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ORIGINAL ARTICLE

Investigation of the temperature responsive behaviors of novel polyaspartamide derivatives bearing alkyl ether-type pendants

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Temperature responsive;
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Abstract Previous works reported that some temperature responsive polyaspartamide derivatives exhibited pH responsiveness, although pH responsive functional groups or pH-cleavable linkages were not introduced during the preparing process, which may be related to the hydroxyl groups of their pendant alkanolamide moieties. In order to investigate whether pH will also affect the temperature responsive behavior of temperature responsive polyaspartamide derivatives without hydroxyl groups, four linear alkyl ether-type amine compounds with one ether oxygen atom and 3 to 6 carbon atoms, denoted as OCx (x represents the number of carbon atoms in alkyl ether-type amine compounds), were selected for the aminolysis reaction of poly(succinimide) to design and synthesize the polyaspartamide derivatives bearing alkyl ether pendants, named PASP-OCx. The chemical structures and molecular weights of the obtained polymers were characterized and confirmed by FTIR, ^{1}H NMR and SEC-MALLS. The water-solubilities, surface tensions and transmittances of PASP-OCx aqueous solutions were also investigated. Results showed that PASP-OCx can be dissolved in deionized water when x is less than or equal to 5. The surface tensions of PASP-OC4 and PASP-OC5 solutions exhibited obvious turning points. Interestingly, transmittances results showed that PASP-OC5 exhibited temperature responsive behaviors under acidic, neutral or alkaline conditions (pH $\leq$ 10.0); PASP-OC4 only exhibited temperature responsive behaviors under acidic condition (pH $\leq$ 3.0); while PASP-OC3 did not exhibit temperature responsive behavior. The LCST value of PASP-OC5 and PASP-OC4 can be turned by adjusting the pH value of their solutions, and decreases with decreasing the pH value. Additionally, the influence of pH on the LCST values of PASP-OC4 and PASP-OC5 was investigated by zeta potential, and the results showed that the decrease of LCST values may be due to the reducing of electrostatic repulsion with

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pH decreasing. Therefore, results showed that pH can also affect the temperature responsive behavior of temperature-responsive polyaspartamide derivatives that do not contain hydroxyl groups.

1. Introduction

In the past decades, the interest in stimuli-responsive polymers has increased significantly in various fields such as biosensor, tissue engineering and drug/gene delivery system (Wei et al., 2017; Wang et al., 2021). Stimuli-responsive polymers usually can respond to a variety of stimuli, e.g., temperature (Ma et al., 2021; Stetsyshyn et al., 2021), pH (Surya et al., 2020), redox (Hsu and Almutairi, 2021) and light (Mehta et al., 2020). Of the above mentioned responses to stimuli, temperature response is one of the most well studied responses in stimuli-responsive polymers (Zarrintaj et al., 2019; Hoang et al., 2021). For example, poly(*N*-isopropylacrylamide) (PNIPAAm) (Heskins and Guillet, 1968; Schild, 1992; Halperin et al., 2015), which consists of acrylamide as backbone and isopropyl group as side chain, is one of the best-known temperature responsive polymers with lower critical solution temperature (LCST) around 32 °C (Pilipenko et al., 2020; Zhang et al., 2015b). In recent years, significant efforts have also been focused on the development of temperature responsive polymers for biomedical applications. For instance, temperature responsive polyacrylates such as poly(n-butyl acrylate) and poly(n-butyl methacrylate have been reported as coatings for temperature-controlled orientation of proteins (Awsiuk et al., 2019). However, although the carbon–carbon backbone of PNIPAAm and polyacrylates is conducive to the stability of polymers, it also limits their applications in biomedical fields due to its non-biodegradability. Therefore, amino acid-based polymers have attracted great attentions due to their biodegradable backbone and excellent biocompatible properties (Song et al., 2017; Bauri et al., 2018; Thompson and Scholz, 2021).

Polyaspartamide derivatives, whose backbone is composed of aspartic acid, are one of the most intensively studied amino acid-based polymers in biomedical fields due to their excellent biocompatibility, biodegradability and non-toxicity of their degradation products (Naito et al., 2019; Dang et al., 2020). Three polyaspartamide derivatives have already entered clinical research as drug carrier (NK911, NK105, NC6300) for cancer treatment up to date (Matsumura et al., 2004; Fujiwara et al., 2019; Chawla et al., 2020). The aminolysis reaction of poly(succinimide) (PSI) with amine compounds is a common synthetic strategy for synthesizing polyaspartamide derivatives (Craparo et al., 2020) and also can be used for preparing polyaspartamide derivatives containing stimuli responsiveness (Nguyen and Kim, 2020). For instance, inspired by the chemical structure of the side chains of PNIPAAm, a series of temperature responsive polyaspartamide derivatives containing isopropylamide (Gu et al., 2013; Vega-Chacon et al., 2019), diisopropylamide (Liu et al., 2015) or *N*-isopropylethylenediamide (Moon et al., 2009) pendants were designed and synthesized by the aminolysis reaction of PSI. In fact, temperature responsive polyaspartamide derivatives can also be prepared even without isopropyl moieties in their side chains. For example, a series of novel polyaspartamide derivatives containing temperature responsiveness were successfully synthesized via the aminolysis reaction of PSI with a mixture of alkanolamines (5-aminopentanol (C5OH)/6-aminohexanol (C6OH) (Tachibana et al., 2003); 4-aminobutanol (C4OH)/C6OH (Hsu et al., 2012)). In addition, based on the reaction product of PSI and C5OH, denoted as PASP-C5OH, phenethyl alcohol (PEA) was successfully introduced into the pentanol side chains of PASP-C5OH via carbonate linkage, and the obtained PASP-C5OH-g-PEA showed temperature responsiveness with tunable LCST that can be adjusted by varying the percentage of grafted "PEA" moieties (Ma et al., 2014). Moreover, temperature responsive behavior can also be observed when part of the pendant C5OH moieties in PASP-C5OH were replaced by 2-azidoethylamide moieties (Zhang et al., 2015a).

Interestingly, the temperature responsive polyaspartamide derivatives prepared by the aminolysis reaction of PSI with a mixture of C4OH/C6OH exhibited pH responsiveness, although pH responsive functional groups or pH-cleavable linkages were not introduced in the preparing process (Hsu et al., 2012). In addition, the phase transition temperature increased with increasing the pH value of polymer solution. This peculiar phenomenon was also reported in other temperature responsive polyaspartamide derivatives containing C5OH moieties in 2019 (Zhang et al., 2019). Moreover, we designed a temperature/pH dual responsive polyaspartamide derviatives P(Asp-Az)$_{39}$-HPA-IMZ containg imidazole rings as pH-responsive moieties in 2015, but these polymer showed two phase transition process with pH increase. With increasing the pH value of polymer solution, the first phase transition process was observed at pH range of 3.7–4.7, which corresponding to the deprotonation of imidazole rings. The second phase transition process was observed in a higher pH. Zeta potential results showed that P(Asp-Az)$_{39}$-HPA-IMZ polymer solution can change from positive charge to negative charge with pH increase (Zhang et al., 2015a). It is very reminiscent of zwitterionic pH-responsive polymers, but what kind of group exsited in above metioned temperature responsive polyaspartamide derivatives can cause negative charge? Perhaps the negative charge is related to the hydroxyl of their pendant alkanolamide moieties (Hsu et al., 2012). Although there are indeed a large number of alcoholic hydroxyl groups in the above mentioned polymers, it is hard to explain why alcoholic hydroxyl can generate negative charge even under acidic conditions (pH 5.0). Motived by these peculiar phenomena, we we want to know whether pH will also affect the temperature responsive behavior of temperature-responsive polyaspartamide derivatives without hydroxyl groups.

Biocompatible oligo(ethylene glycol) (OEG) pendants are usually chosen for preparing temperature responsive polymers (Weber et al., 2012; Stetsyshyn et al., 2017). For instance, polymers of various oligo(ethylene glycol) methacrylates generally exhibit a LCST in aqueous solution when the number of EO units in oligo(ethylene glycol) methacrylates is great than or equal to 2 and less than 10 (Czaderna-Lekka et al., 2021). The ether oxygens of OEG pendants in poly(oligo (ethylene glycol) methacrylates) can form hydrogen bonds with water to keep the balance between polymer-water interaction and

polymer–polymer interaction below the LCST, but this balance is disrupted due to the breaking up of hydrogen bonds between polymer and water above LCST (Lutz, 2008). Other temperature responsive polymers bearing OEG pendants were also designed and prepared using polynorbornene (Jia and Zhu, 2015), poly(pentafluorostyrene) (Pelosi et al., 2021), cellulose (Porsch et al., 2011), or polyglutamate (Zhou et al., 2020) as backbone in recent years. Thus, ether oxygen plays an important role in the temperature responsive behavior of polymers. Maybe alkyl ether-type amine compounds are a good choice to replace alkanolamines for preparing temperature responsive polyaspartamide derivatives without hydroxyl groups to investigate above peculiar phenomena.

So far, no work has reported temperature responsive polyaspartamide derivatives bearing alkyl ether-type pendants, or investigated the relationship between the chemical structures of alkyl ether-type moieties and the temperature responsive behaviors of corresponding polyaspartamide derivatives. In the present study, four linear alkyl ether-type amine compounds containing methoxyl, ethyoxyl or propoxyl group, denoted as OCx (x represents the number of carbon atoms in linear alkyl ether-type amine compounds), were utilized for opening the succinimide rings of PSI via aminolysis reaction to synthesize a series of polyaspartamide derivatives bearing alkyl ether-type pendants as depicted in Scheme 1. The preparation, chemical structural characterization, water solubility and the relationship of pH, zeta potential and temperature responsive behavior of the obtained polyaspartamide derivatives were investigated.

2. Experimental

2.1. Materials

2-Methoxyethylamine (OC3) and 3-ethoxypropylamine (OC5) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (≥98%, Shanghai, China). 2-Ethoxyethylamine (OC4) and 3-propoxypropylamine (OC6) were purchased from TCI (Shanghai) Chemical Industry Development Co., Ltd. (≥98%, Shanghai, China). *L*-aspartic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and used as received. PNIPAAm was purchased from Sigma-Aldrich (Shanghai) Trading Co., Ltd (M_n = 30,000, Shanghai, China). All other reagents and solvents were of analytical grade and used without further purification.

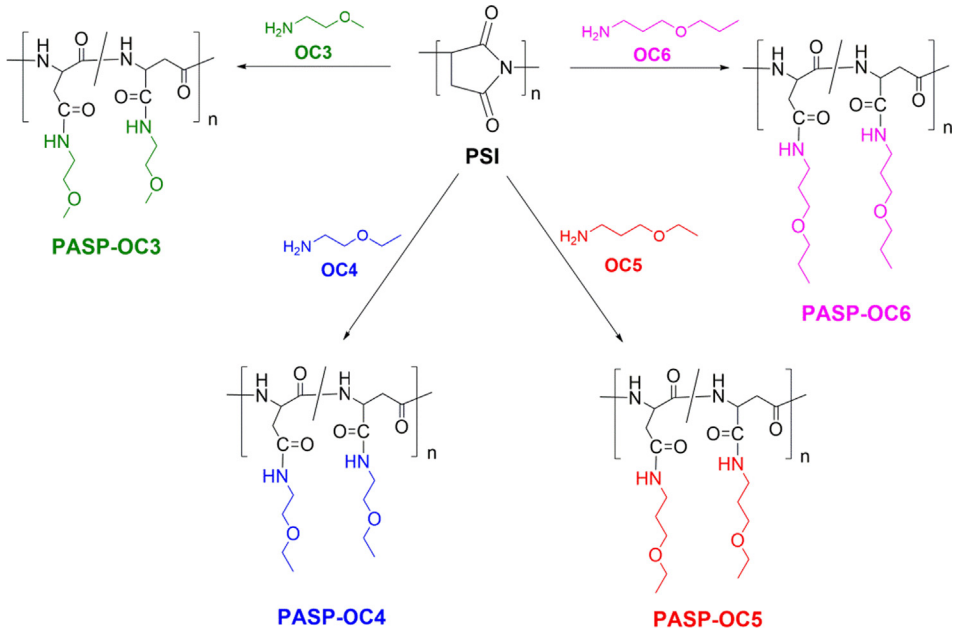
2.2. Synthesis of polyaspartamide derivatives

Poly(succinimide) (PSI) was prepared using *L*-aspartic acid as monomer and 85% phosphoric acid as catalyzer under reduced pressure at 180 °C according to previous work (Cheng et al., 2009). The weight-average molecular weight (M_w) and the molecular weight distribution (MWD) of PSI evaluated by size-exclusion chromatography combined with multi-angle laser light scattering (SEC-MALLS) were 27.6 kDa and 1.5, respectively.

Polyaspartamide derivatives containing pendant alkyl ether-type moieties, denoted as PASP-OCx, were synthesized via aminolysis reaction between PSI and various linear alkyl ether amine compounds (OCx) in *N,N*-dimethyllformamide (DMF) according to the following procedure: PSI (200 mg, 1 equiv.) was dissolved in 3 mL DMF, and then a solution of OCx (2 equiv.) in DMF was added. Next, the obtained reaction mixture was stirred at room temperature for 4 days. The resulting solution was purified by dialysis against deionized water for one week to remove unreacted OCx (MWCO 3.5 kDa). The white flocculent product PASP-OCx was collected after freeze-drying.

2.3. Characterizations

Fourier transformed infrared (FTIR) spectra of PASP-OCx were recorded with Nicolet 6700 spectrometer (Thermo Fisher Scientific, USA). ^{1}H nuclear magnetic resonance (^{1}H NMR)



Scheme 1 Chemical structures of four linear alkyl ether amine compounds (OCx) and their corresponding polyaspartamide derivatives PASP-OCx.

spectra of PASP-OCx were determined by AVANCE III HD spectrometer (400 MHz) (Bruker, USA) in D_2O or DMSO d_6. The molecular weights and MWD of the obtained PASP-OCx were evaluated by SEC-MALLS system consisting of a Waters e2695 separations module, an Optilab rEX refractive index detector (RI), a Wyatt DAWN EOS detector (MALLS) and a set of Waters Styragel HR columns (7.8 × 300 mm). DMF containing 10 mM LiBr was used as the eluent at a flow rate of 1.0 mL/min at 25 °C. The data were processed with Astra V software (Wyatt Technology Corp., USA).

2.4. Water-solubility and surface tension

PASP-OCx (about 15 mg) was weighed, and then diluted with deionized water to the concentration of 5.0 mg/mL using a vortex mixer to promote the dissolution of PASP-OCx. The optical photographs of the obtained PASP-OCx aqueous solutions under different temperature were recorded with a camera in glass bottles with a diameter of 18 mm. The surface tensions of PASP-OCx aqueous solutions were measured by a QBZY-3 fully automatic surface tension meter (Shanghai Fangrui Instrument Co., Ltd., China) based on the platinum plate method in a water bath at 20 °C. The critical micelle concentration (CMC) was calculated based on the surface tension method (Shang et al., 2019).

2.5. Stimuli responsive behavior and zeta potential

Stimuli responsive behaviors of PASP-OCx aqueous solutions (polymer concentration: 5.0 mg/mL) were evaluated by light transmittance measurement at 500 nm using a Shimadzu UV-2550 UV/Vis spectrometer (Shimadzu, Japan). The heating and cooling rate for the measurement of the optical transmittance curve of PASP-OCx was 0.4 °C/min, and the holding time for each test temperature was 1 min. In this study, the light transmittance of deionized water was defined as 100%, and the LCST was defined as the temperature corresponding to a 50% reduction in the original transmittance of the solution. The pH values of PASP-OCx aqueous solutions were adjusted with HCl solutions and NaOH solutions, and measured by a PB-10 pH meter (Sartorius, Germany). The light scattering intensity was evaluated by Zetasizer Nano ZS (Malvern, UK). Zeta potential was determined using a ZetaPlus Zeta Potential Analyzer (Brookhaven, USA) at 25 °C.

3. Results and discussion

3.1. Synthesis and characterization of PASP-OCx

In previous works, the mixture of alkanolamines containing 4–6 carbons was used for preparing temperature responsive polyaspartamide derivatives successfully (Tachibana et al., 2003; Hsu et al., 2012). However, PASP-C5OH was completely soluble in water without any temperature responsive behavior at the test temperature range of 0–100 °C, while PASP-C6OH (the reaction product of PSI with C6OH) was completely insoluble in water (Tachibana et al., 2003). Taking into account the difference of hydrophilicity between hydroxyl group and ether oxygen, four linear alkyl ether amine compounds were selected to synthesize polyaspartamide derivatives for investigating the influence of carbon number in OCx on the water-solubility and stimuli responsive behavior of PASP-OCx in the present study. The number of ether oxygen and carbon atoms in alkyl ether-type pendants of PASP-OCx was summarized in Table 1.

Polyaspartamide derivatives PASP-OCx were synthesized with a fixed molar feed ratio of 2:1 (OCx: succinimide units of PSI) to ensure the succinimide rings of PSI reacted completely. The yields of the PASP-OCx polymers were above 90% and summarized in Table 1. The chemical structures of the obtained PASP-OCx were characterized by FTIR (Fig. 1). From the FTIR spectra in Fig. 1, PASP-OCx can be distinguished from PSI clearly. Compared with the FTIR spectrum of PSI (Fig. 1**A**), new broad absorption peaks at 3310 cm^{-1} (Fig. 1**B-E**) were observed in FTIR spectra of PASP-OCx, which can be ascribed to the N-H stretch. The peak corresponding to the stretching vibrations of C = O also shifted from 1713 cm^{-1} (PSI, Fig. 1**A**) to 1655 cm^{-1} (PASP-OCx, Fig. 1**B-E**) after aminolysis. In addition, the new intense absorption peak was observed at 1540 cm^{-1} in accordance with the bending vibrations of N-H in –CONH–. These changes confirmed the formation of secondary amide groups in PASP-OCx. Moreover, a new strong absorption peak at 1120 cm^{-1} corresponding to the asymmetric C–O–C stretching vibration was also detected in all obtained PASP-OCx polymers, which indicates the presence of alkyl ether-type moieties. The absorption peaks at 2930 cm^{-1} and 2860 cm^{-1} may be ascribed to the stretching vibrations of $–CH_2–$. As the increasing of carbon number of OCx from 3 to 6, the intensities of absorption peaks corresponding to $–CH_2–$ increased in PASP-OCx. The FTIR analysis implies that PSI has reacted with OCx.

Besides FTIR, the chemical structures of PASP-OCx were also characterized by ^1H NMR (Fig. 2). Proton NMR chemical shift assignments for PASP-OC3, PASP-OC4 and PASP-OC5 in D_2O are shown in Fig. 2**A, 2B and 2C**, respectively. Proton NMR chemical shift assignments for PASP-OC6 in DMSO d_6 are shown in Fig. 2**D**. Compared with the NMR spectrum of PSI (**Fig. S1**), no peak was observed at 5.3 ppm (corresponding to the methine proton (C*H*) of the succinimide unit of PSI) for all the obtained PASP-OCx (Fig. 2**A-D**), which indicates that no unreacted succinimide rings remained after aminolysis and PSI has been reacted completely. The peak observed at 7.8–8.5 ppm in Fig. 2**D** was the protons signal

Table 1 The number of carbon atoms in alkyl ether-type pendants, as well as the yields, molecular weights and water solubility of PASP-OCx.

Polymer	Number of carbon in alkyl ether-type pendants *	Yield	M_w (kDa)	M_w/M_n	Watersolubility
PASP-OC3	3	92%	30.7	1.6	soluble
PASP-OC4	4	91%	32.1	1.7	soluble
PASP-OC5	5	92%	33.4	1.8	soluble
PASP-OC6	6	94%	35.5	1.5	Insoluble #

* The number of ether oxygen in alkyl ether-type pendants for the obtained four polymers is the same, which is 1.
# Evaluated at pH range of 2.0–12.0.

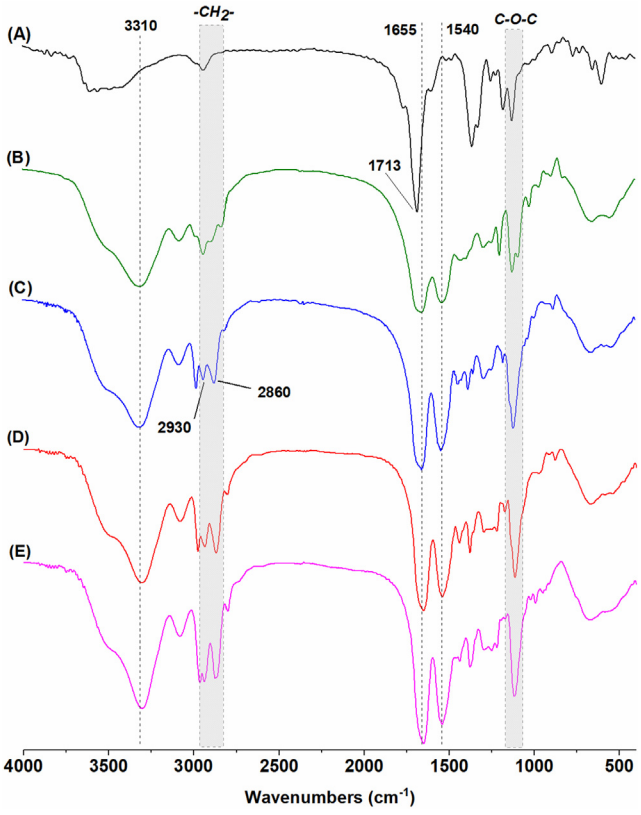


Fig. 1 FTIR spectra of (A) PSI, (B) PASP-OC3, (C) PASP-OC4, (D) PASP-OC5 and (E) PASP-OC6 in KBr.

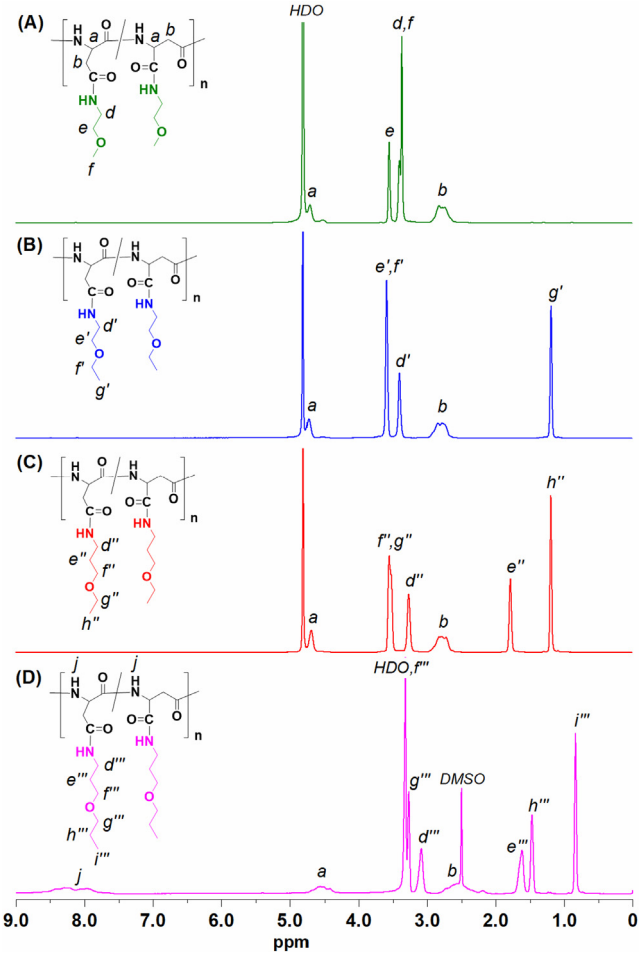


Fig. 2 ^{1}H NMR spectra of (A) PASP-OC3, (B) PASP-OC4, (C) PASP-OC5 in D_2O and (D) PASP-OC6 in DMSO d_6.

of –CON*H*– in PASP-OCx, which was not observed in Fig. 2-**A-C** because D_2O can exchange the active hydrogen of amide in PASP-OCx. The grafting ratios of OC3, OC4, OC5 and OC6 to PSI were further calculated based on the peak-area ratio of peak e/peak b (Fig. 2**A**), peak d'/peak b (Fig. 2**B**), peak d''/peak b (Fig. 2**C**), peak d'''/peak a (Fig. 2**D**) for PASP-OC3, PASP-OC4, PASP-OC5 and PASP-OC6, respectively, and the results confirmed that all the grafting ratios of OCx in PASP-OCx were nearly 100%.

The molecular weights and MWDs of PASP-OCx were determined by SEC-MALLS and summarized in Table 1. The weight average molecular weights of PASP-OC3, PASP-OC4, PASP-OC5 and PASP-OC6 are 30.7 kDa, 32.1 kDa, 33.4 kDa and 35.5 kDa, respectively, obvious higher than that of PSI, which also indicates the successful occurring of aminolysis reaction between linear alkyl ether-type amine compounds and PSI. With all FTIR, ^{1}H NMR and SEC-MALLS analysis results taken together, it can be concluded that the synthesis of PASP-OCx was successful.

3.2. Water-solubility and surface tension

The photographs of PASP-OCx aqueous solutions at 20 °C and 60 °C were shown in Fig. 3. As shown in Fig. 3**A**, PASP-OCx was soluble in deionized water at 20 °C when the carbon number of OCx is smaller than or equal to 5, PASP-OC6 was insoluble in deionized water. As the temperature of PASP-OCx aqueous solutions increased to 60 °C, PASP-OC3 and PASP-OC4 aqueous solutions were still clear, but PASP-OC5 aqueous solution became turbid (Fig. 3**B**). PASP-OC6 was aggregated together and floated on water. When the temperature drops to 20 °C, PASP-OC5 aqueous solution can revert transparent again. These points imply that PASP-OC5 exhibit temperature responsiveness.

The surface tensions of different concentrations of PASP-OCx aqueous solutions were measured at 20 °C (Fig. 4**A**). As the increasing of concentration of PASP-OCx from 4.12 mg/mL to 1000 mg/mL, the surface tensions of both PASP-OC4 and PASP-OC5 aqueous solutions exhibited obvious turning point, and decreased from 72.5 mN/m to 52.8 mN/m and 42.7 mN/m respectively; while the surface tension of PASP-OC3 aqueous solutions only decreased from 72.5 mN/m to 65.9 mN/m without obvious turning point. The CMC values of PASP-OC4 and PASP-OC5 calculated based on the surface tension method were 110.36 mg/L and 30.19 mg/L, respectively (Fig. 4**B**). With the results of water-solubilities and surface tensions of PASP-OCx, it indicates that the carbon number of OCx pendants has significant influence on the hydrophilicity and CMC of PASP-OCx. The smaller the carbon number of OCx pendants is, the better hydrophilicity of PASP-OCx is and the larger CMC is.

3.3. Stimuli responsive behavior

Temperature responsive behavior of PASP-OC5 in deionized water was further evaluated in both heating and cooling pro-

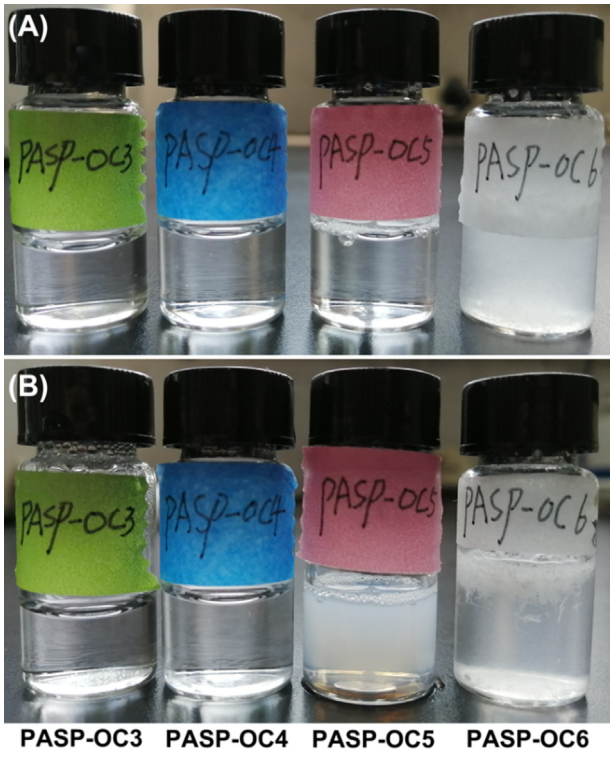


Fig. 3 The optical pictures of PASP-OCx aqueous solutions at (A) 20 °C and (B) 60 °C (polymer concentration: 5.0 mg/mL).

cess by UV/Vis at 500 nm (Fig. 5). It can be seen from Fig. 5**A** that PASP-OC5 solution exhibited temperature responsive behavior with a LCST of around 54 °C in deionized water and the phase transition of PASP-OC5 solution is reversible. The thermodynamic phase transition of PASP-OC5 was also evaluated by dynamic light scattering (Fig. 5**D**). As shown in Fig. 5**D**, the LCST was around 54 °C, which is consistence with the value measured by transmittance. Interestingly, an obvious hysteresis was observed in the cooling process for PNIPAAm in deionized water (**Fig. S3**); while the temperature-dependent transmittance curves of heating and cooling process are almost similar without significant hysteresis for PASP-OC5, which is not in accordance with PNIPAAm (Cheng et al., 2006; Lutz et al., 2006). A possible reason is that there is only one ether oxygen atom for each grafted OC5 moiety of PASP-OC5. Thus, there are no strong and stabilizing intramolecular and intermolecular H-bonding interactions formed in the collapsed state for PASP-OC5. Hence, the temperature-transmittance curve of PASP-OC5 does not exhibit obvious hysteresis due to no additional interactions hindering the rehydration during the cooling process. PASP-OC3 and PASP-OC4 in deionized water were also investigated by measuring the transmittance at 500 nm. The transmittances of both PASP-OC3 and PASP-OC4 aqueous solutions were around 100% at the temperature range of 42–70 °C as shown in Fig. 5**B**, and always kept transparent appearances even at 100 °C. Thus, no temperature responsive behavior was observed for both PASP-OC3 and PASP-OC4 in deionized water. Effect of polymer concentration on the temperature responsive behavior of PASP-OC5 was also investigated, and results showed that the LCST of PASP-OC5 showed an increase from 54 °C to 59 °C with decreasing polymer concentration from 5.0 mg/mL to 2.0 mg/mL (**Fig. S4**). It can be explained that a lower polymer concentration is not conducive to the intermolecular aggregation, which influences the LCST (Cheng et al., 2011).

Since PASP-OC5 exhibited temperature responsiveness, the temperature responsive behavior of PASP-OC5 was further investigated in PBS (pH: 7.0, ionic strength: 150 mM) as shown in Fig. 5**C**. The temperature-dependent transmittance curves of PASP-OC5 on heating and cooling process are also similar in PBS, but the LCST decreased to 41 °C. This may be ascribed to the high ionic strength due to the presence of NaCl in PBS. Thus, the effect of NaCl concentration on the LCST of PASP-OC5 was also evaluated (**Fig. S6**), and results showed that the LCST of PASP-OC5 decreased with increasing the concentration of NaCl. The LCST of PASP-OC5 in water (containing 150 mM NaCl) is very close to that in PBS (pH: 7.0, ionic strength: 150 mM). This "salting-out" effect was also reported in other studies (Cheng et al., 2011).

Previous works reported that pH has great influence on the temperature responsive behaviors of some polyaspartamide derivatives (Hsu et al., 2012). Thus, the effect of pH on the temperature responsive behavior of PASP-OCx was investigated in the present study. Although PASP-OC3 solution

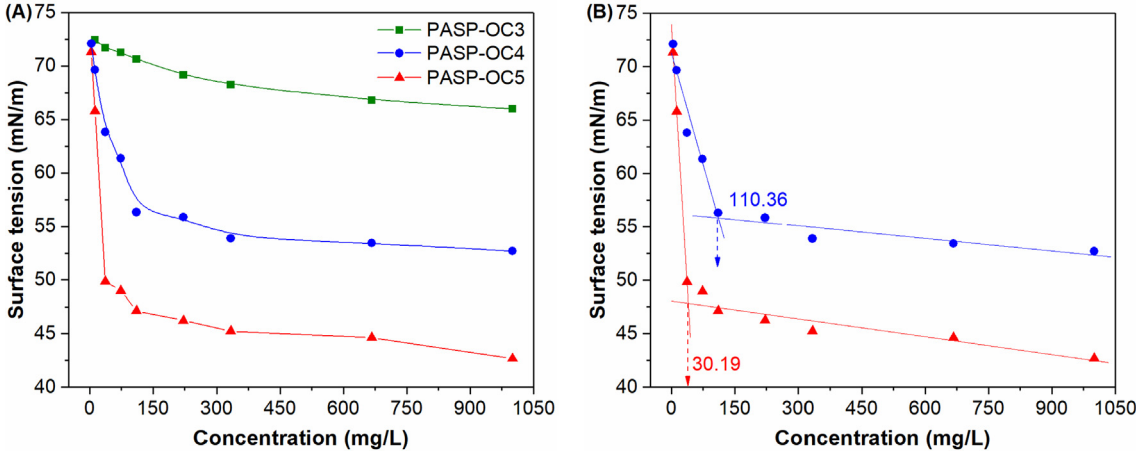


Fig. 4 (A) Surface tensions of different concentrations of PASP-OCx aqueous solutions at 20 °C, and (B) the CMC of PASP-OC4 and PASP-OC5 measured by the surface tension method.

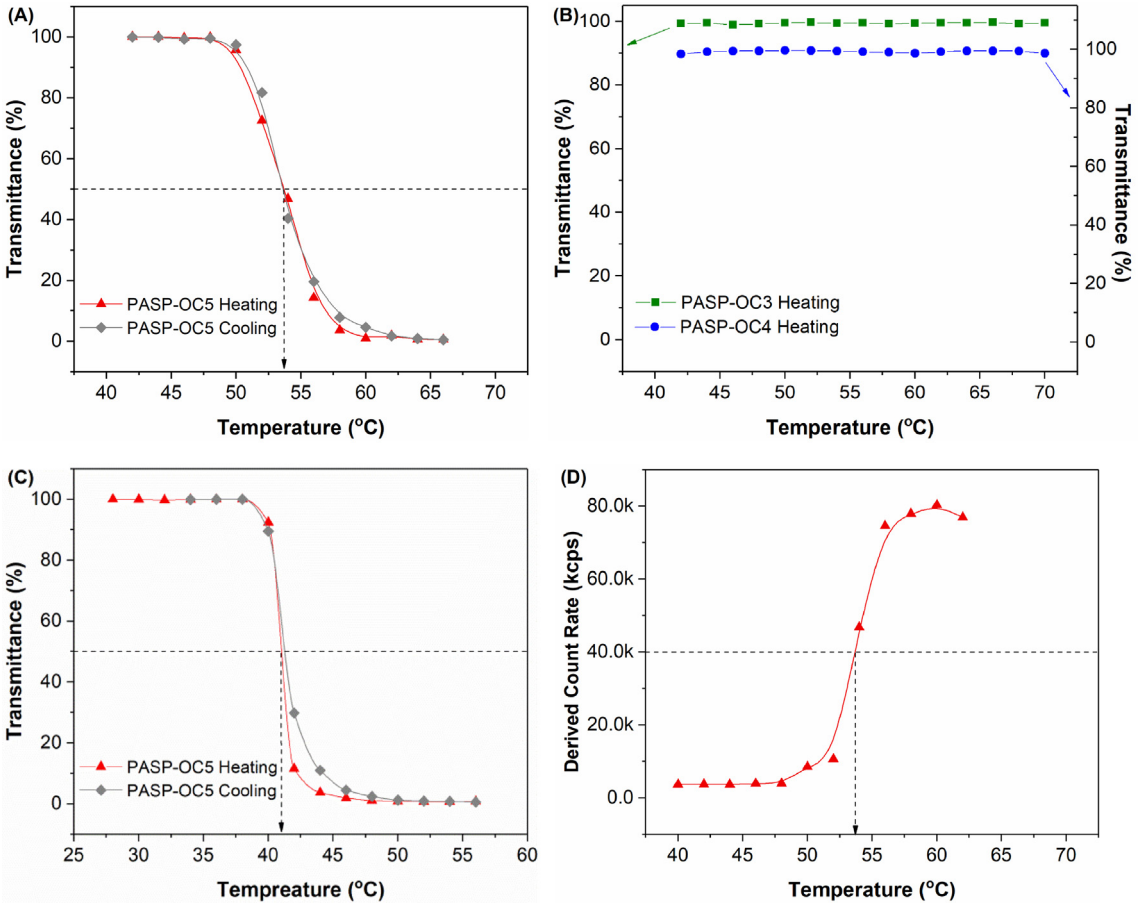


Fig. 5 Temperature dependence of the light transmittance for (A) PASP-OC5 in deionized water on the heating and cooling process; (B) PASP-OC3 and PASP-OC4 in deionized water on the heating process, and (C) PASP-OC5 in PBS (pH: 7.0, ionic strength: 150 mM) on the heating and cooling process measured by UV/Vis at 500 nm. (D) The temperature responsive behavior of PASP-OC5 in deionized water on the heating process measured by dynamic light scattering. (polymer concentration: 5.0 mg/mL).

was investigated by transmittance at the pH range of 2.0–7.0, no temperature responsive behavior was detected. In the case of PASP-OC6, sample was observed to be insoluble in water at the pH range of 2.0–12.0 (Fig. S2). Thus, there is no PASP-OC6 polymer solution suitable for the investigation of the temperature behavior at different pH values by transmittance. In the case of PASP-OC5 and PASP-OC4, the effect of pH on the temperature responsive behavior was significant (Fig. 6), and the LCSTs of PASP-OC4 and PASP-OC5 at different pH values were summarized in Table 2.

For PASP-OC4, the temperature responsive behavior was observed when the pH value of PASP-OC4 aqueous solution was below or equal to 3.0 (Fig. 6**A**); the LCST of PASP-OC4 solution decreased with decreasing the pH value of its solution. For PASP-OC5, the temperature responsive behavior was observed in the range of pH 2.0–10.0 (Fig. 6**B**), and its LCST increased with increasing pH showing a minimum LCST value of around 37 °C and a maximum LCST value of around 67 °C (Table 2). Interestingly, in the case of PASP-OC5, there appears to be a broadening LCST transition range with the increase of pH. So, what causes the increase of LCST and the broader phase transition range of PASP-OC5?

The degradation of PASP-OC5 under alkaline condition seems to be a possible reason, because the degradation of PASP-OC5 will not only affect its molecular weight but also its hydrophilicity. The hydrolysis of –CONH– will increase its hydrophilicity due to the generation of carboxyl and amino groups. This may lead to the increase of its LCST. Thus, two PASP-OC5 samples were treated at pH 2.0 and 12.0 for 24 h respectively for evaluating their molecular weights. However, SEC traces of treated and untreated PASP-OC5 samples showed that no significant degradation was observed (**Fig. S5**). Considering the entire preparation and measuring process for each PASP-OC5 polymer solution with different pH values does not exceed 6 h, the degradation of PASP-OC5 does not seem to be the main factor.

Previous work reported that zeta potential may be a possible cause of the increase in LCST (Hsu et al., 2012; Zhang et al., 2019). Therefore, the zeta potentials of PASP-OC5 and PASP-OC4 at different pH values were further investigated.

3.4. Zeta potential

The zeta potentials of PASP-OC5 and PASP-OC4 aqueous solutions were measured at different pH values. As shown in Fig. 7, the zeta potentials of both PASP-OC5 and PASP-OC4 solutions decreased with increasing pH. The pH value corresponding to zero potential point was around 3.3 for both

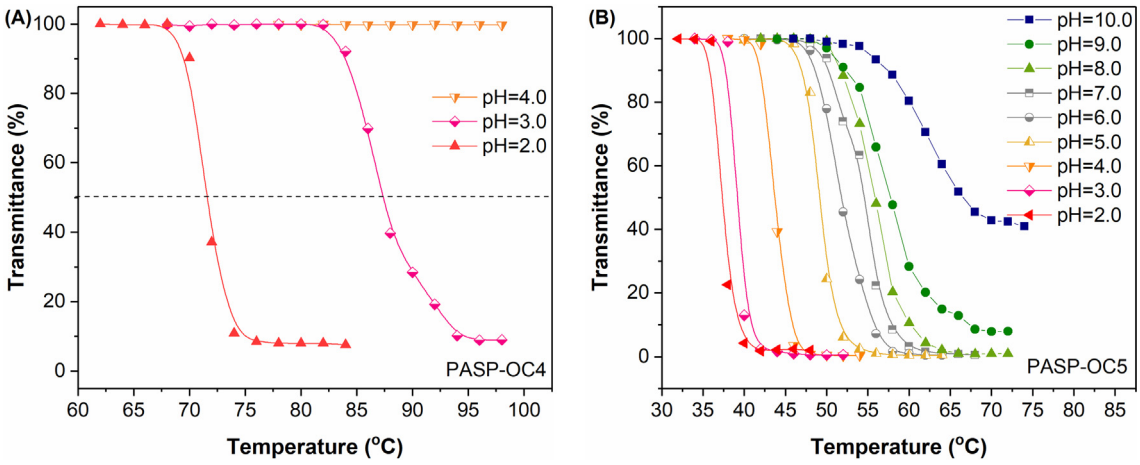


Fig. 6 Effect of pH on the transmittance of (A) PASP-OC4 and (B) PASP-OC5 aqueous solutions on the heating process measured by UV/Vis at 500 nm (polymer concentration: 5.0 mg/mL).

Table 2 The LCSTs of PASP-OC5 and PASP-OC4 at different pH values.

pH	LCST (°C)	
	PASP-OC5	PASP-OC4
2.0	37	72
3.0	39	87
4.0	43	–
5.0	49	–
6.0	52	–
7.0	54	–
8.0	56	–
9.0	58	–
10.0	67	–

PASP-OC5 and PASP-OC4 solutions. When the pH value of PASP-OCx solution is below or equal to 3, the solution shows a very weak positive charge. As the PASP-OCx solution is alkalized, the negative charge becomes stronger with a maximum value of around −16 mV. The strong negative charge enhanced the electrostatic repulsion, which hinders the aggregation of PASP-OCx molecules. Thus, the LCST of PASP-OCx solution increased with increasing pH value as shown in Fig. 6. In addition, as the pH rises from 4.0 to 10.0, the strength of the negative charge becomes stronger, so the resistance to molecular aggregation becomes greater, resulting in a broader phase transition range and a higher LCST. The charge repulsion (-12 mV) of PASP-OC5 may be the reason why the optical transmittance curve of PASP-OC5 (Fig. 5**A**) is relatively broad compared with PNIPAAm. Additionally, the change of zeta potential of PASP-OC5 in deionized water from temperature was also investigated, but no significant changes in zeta potential from temperature were observed (**Fig. S7**).

Although the effect of pH value on the LCST of PASP-OCx solution can be explained by the increasing negative charge, the reason for the zeta potential change is not completely clear. Hsu's group explained that the increasing negative charge was related to the presence of hydroxyl groups, and many hydroxyl groups do indeed exist in previous reported temperature polyaspartamide derivatives containing alkanolamides pendants (Hsu et al., 2012; Zhang et al., 2015a, 2019). However, hydroxyl group does not exist in PASP-OCx. So, what causes the zeta potential change of PASP-OCx solution? Maybe it is related to the end groups

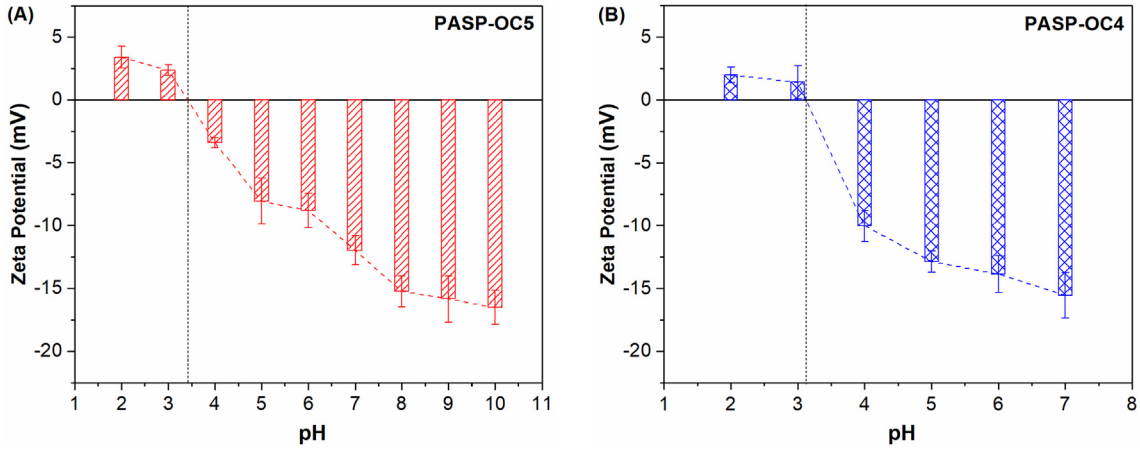


Fig. 7 Effect of pH on the zeta potentials of (A) PASP-OC5 and (B) PASP-OC4 aqueous solutions at 25 °C (polymer concentration: 5.0 mg/mL).

(two carboxyl groups and one amine group) (Park et al., 2011) at the backbone of PASP-OCx. Thus, more polyaspartamide derivatives are needed to be designed for investigating and completely understanding the cause of the zeta potential change. This work is ongoing and will be reported in the next article.

4. Conclusions

In the present study, polyaspartamide derivatives bearing alkyl ether-type pendants, PASP-OCx, were synthesized by the aminolysis reaction between PSI and four linear alkyl ether-type amine compounds with varying carbon atoms, and then characterized by FTIR, ^{1}H NMR and SEC-MALLS. In the obtained four polymers, PASP-OC3, PASP-OC4 and PASP-OC5 can be dissolved in water. The CMC values of PASP-OC4 and PASP-OC5 at 20 °C were 110.36 mg/L and 30.19 mg/L, respectively. Results showed that PASP-OC3 did not exhibit any temperature responsive behavior; while PASP-OC5 and PASP-OC4 exhibited temperature responsive behaviors. The LCST values of PASP-OC5 and PASP-OC4 can be tunable by adjusting the pH value of their solutions, and both decreased with decreasing the pH value of their solutions. Therefore, pH can also affect the temperature responsive behavior of temperature-responsive polyaspartamide derivatives that do not contain hydroxyl groups.

CRediT authorship contribution statement

Guangyan Zhang: Conceptualization, Methodology, Supervision, Writing - review & editing. **Chenhui Bao:** Investigation, Data curation, Writing - original draft. **Hui Yi:** Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103287.

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