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RESEARCH PAPER

Investigation on properties of P((MAA-co-DMAEMA)-g-EG) polyampholyte nanogels

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Abstract P((MAA-co-DMAEMA)-g-EG) polyampholyte nanogels (PANGs) were prepared by distillation-dispersion copolymerization of poly(ethylene glycol) methyl ether methacrylate (MPEGMA), *N*,*N*-dimethylaminoethyl methacrylate (DMAEMA), and methacrylic acid (MAA) using acetonitrile (AN) as dispersion medium. The results of FTIR spectra indicate that the composition of P((MAA-co-DMAEMA)-g-EG) PANGs is consistent with the designed structure. The results of TEM and laser particle size analyzer (LPSA) show that P((MAA-co-DMAEMA)-g-EG) PANGs present spherical morphology and a bimodal size distribution after and before swelling. P((MAA-co-DMAEMA)-g-EG) PANGs have typically amphoteric characters responding to pH, whose isoelectric point (IEP) increases with decreasing the ratio of MAA/DMAEMA and equilibrium swelling degree (ESD) is greater than that at IEP when the pH value is distant from IEP. P((MAA-co-DMAEMA)-g-EG) PANGs also represent ionic strength sensitivity. Using the water-soluble chitosan (CS, Mn = 5 kDa) as model drug, in vitro release indicates that CS can be effectively incorporated into PANGs and the release rate of CS at pH 1.89 is an order of magnitude greater than that at pH 8.36. P((MAA-co-DMAEMA)-g-EG) PANGs may be useful in biomedicine, especially in oral drug delivery of biomacromolecule.

Keywords Polyampholyte · Nanogels · pH-sensitive · Controlled release · Nanostructure · Distillation-dispersion polymerization

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Introduction

Therapeutic peptides and proteins are administered mainly by intramuscular or intravenous injections due to their delicate physicochemical characteristics in aqueous solutions and susceptibility to be degraded by proteolytic enzymes in biological fluids. Hydrogels with water-swollen polymer networks are suitable candidates for oral drug delivery of biomacromolecular drugs due to their ability to respond to environments. In recent years, hydrogels have received attention for use as components in chemical separation systems, drug delivery systems, soft contact lenses, and artificial muscles or switches (Hoffman 2002; Peppas et al. 2000; Qiu et al. 2001). Polyampholyte hydrogels are hydrogels with both positively and negatively charged units (Yao et al. 2003; Zhai et al. 2004). The net charge in polyampholyte hydrogels can be adjusted by changing the monomer content of feed solution in preparing hydrogels or by changing pH of the solution. Polyampholyte hydrogels are of different physical and chemical behavior compared with cationic, nonionic,

or anionic hydrogels (Valencia and Piérola 2007; Zhai et al. 2004). For example, polyampholyte hydrogels show the antipolyelectrolyte effect at IEP, viz. ESD increases with the increment of ionic strength. Therefore, the study on the polyampholyte hydrogels is very helpful in understanding the properties of polyelectrolyte hydrogels and their applications as a new kind of biomaterial (Chen et al. 2004; Ferruti et al. 2005, 2006; Guo et al. 2007; Xu et al. 2006).

Nanogels (NGs) are sub-micron size, internally crosslinked particles of hydrophilic polymers (Rosiak et al. 2003; Ulański et al. 2002; Vinogradov et al. 2002), which can be synthesized in absence of drug, and then loaded with the drug, resulting in a gel collapse and formation of nanoparticles (Choi et al. 2006; Huang et al. 2004; Lemieux et al. 2000; Liu et al. 2005; Sahiner et al. 2007; Shin et al. 2001). These properties make NGs useful in oral drug delivery of biomacromolecule such as proteins, peptides, and DNA (Bharali et al. 2003; Jana et al. 2002; Na et al. 2004; Sahoo et al. 1998; Torres-Lugo and Peppas 2002). The thermosensitive NGs of poly(ethylene glycol) and *N*-isopropyl acrylamide copolymers show a high loading efficiency for insulin and protect insulin from high temperature (Kim et al. 2005; Leobandung et al. 2002). Poly (methacrylic acid-grafted-poly(ethylene glycol)) (P(MAA-g-EG)) NGs, a kind of pH-sensitive anionic hydrogel, can protect the protein drugs from degradation at low pH values as they travel through the stomach and make the drug release at high pH values (Deng et al. 2007; Morishita et al. 2002; Robinson et al. 2002). As effective non-viral gene delivery vehicles, the cationic NGs such as poly (ethylene oxide) (PEO)/polyethyleneimine (PEI) (PEO-cl-PEI) NGs (Lungwitz et al. 2005; Ogris et al. 2003; Vinogradov et al. 1998, 1999) has become hot issue. Interaction of anionic oligonucleotides with PEO-cl-PEI results in formation of nanocomposite materials.

PANGs are a kind of NGs that contain both positive and negative charges along the polymer chain (Das and Kumacheva 2006; Ogawa et al. 2003). From a scientific perspective, PANGs are fascinating systems, due to the multiple interactions acting in parallel with or competing against each other within the NGs interior. The diameter of PANGs depends on pH and shows a minimum value at IEP. The antipolyelectrolyte behaviors are also observed at IEP, viz. an increase in salt concentration causes swelling of PANGs. Because of the characteristics of polyampholyte hydrogels and NGs, PANGs can be broadly applied in drug-controlled delivery systems, especially oral drug delivery systems of biomacromolecule. However, only a few studies on PANGs have been reported (Das and Kumacheva 2006; Kokufuta 2007; Ogawa et al. 2003, 2003; Tan et al. 2006, 2007). Therefore, we prepared PANGs from MAA and DMAEMA in this article. PDMAEMA is a water-soluble synthetic polymer and a weak polybase (pKa ~ 7.0) (Bütün et al. 2001; Wakebayashi et al. 2004). It has been used in polyelectrolyte complexes (PECs) as DNA-binding agent for non-viral gene delivery systems (Paneva et al. 2006; Wakebayashi et al. 2004).

Emulsion polymerization (Tobia et al. 2000; Vinogradov et al. 2002), radiation polymerization (Rosiak et al. 2003; Ulański et al. 1998, 1999), dispersion polymerization (Capek 2000), distillation-precipitation polymerization (Bai et al. 2004, 2005, 2006) and self-assembly method (Gu et al. 2003; Herrero-Vanrell et al. 2005) are often used to prepared polymer particles. In this article, a novel polymerization technology, distillation-dispersion polymerization, was explored to prepare P((MAA-co-DMAEMA)-g-EG) PANGs. P((MAA-co-DMAEMA)-g-EG) PANGs were formed simultaneously through a dispersion polymerization manner during the distillation of AN off the reaction system without agitation. The properties of P((MAA-co-DMAEMA)-g-EG) PANGs were mainly investigated.

Materials and methods

Materials

MPEGMA (Mn = 2,080 Da) was obtained from Sigma Chemical Co. DMAEMA and Tetra(ethylene glycol) diacrylate (TEGDA) were purchased from Aldrich Chemical Co. MAA was provided by Beijing Chemical Factory (China). 2,2′-azobisisobutyronitrile (AIBN) and AN were purchased from Tianjin Chemical Reagent Factory (China). Water-soluble chitosan (CS, Mn = 5 kDa, deacetylation degree of 92%) was purchased from Yuhuan Ocean Biochemical Co. Ltd. (China). All reagents were the analytical grade and used as received.

Synthesis of P((MAA-co-DMAEMA)-g-EG) PANGs

P((MAA-co-DMAEMA)-g-EG) PANGs were prepared by distillation-dispersion polymerization. DMAEMA

and MAA were used as monomer and MPEGMA was used as macromonomer, i.e., reactive stabilizer. The monomers were mixed in a certain mass ratio of MAA/DMAEMA/MPEGMA and the concentration of total monomers in AN varied from 10–100 mg/ml. TEGDA, the crosslinker, was dissolved in the monomer mixture in the amount of 2 mol% of total monomers. Nitrogen was bubbled through the mixture for 20 min to remove the dissolved oxygen. When the temperature of the reaction system reached boiling point, AIBN, the initiator, was added into the reaction system in the amount of 2.6 mol% of total monomers and then the solvent began to be distilled. The initially homogeneous reaction mixtures became milky white after boiling for about 15 min. Water was dropped into the reaction system when two-thirds of AN was distilled and the reaction was ended after complete distillation of AN. The resulting dispersion was centrifuged by a centrifuger (LD5-2A, Beijing, China) at 5,000 rpm for 15 min and the large gel aggregates (about 5% volume fraction) were removed. In order to eliminate any unreacted monomer, oligomer, and non-crosslinked polymer chains from the dispersion, the dispersion was dialyzed opposite to water for approximately 3 days by using a dialysis membrane (12 KDa molecular weight cut-off) by changing water daily. The obtained dispersion could be used directly or frozen and lyophilized into freeze-dried powder of P((MAA-co-DMAEMA)-g-EG) PANGs, which can easily re-disperse into water and form PANGs dispersion. The yield of P((MAA-co-DMAEMA)-g-EG) PANGs was 70–90%. The P((MAA-co-DMAEMA)-g-EG) PANGs prepared in this article are listed in Table 1.

Characterization of P((MAA-co-DMAEMA)-g-EG) PANGs

Fourier transform infrared (FTIR) spectroscopy (FT3000, Bio-Rad, Hercules, CA) was used to confirm the composition of P((MAA-co-DMAEMA)-g-EG) PANGs at a resolution of 2 cm^{-1} at room temperature. Polymer samples were pressed into KBr pellets (1:100 copolymer/KBr ratio) and analyzed with IR data manager software.

Size and distribution of P((MAA-co-DMAEMA)-g-EG) PANGs were determined by LPSA (BI-90Plus, Brookhaven Instruments, USA). In all cases, λ of measurement was 678 nm, detected angle was 90°, and temperature was 25 °C.

Table 1 Effects of the ratio of MAA/DMAEMA in the feed solution on IEP of P((MAA-co-DMAEMA)-g-EG) PANGs

P((MAA-co-DMAEMA)-g-EG) PANGs	Ratio of MAA/DMAEMA (mol/mol)	IEP
PANGs-16	1/6	8
PANGs-11	1/1	4
PANGs-21	2/1	3.8
PANGs-61	6/1	/

Transmission electron microscopy (TEM) specimens for P((MAA-co-DMAEMA)-g-EG) PANGs were observed under a JEM-100CX II instrument. The samples were prepared by adding a drop of P((MAA-co-DMAEMA)-g-EG) PANGs dispersion on the Formvar-coated copper TEM grid, and then dyed by phosphatotungstic acid.

Swelling behaviors of P((MAA-co-DMAEMA)-g-EG) PANGs

The equilibrium swelling was performed to characterize the environment-responsive behavior of P((MAA-co-DMAEMA)-g-EG) PANGs. To determine the equilibrium swelling behavior, 100 mg freeze-dried P((MAA-co-DMAEMA)-g-EG) PANGs was dispersed in 10 ml buffer solution at the pH values of 1.89–12.00. The buffer solutions were prepared with 0.01 mol/L sodium hydroxide solution, 0.1 mol/L phosphatic acid solution, 0.1 mol/L potassium dihydrogen phosphate solution, and 0.1 mol/L disodium hydrogen phosphate solution. The ionic strength of buffer solution was adjusted to 0.5 with solid NaCl. The pH values of solutions were determined by a pH meter ((PHS-3C, Shanghai LeiCi Technology Co, China). The size of P((MAA-co-DMAEMA)-g-EG) PANGs was measured by LPSA before and after swelling. ESD of P((MAA-co-DMAEMA)-g-EG) PANGs was defined as the volume ratio of nanogels at the equilibrium and before swelling. The pH at the minimal ESD is defined as IEP of P((MAA-co-DMAEMA)-g-EG) PANGs.

In vitro release of the drug-loaded PANGs

The drug-loaded PANGs were prepared with P((MAA-co-DMAEMA)-g-EG) PANGs as carriers and CS as model drug. CS was added into the swelled PANGs dispersion and the dispersion was frozen and

lyophilized after agitating for 24 h. 5 mg freeze-dried PANGs was well dispersed in a phosphate buffer solution (5 ml of PBS) and then placed in a dialysis bag (12 KDa molecular weight cut-off), which was immersed in 25 ml receptor fluid (PBS). In vitro release was carried out in incubator shaker (SHZ-88, Jintan Medical Treatment Instruments manufactory, Jiangsu, China) at 130 rpm and 37 °C. At the appropriate time intervals, 25 ml receptor fluid was replaced by 25 ml fresh PBS. The pH values of PBS were varied to observe the effects of pH on in vitro release of CS.

The standard curve of CS was calibrated at 222 nm by UV (WFZ-26A UV–Visible spectrophotometer, Tianjin Science Instrument Plant, China). The concentration of CS in the receptor fluid was determined by UV and the accumulated release was calculated as follows:

$$E = \frac{25\sum_{1}^{n} C_i}{m_{\text{drug}}}$$

where E is the accumulated release (%) of CS, C_i is the concentration of CS in the receptor fluid, m_{drug} is the mass of CS in the drug-loaded PANGs.

Results and discussion

Characterization of P((MAA-co-DMAEMA)-g-EG) PANGs

Analysis of FTIR spectra

The FTIR spectra of P((MAA-co-DMAEMA)-g-EG) PANGs are illustrated in Fig. 1. As shown in Fig. 1a, the spectra of P((MAA-co-DMAEMA)-g-EG) PANGs present the characteristic peaks of MAA, DMAEMA, and MPEGMA. The peak at 2,877 cm^{-1} corresponds to methyl and methylene vibrations. The peak at 1,110 cm^{-1} features asymmetric vibrations of C–O–C. The peaks at 1,720 cm^{-1} and 1,160 cm^{-1} are respectively the characteristic peaks of C = O and C–O of ester group. The wide peak at 3,422 cm^{-1} is the characteristic peak of –COOH. Compared with curve **b**, **c**, and **d** (presenting MAA, MPEGMA and DMAEMA respectively), the disappearance of the peak at 1,634 cm^{-1} assigned to C = C in curve **a** suggests that the copolymerization of MAA, DMAEMA, and MPEGMA has taken place. A new peak occurs at 1,570 cm^{-1}, which may result from the interaction between carboxyl groups and amino groups.

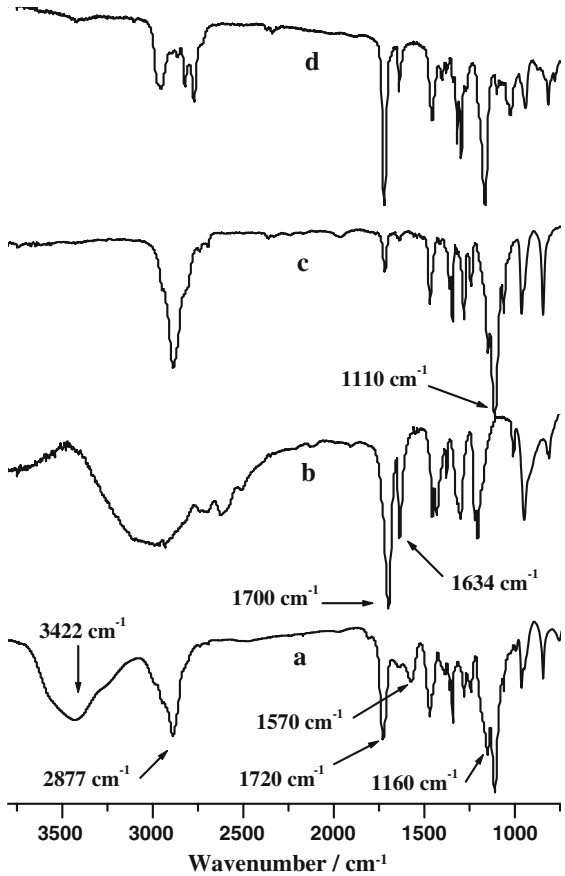


Fig. 1 FTIR spectra of PANGs-11 (**a**), MAA (**b**), MPEGMA (**c**), and DMAEMA (**d**)

In order to further illuminate the compositions of PANGs, P((MAA-co-DMAEMA)-g-EG) PANGs reacted with HCl and NaOH, respectively, and then the excess HCl or NaOH was removed. The FTIR spectra of PANGs·HCl and PANGs·NaOH are shown in Fig. 2. The multipeaks at 2,250–3,000 cm^{-1} in Fig. 2b and wide peak in Fig. 2c further prove the existence of amino groups and carboxyl groups in P((MAA-co-DMAEMA)-g-EG) PANGs, which indicates that the composition of copolymers is consistent with that of designed polymer.

Particle morphology and size distribution

The TEM micrograph of P((MAA-co-DMAEMA)-g-EG) PANGs is shown in Fig. 3. The TEM

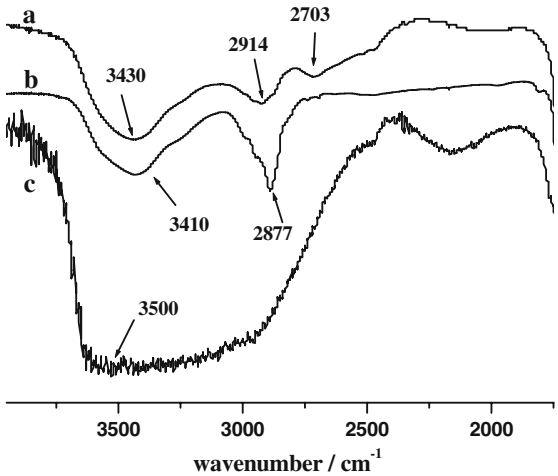


Fig. 2 FTIR spectra of PANGs-11·HCl (**b**) and PANGs-11·NaOH (**c**)

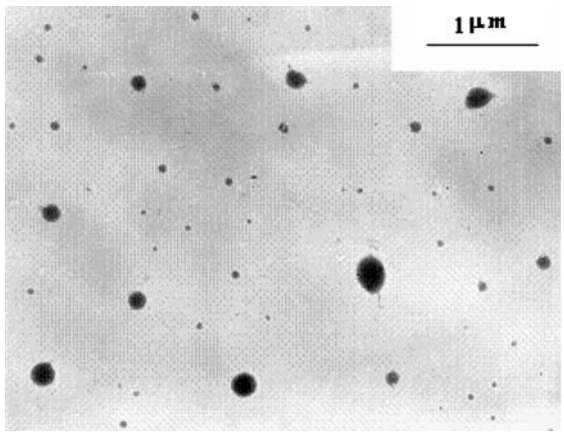


Fig. 3 TEM micrograph of PANGs-11 before swelling

micrograph proves that the spherical PANGs-11 are formed during the distillation-dispersion polymerization of MAA, DMAEMA and MPEGMA. The diameters of PANGs-11 vary approximately in the range of 50 ~ 200 nm. PANGs-11 possess a bimodal size distribution, which accords with the result obtained by LPSA, viz. a bimodal size distribution at about 60 and 380 nm, respectively (Fig. 4). The mean size of PANGs-11 is 270 nm and the polydispersity index is 0.286. Compared to the results of LPSA, the size of PANGs-11 measured by TEM is smaller. This might be because the specimens of PANGs-11 for TEM were in dry state and PANGs-11 shrinked.

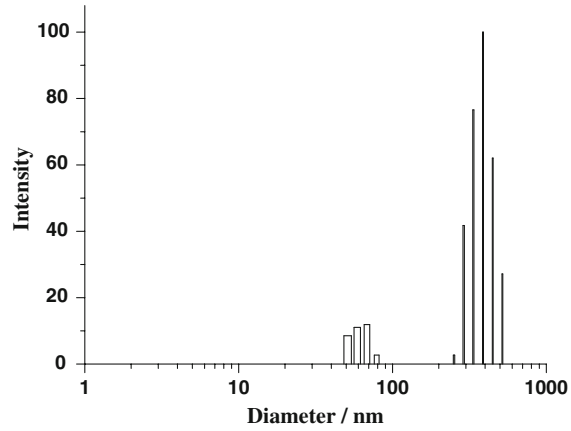


Fig. 4 Particle size distribution of PANGs-11 in ethanol dispersion before swelling

The Morphology of P((MAA-co-DMAEMA)-g-EG) PANGs after swelling in PBS of pH 1.89 was observed by TEM as shown in Fig. 5. After swelling for 24 h, the sizes of PANGs-11 reach 200 ~ 500 nm and PANGs-11 present core-shell structure, which indicate that PANGs are obviously swelled. Similar to PANGs shown in Fig. 3, the swelled PANGs-11 still possess a bimodal size distribution. As shown in Fig. 5b, after swelling for 48 h, the core-shell structure of PANGs-11 is more obvious and there are few changes in the size of nanogels, which suggests that the swelling behavior of PANGs reaches the equilibrium state after swelling for 24 h. Figure 5c is the amplified TEM micrograph of particle in Fig. 5b. It can be seen that the swelled P((MAA-co-DMAEMA)-g-EG) PANGs are composed of hollow core and crosslinking network shell, which suggests that the hollow P((MAA-co-DMAEMA)-g-EG) PANGs are formed via osmotic swelling.

The conductance of PANGs-11 dispersion was measured to further investigate the swelling behavior of PANGs. As shown in Fig. 6, the conductance changes were divided into three phases during swelling process. (1) From a to b, the sharp increment of the conductance indicates that the surface charges of P((MAA-co-DMAEMA)-g-EG) PANGs rapidly transfer with the hydrophilic chain stretching as soon as the dry PANGs disperse in water. (2) In the b–c phase, the increase rate of the conductance is nearly invariable. In this phase, the transference of charges mainly depends on the stretching of segments and the osmotic pressure with the networks is relatively

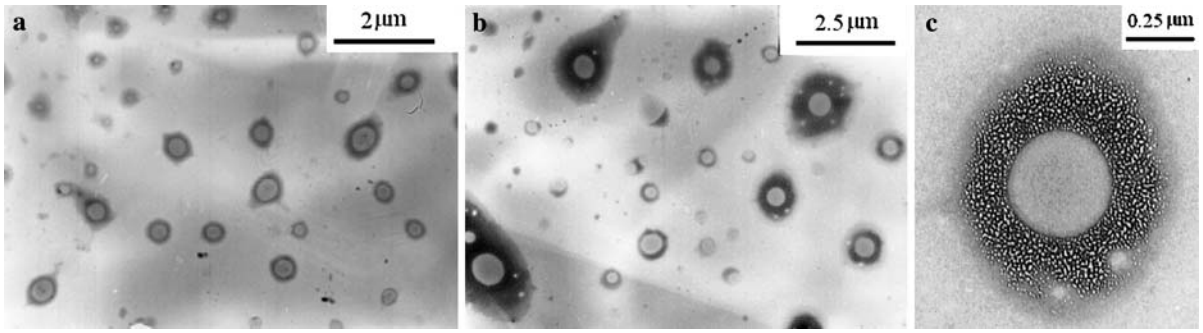


Fig. 5 TEM micrographs of PANGs-11 after swelling in PBS (pH 1.89) at 30 °C. PANGs-11 were swelled for 24 h (**a**) and 48 h (**b** and **c**), respectively. The concentration of PANGs-11 in aqueous solution was 10 mg/ml and ionic strength of solution was 0.5

small. Therefore, this phase is the constant-rate increase period. (3) After swelling for 24 h, viz. c–d phase, the conductance of P((MAA-co-DMAEMA)-g-EG) PANGs dispersion level off, which suggests that PANGs are completely swelled.

Environment sensitivity of P((MAA-co-DMAEMA)-g-EG) PANGs

The swelling equilibrium of polyelectrolyte hydrogel is determined by a balance of three primary forces: (1) the elastic retractile response of the networks; (2) the net osmotic pressure with the networks resulting from the mobile counterions surrounding the fixed charge groups; (3) the free energy of mixing of the network chains with solvent (Zhai et al. 2004). When all these factors in hydrogels are in an equilibrium state, swelling equilibrium is reached. Because there is equal amount of anionic and cationic units on their backbone at IEP, P((MAA-co-DMAEMA)-g-EG) PANGs are of unique characters.

Effects of pH on ESD

As shown in Fig. 7 and Table 1, ESD of P((MAA-co-DMAEMA)-g-EG) PANGs depends strongly on pH. As pH increases from 2, ESD of PANGs-11 firstly decreases steadily and reaches minimum value at IEP, and then rises with further increase of pH. The ionic strength of buffer solution was fixed at 0.5. Near IEP, the Coulombic interaction between opposite charges on the networks of nanogels makes them collapse. However, the nanogels can maintain electrical neutrality with fewer electrolytes present. The osmotic pressure with the networks of P((MAA-co-DMAEMA)-g-EG) PANGs is smallest at IEP.

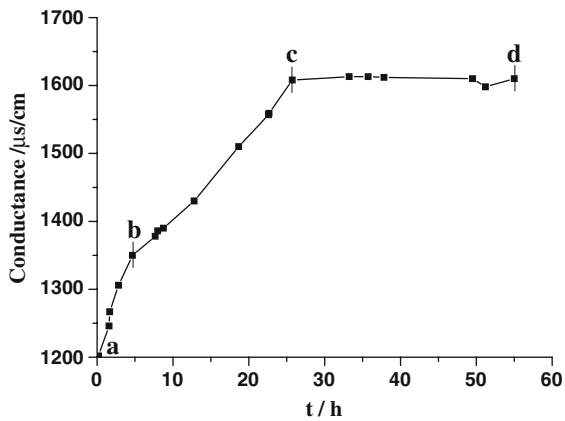


Fig. 6 Conductance change curve of PANGs-11 dispersion (pH 6.0) during swelling process at 30 °C. The concentration of PANGs-11 in aqueous solution was 10 mg/ml and ionic strength of solution was 0.5

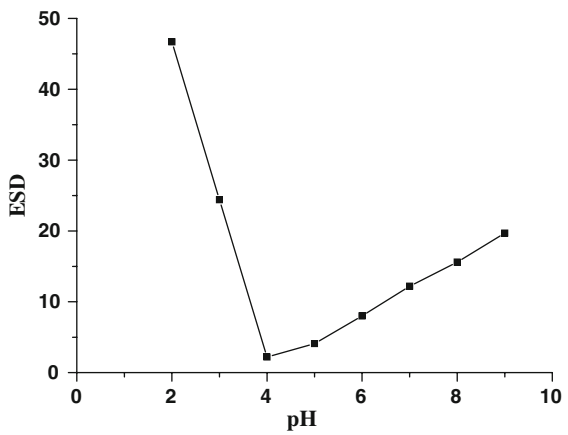
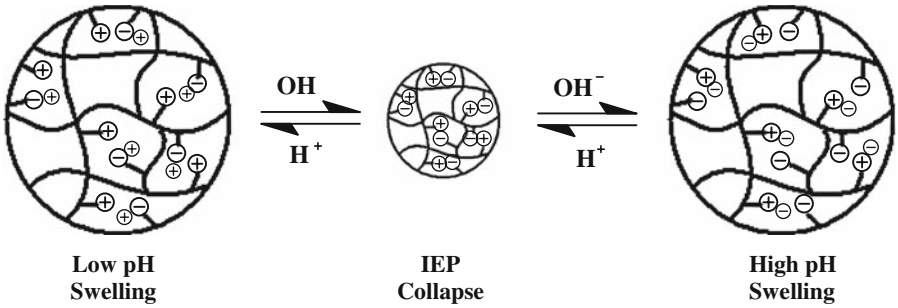


Fig. 7 Effect of pH on ESD of PANGs-11 after swelling at 30 °C for 24 h. The concentration of PANGs-11 in aqueous solution was 10 mg/ml and ionic strength of solution was 0.5



Scheme 1 Hypothetical scheme of swelling-collapse network structure of P((MAA-co-DMAEMA)-g-EG) PANGs in solutions of different pH value

These two factors lead to the minimum values of ESD at IEP. At the pH values lower than IEP, the tertiary amine groups of DMAEMA units on the networks of P((MAA-co-DMAEMA)-g-EG) PANGs become protonated. Therefore, there will then be more cations than anions on the chains, which will lead to the increase of charge density on the networks. The internal osmotic pressure will increase when mobile counterions come into the nanogels to balance the charge of side groups. Thus PANGs have a larger ESD at a pH lower than IEP. At a pH higher than IEP, there will be more ionized carboxyl groups of MAA in the network of PANGs. Similar to the situation below IEP, ESD increases with pH. The explanation is schematically shown in Scheme 1.

From Table 1, it also can be seen that IEP of P((MAA-co-DMAEMA)-g-EG) PANGs will move toward lower pH values with increasing the ratio of MAA/DMAEMA in the feed solution used to prepare PANGs. When the ratio of MAA/DMAEMA is greater than 1/1 (mol/mol), IEP of P((MAA-co-DMAEMA)-g-EG) PANGs changes unobviously. When the ratio of MAA/DMAEMA is 6/1 (mol/mol), P((MAA-co-DMAEMA)-g-EG) PANGs present the properties of polyanion. When the ratio of MAA/DMAEMA is 1/6 (mol/mol), there are more tertiary amine groups of DMAEMA in the network of PANGs, which need more ionized carboxyl groups to form electron pairs. Therefore, IEP of P((MAA-co-DMAEMA)-g-EG) PANGs is high when the ratio of MAA/DMAEMA is 1/6 (mol/mol). With increasing the ratio of MAA/DMAEMA, the amount of tertiary amine groups in PANGs decreases, which makes IEP of P((MAA-co-DMAEMA)-g-EG) PANGs move toward lower pH values. Thus, by changing the monomer ratio in the feed solution, P((MAA-co-DMAEMA)-g-EG) PANGs with different IEP could easily be prepared.

Effects of ionic strength on ESD of PANGs

The freeze-dried P((MAA-co-DMAEMA)-g-EG) PANGs were dispersed in 10 ml buffer solution at different pH values at 30 °C and the total ionic strength of the buffer solution was adjusted to a desired level with calculated amount of NaCl. Figure 8 shows the effects of ionic strength on ESD of PANGs-11 at different pH values. At pH of 6.0, PANGs-11 will behave like polyanion because IEP of PANGs-11 is 4.0. Increase of ionic strength will only lead to the decrease of osmotic pressure of the networks. Therefore, ESD of PANGs-11 decreases with increasing the ionic strength. However, ESD of PANGs-11 increases when the ionic strength is higher than 0.8. Because hydrogen bonds between PEG and water are destroyed, solubility of PEG in water decreases and interactions among PEG chains change to attractive forces, the aggregation of particles makes the size of PANGs-11 increase.

As shown in Fig. 8b, an 'antipolyelectrolyte' phenomenon was observed at pH of 4.0, i.e., IEP of PANGs-11. ESD of PANGs-11 increases as the ionic strength increases. According to Donnan equilibrium theory, when the ionic strength in solution increases, there will be more electrolytes entering the hydrogels. These low-molecular weight electrolytes will then screen opposite charges on the gel chains. At IEP, increase of ionic strength will weaken the electrostatic attractive forces between charges on the gel chains. Polyampholyte chains tend to expand, which make ESD increase with increaseof ionic strength. Once higher than 0.6, the ionic strength begins to

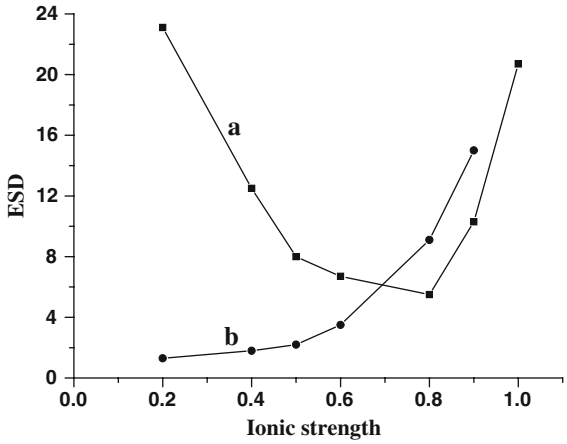


Fig. 8 Effect of ionic strength on ESD of PANGs-11. The concentration of PANGs-11 in aqueous solution was 10 mg/ml and PANGs-11 was swelled at 30 °C for 24 h at the pH value of 6.0 (**a**) and 4.0 (**b**), respectively

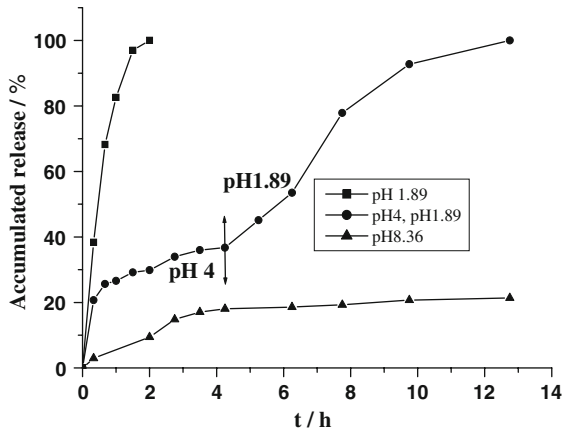


Fig. 9 In vitro release profiles of the water–soluble chitosan from the drug-loaded PANGs. PANGs-11 were used as the carrier materials. The drug-loaded content was 5%. In vitro release was carried out in incubator shaker at 130 rpm and 37 °C

exert the effects on hydrogen bonds between PEG and water. Therefore, ESD of PANGs-11 sharply increases with increasing the ionic strength till PANGs-11 precipitate out of the dispersion.

In vitro release

The drug-loaded PANGs were prepared with PANGs-11 as carriers and CS as model drug. In vitro release profiles of CS from the drug-loaded PANGs-11 are shown in Fig. 9. Upon increment of pH, the release rate and accumulated release decrease. At pH of 1.89, the release rate of CS is very high and the accumulated release reaches 100% after 2 h. However, at pH of 8.36, the release rate of CS is very low and the accumulated release is only 21% after 13 h. The release rate of CS at pH of 1.89 is an order of magnitude greater than that at pH of 8.36. In order to prove influences of pH on the release rate of CS from PANGs-11, the pH value of acceptor fluids was adjusted to 4. The result shows that the accumulated release of CS reaches to 34% after 3.5 h and is not obviously varied after 4 h. If the pH value of acceptor fluids is adjusted from 4 to 1.89 after 4 h, the release rate of CS will become greater again and the accumulated release reaches 100% quickly. CS is loaded by P((MAA-co-DMAEMA)-g-EG) PANGs via electrostatic interaction with the carboxyl groups of MAA units in PANGs. At pH 1.89, the carboxyl groups are not ionized, which weakens the influences of electrostatic interaction between CS and MAA and makes CS release rapidly from PANGs. With pH value increasing, the degree of ionization of MAA increases, which enhances the electrostatic interaction between CS and MAA and makes the release rate of CS slow. Thus, P((MAA-co-DMAEMA)-g-EG) PANGs have a potential application in drug-controlled delivery system, especially biomacromolecular drugs.

Conclusions

P((MAA-co-DMAEMA)-g-EG) PANGs, a new kind of polyampholyte nanogels, were prepared by distillation-dispersion copolymerization. P((MAA-co-DMAEMA)-g-EG) PANGs which can redisperse into water after freeze-drying are of spherical morphology and presents a bimodal size distribution. P((MAA-co-DMAEMA)-g-EG) PANGs also exhibit good swelling ability and can form hollow particles during swelling process. When pH value deviates from IEP, P((MAA-co-DMAEMA)-g-EG) PANGs behave like polycation or polyanion. With the ionic strength increasing, ESD of P((MAA-co-DMAEMA)-g-EG) PANGs firstly increases and then decreases. At IEP, ESD of P((MAA-co-DMAEMA)-g-EG) PANGs is minimal and behaves 'antipolyelectrolyte' character, viz. ESD increases with the increment of ionic strength. The investigation on in vitro release of

drug-loaded PANGs indicates that P((MAA-co-DMAEMA)-g-EG) PANGs have potential applications in drug-controlled delivery systems, especially oral drug-controlled delivery systems of biomacromolecular drugs.

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