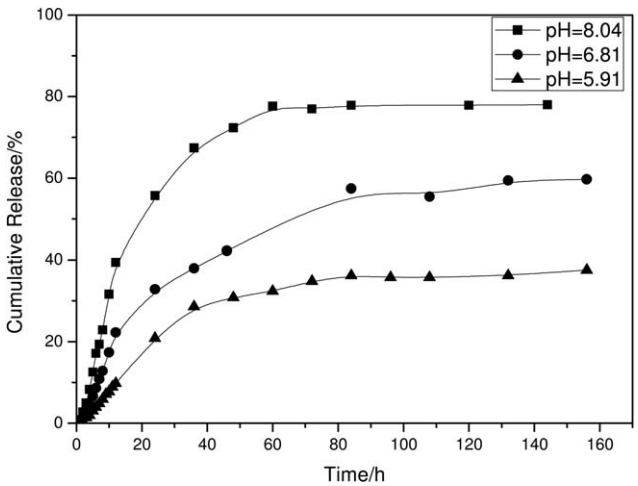


Figure 7. *In vitro* drug release profiles from the polymer assemblies of sample 6 at pH 5.91, 6.81 and 8.04 PBS solutions.

tertiary amine based block copolymer micelles.[37] In their report, the *in vitro* DOX release from MPEG-b-(PLA-co-PAE) micelles was significantly reduced when solution pH increased from 5.0 to 7.4, which was attributed to the tight micelle structure due to the deprotonation of amino groups in PAE moieties at higher pH values. In present work, however, the increase of solution pH from 6.81 to 8.04 not only resulted in the formation of more compact and tight unimolecular particles (negative effect on the drug release), but also enhanced the solubility of indomethacin (positive effect on the drug release). The result suggested that the latter effect played the dominant role on the release of indomethacin from the branched copolymer micelles.

CONCLUSIONS

A series of novel pH-responsive, amphiphilic branched copolymers based on PEGMA, DEAEMA, and tBAEMA were successfully synthesized via a thiol-modified free radical solution polymerization. In aqueous solution at acidic pH, these copolymers formed soft swollen microgel-like particles with hydrodynamic diameter ranging from 125 to 145 nm. On increasing the solution pH the swollen particles collapsed and became more compact and tight. The hydrodynamic diameter of particles decreased to 5–30 nm due to the dehydration of PDEAEMA residues. The drug load and release experiments indicated that the final encapsulation efficiency and the rate of indomethacin release were highly affected by the solution pH.

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