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Novel thermo- and pH-responsive hydroxypropyl cellulose- and poly (L-glutamic acid)-based microgels for oral insulin controlled release

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ABSTRACT

Novel smart microgel particles made of poly (L-glutamic acid-2-hydroxylethyl methacrylate) (PGH) and hydroxypropyl cellulose-acrylic acid (HPC-AA) have been successfully prepared *via* emulsion polymerization. The dynamic light scattering measurement reveals that the average hydrodynamic radius $\langle R_h \rangle$ and hydrodynamic radius distributions $f(R_h)$ of the microgel particles depend on the temperature and pH value thus the microgel particles exhibit both pH- and temperature-sensitivity. *In vitro* release study shows that the amount of insulin released from microgels in the gastric juice (at pH 1.2) is significantly less than that in the intestinal fluid (at pH 6.8). These results indicate that the resultant microgels are of potential for use in intelligent oral drug delivery systems.

1. Introduction

The design and preparation of "smart" synthetic materials which are able to respond to external or internal stimuli such as heat, light, pH, magnetic and electric fields and chemical substances have attracted a great deal of interest for their potential applications in tissue engineering, biomedical implants, drug delivery, and bionanotechnology (Li, Luo, & Lam, 2009; Ma, Fan, Liang, & Xu, 2010; Ma, Liu et al., 2010; Pasparakis & Vamvakaki, 2011; Zhang et al., 2010). Microgels, as intramolecular cross-linked polymeric particles of colloidal size, can swell and deswell in response to stimuli more rapidly than bulk gels, are the most commonly studied types, especially in the field of controlled release (Heyes & Branka, 2009; Hoare & Pelton, 2008; Liu, Luo, Guan, & Zhang, 2010; Nur, Pinkrah, Mitchell, Benée, & Snowden, 2010). Poly (N-isopropylacrylamide) (PNIPAM), as the first synthesized thermal-responsive polymers for bio-related applications, exhibits a reversible volume phase transition around the lower critical solution temperature (LCST, approximately 32 °C) (Chen et al., 2010; Liu, Yu, Tian, Sun, & Fan, 2009). However, the poor biocompatibility of PNIPAM may be a limitation to be used *in vivo*, especially as drug vehicles (Pelton, 2000). Therefore, considerable efforts have been made to explore nontoxic and biocompatible natural polysaccharides as suitable candidates including starch, dextrans, chitosan.

Hydroxypropylcellulose (HPC), as another class of thermal-responsive polymer, approved by FDA is a derivative of natural polysaccharide cellulose and widely used in food, drug and cosmetics areas (Heitfeld, Guo, Yang, & Schaefer, 2008). This amphiphilic polymer, potentially alternative to PNIPAM, exhibits a LCST around physiological temperature (about 42 °C) due to the hydrophilic/hydrophobic balance (Lu, Hu, & Gao, 2000; Xia, Tang, Lu, & Hu, 2003). This kind of phase transition behavior is similar to that of PNIPAM, which makes it feasible to use HPC as a biodegradable and biocompatible substitute of PNIPAM. Ma et al. studied the self-assembly properties of HPC-graft-(2-(dimethylamino)ethyl methacrylate) DMAEMA copolymers in aqueous solutions and showed that the LCST onset could be precisely tuned (Ma, Fan, et al., 2010; Ma, Liu et al., 2010).

Very recently, some multi-responsive micro/nanoparticles with controlled architecture have been designed for biomedical imaging, drug delivery and intracellular biosensing (George & Maria, 2011). Combining thermo-responsive polymer with pH-sensitive moiety containing pendent carboxylic groups such as acrylic acid makes it possible to successfully prepare dual stimuli-responsive microgels which can respond to not only temperature but also pH stimuli due to changing the ionization–deionization equilibrium by adjusting the structure and conformation (Dou, Yang, Tao, Li, & Sun, 2010; Wu et al., 2010). To advantage for the difference between gastric juice and intestinal juice *in vivo*, these double-responsive micro/nanoparticles will be prominent candidates for

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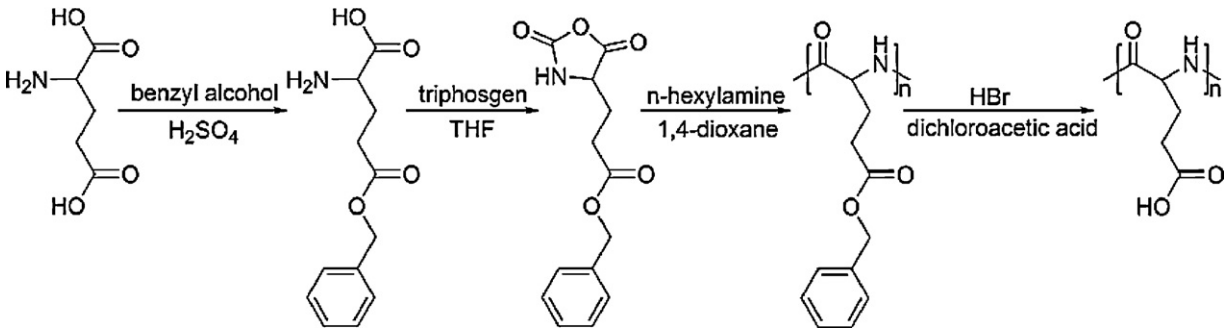


Scheme 1. Synthetic route of PGA.

protein deliveries, especially on insulin release systems for the diabetes treatment (Leobandung, Ichikawa, Fukumori, & Peppas, 2002; Schmaljohann, 2006). These "smart" delivery systems may protect the drugs against a harmful gastric environment (chemical degradation), encapsulating insulin in particulate carriers can prevent enzymatic degradation, while controlling the drug release and increasing their absorption in the small intestine.

However, the above mentioned pH-sensitive polymers are not biodegradable and not suitable for application *in vivo*. Therefore, using biodegradable pH-sensitive materials to develop oral insulin-delivery systems has received considerable attention. Poly (L-glutamic acid) (PGA) is one of the most widely used pH-responsive polypeptides in the biomedical area due to their biocompatibility and biodegradability (Murakami & Aoki, 2006). The γ-PGA-based pH-responsive nanoparticles have been successfully prepared and used as the delivery of insulin (Sonaje et al., 2010).

Accordingly, in this article a series of novel pH- and thermal-responsive microgels composed of HPC-AA and PGH were synthesized by the emulsion polymerization. The influences of pH and temperature on the swelling behaviors of the microgels were investigated. The microgels exhibited special pH-dependent temperature-sensitivity because of the increase of LCST with pH value. Based on this property, the intelligent microgels have interesting potential application in insulin controlled release *via* the reversible swelling/shrinking transition.

2. Experimental

2.1. Materials

Hydroxypropyl cellulose (HPC) (average $M_w = 1 \times 10^5$) was purchased from Acros Organics and used directly. Acrylic acid (AA) was obtained from Shanghai Chemical Reagent Co. (China) and distilled under reduced pressure to remove inhibitors before use. 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC·HCl, Shanghai GL Biochem, China) and 4-dimethylaminopyridine (DMAP 98% Fluka) were used as received. γ-Benzyl-L-glutamate was purchased from Sigma. n-Hexylamine (Aldrich, 99%) was used as received. Hydroxyethyl methacrylate (HEMA 96% Acros) was distilled under reduced pressure before use. Ammonium persulfate (APS) and N,N,N′,N′-tetramethylethylenediamine (TEMED) were obtained from Shanghai Chemical Reagent Co. (China) and used directly. Trichloromethane and dimethyl sulfoxide (DMSO) were dried over CaH_2, followed by distillation under reduced pressure prior to use. Distilled water was used throughout.

Artificial gastric juice and artificial intestinal liquid were prepared as follows. 7 ml of hydrochloric acid (37 wt%) and 2.0 g sodium chloride were dissolved into 1000 ml of deionized water to prepare artificial gastric juice. 6.8 g potassium dihydrogen phosphate was dissolved into 500 ml of deionized water, and then the pH value was adjusted to 6.8 and the solution was diluted to 1000 ml to get artificial intestinal liquid.

Oil phase of emulsion polymerization was prepared as follows. 1.35 g Span-80 and 0.15 g Tween-80 (Span-80/Tween-80 = 9, w/w) were dispersed into 240 ml of n-hexane and 60 ml of paraffin (n-hexane/paraffin = 4, v/v) and then the mixture was magnetically stirred uniformly for later.

2.2. Synthesis of Poly (L-glutamic acid) (PGA)

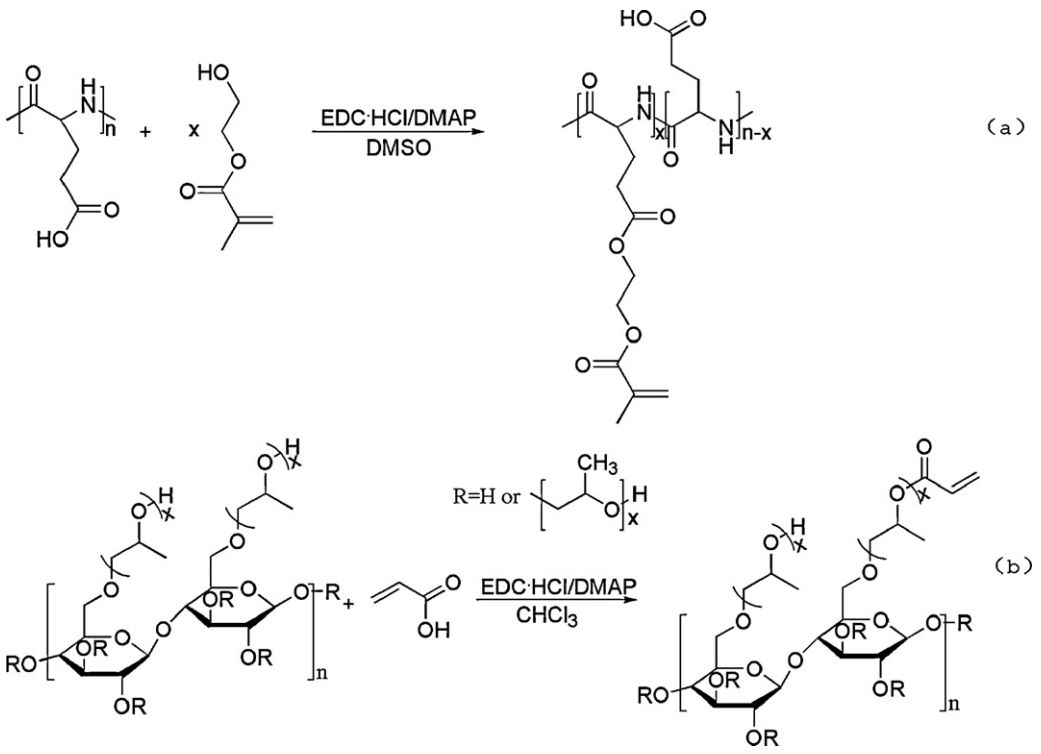
Firstly, γ-benzyl-L-glutamate N-carboxyanhydride (BLG-NCA) was prepared as follows. 10 g of γ-benzyl-L-glutamate and 6 g of triphosgene were suspended into 100 ml of dry THF under a flow of nitrogen. The mixture was stirred at 60 °C with nitrogen bubbling until it turned into a transparent solution. The solution was poured into 1000 ml of n-hexane to yield white precipitate and the crude product was recrystallized from the ethyl acetate/n-hexane mixed solution for three times. The yield was 70%.

Secondly, poly (γ-benzyl-L-glutamate) (PBLG) was synthesized through the ring-opening polymerization (ROP) of BLG-NCA initiated by n-hexylamine. A typical experimental procedure was as follows: BLG-NCA (5 g) was dissolved in 150 ml of anhydrous dioxane, followed by adding 4.75 ml of 0.1 M n-hexylamine in dioxane solution (with a monomer/initiator ratio = 40, mol/mol). After vigorous stirring for 3 days at room temperature, the reaction mixture was added to a 10-fold excess of diethyl ether. The precipitated polypeptide was washed twice with diethyl ether and dried overnight under vacuum, yielding PBLG 4.5 g (90%).

Lastly, poly (L-glutamic acid) was prepared by debenzylation of PBLG using HBr as shown in Scheme 1. Briefly, PBLG (4.5 g) was dissolved in 45 ml of dichloroacetic acid, followed by addition of excessive 33 wt% HBr solution in acetic acid (HBr/benzyl groups = 8, mol/mol). After the solution was stirred at 30 °C for 1 h, it was precipitated into excessive diethyl ether, isolated by filtration, and repeatedly washed using acetone. Then the product was dried under vacuum at room temperature for 24 h. The yield was 77%.

2.3. Synthesis of PGA-HEMA (PGH)

The synthetic route of PGH was shown in Scheme 2(a). 3.0 g PGA and 0.3 ml of HEMA (the molar ratio of HEMA and COOH groups in PGA is 1:10) were dissolved in 90 ml of DMSO. After dissolution, EDC·HCl (0.9 g) and DMAP (0.06 g) were added as the coupling agent and catalyst, respectively. The mixture was mixed thoroughly and then quickly poured into a glass mold. The reaction was allowed to proceed at room temperature for 72 h. The product was collected by precipitating in 10-fold diethyl ether.



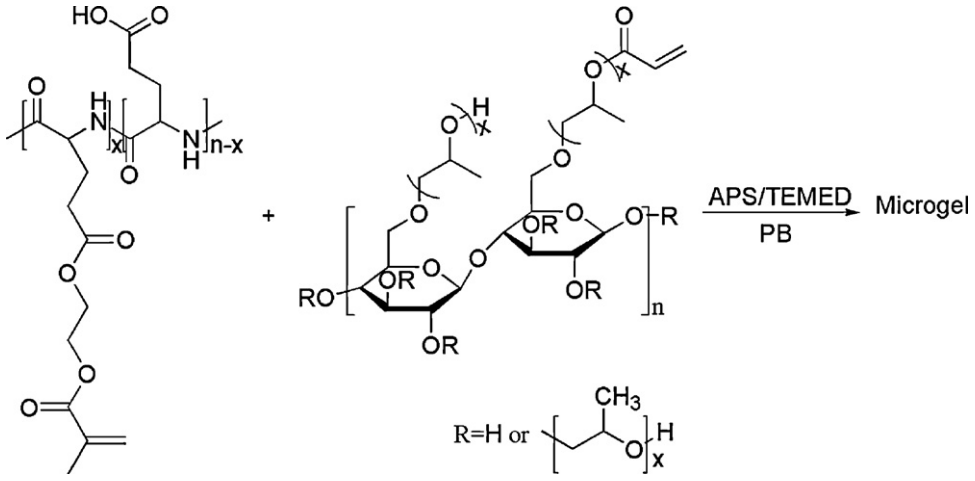
Scheme 2. Synthetic route of PGH (a) and HPC-AA (b).

2.4. Synthesis of HPC-AA

HPC-AA was prepared by esterification reaction of AA in HPC trichloromethane solution in the presence of DMAP as a catalyst and EDC·HCl as the coupling agent, respectively, as depicted in Scheme 2(b). Firstly, 5 g HPC was dissolved into 100 ml of trichloromethane by gentle stirring. Secondly, 0.46 ml of AA (the molar ratio of AA and hydroxyl groups in HPC was 1:10) was added into the mixture. Finally, 0.08 g DMAP and 1.3 g EDC·HCl were subsequently added into the mixture and the reaction was allowed to proceed for 48 h at room temperature. The crude product was precipitated in diethyl ether (1000 ml). Then the precipitate was filtered and dried, followed by dialysis for 7 days against very frequently changed water. HPC-AA was obtained in 75% yield.

2.5. Microgels synthesis

The microgel dispersions were prepared by free-radical emulsion polymerization of HPC-AA and PGH, as shown in Scheme 3. Firstly, HPC-AA and PGH (total amount of 50 mg) at different weight ratios, *e.g.* PGH wt% = 30%, 50% and 70%, were dissolved into 5 ml of phosphate buffer (pH = 7.4, 0.1 M), after dissolution, 5 mg APS was dissolved into the mixture solution. Secondly, 50 ml of pre-prepared oil phase was emulsified for 10 min at 4200 rpm on a homogenizer under ice water bath and then 5 ml of water phase was added dropwise. After continuous emulsification for another 5 min to form w/o emulsion, 5 μl of TEMED as a catalyst was added to initiate the radical cross-linking reaction for 12 h under room temperature. Lastly, the resultant was collected by centrifugation, washed by ethanol, diethyl ether and water and then freeze-dried.



Scheme 3. Synthetic route of microgel.

2.6. Characterization

The FT-IR spectra were recorded with a Bruker Vertex 70 Fourier transform infrared spectrometer using KBr method. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded by a Bruker 300 MHz spectrometer with $CDCl_3$ and D_2O as solvent.

2.7. Dynamic light scattering (DLS) measurements

The hydrodynamic radius distributions of the microgels in aqueous solution were monitored using DLS performed on a Wyatt QELS instrument with a vertically polarized He–Ne laser (DAWN EOS, Wyatt Technology), and 90° collecting optics. All samples were prepared in aqueous solution at a concentration of 0.1 mg/ml and the measurements were carried out at 25, 35 and 60 °C, respectively. Each sample was kept in the thermostat of the apparatus at various temperatures for 30 min to reach the equilibrium prior to measurements.

2.8. Scanning electron microscopy (SEM) measurements

The morphologies of the microgels were investigated by environmental scanning electron microscopy (ESEM) on an XL 30 ESEM FEG Scanning Electron Microscope (Micrion FEI PHILIPS). A drop of the microgel solution (0.1 mg/ml) was deposited onto a silicon chip and air-dried. A layer of gold was sputter-coated on the specimen surface before ESEM measurement.

2.9. Phase transition measurements

Phase transition measurements were performed by a UV-Vis spectrometer (Shimadzu UV-2401PC) equipped with a temperature controller (Shimadzu S-1700). The concentration of the microgel solution used for the determination of phase transition was 0.1 mg/ml. We selected 550 nm as the analyzing wavelength. The heating rate was 0.5 °C/min over the temperature range of 25–60 °C. The LCST was determined as the temperature at which the optical transmittance decreased to 90% of its initial value.

2.10. Insulin load and release

The hydrophilic insulin was loaded into the microgels by a swelling-diffusion method. Insulin, for the treatment of diabetes, has poor solubility at neutral pH values while under acidic conditions it is quite soluble (Nolan, Serpe, & Lyon, 2004). According to this property, the insulin-loaded microgels were prepared as follows. Firstly, 20 mg insulin was dissolved into 200 μl of 0.1 N HCl to prepare stock solution, and then the solution was diluted with 99.6 ml of phosphate buffer solution (pH = 7.4) and normalized with 200 μl of 0.1 M NaOH. The final pH value of the solution with a concentration of 0.2 mg/ml was 7.4. Secondly, 4 mg lyophilized microgel was immersed into 10 ml of the insulin solution at room temperature for 24 h to load the insulin into the microgels. Lastly, after reaching swelling equilibrium the resulting solution was transferred into centrifuge tubes and centrifuged at 12,000 rpm at 25 °C for 10 min to get rid of unloaded insulin. After collecting the supernatant in another container, the precipitate was rinsed with 10 ml of the artificial gastric juice (pH = 1.2) or the artificial intestinal liquid (pH = 6.8) for 10 min to move the drug adsorbed on the surface of the microgels. The unloaded insulin solution and the artificial gastric juice/artificial intestinal liquid used to rinse the insulin-loaded microgels were collected together. The concentration of insulin was monitored by a UV–Vis spectrophotometer (Shimadzu UV-2401PC) at a wavelength of 280 nm.

To study the release behavior of insulin from the microgels, insulin-loaded microgels were dissolved into 1 ml of the artificial gastric juice or the artificial intestinal liquid and the microgel solution was poured into a dialysis bag (cut-off 7000) placed in a beaker containing 10 ml of the artificial gastric juice and the artificial intestinal liquid as release medium for 10.5 h, respectively. The beaker was shaken at 100 rpm at 37 °C. At predetermined intervals 1 ml of medium was taken out and replaced with 1 ml of the fresh solution to keep constant volume. The concentration of the insulin in the medium was determined by the UV–Vis spectrophotometer at 280 nm. All the release experiments were carried out in triplicate, and the averaged results were obtained.

3. Results and discussion

3.1. Preparation of polymers and microgels

PGA was synthesized by ring-opening polymerization of BLG-NCA using n-hexylamine as the initiator followed by debenzylation in dichloroacetic acid using 33 wt% HBr of acetic acid solution at 30 °C. The degree of polymerization (DP) is 36 calculated from the intensities of ^{1}H NMR signals at 0.68 ppm of terminal $-CH_3$ and 4.14 ppm methine proton (H) (—NHC*H*CO—) of PGA. And then PGH was prepared by esterification between PGA and HEMA in the presence of EDC·HCl and DMAP. The absorption peaks appearing at 5.46 ppm and 5.15 ppm (2H, CH_2=CCH_3—) (Fig. 1A) verifies the successful coupling of HEMA to PGA. The coupling ratio of HEMA is calculated from the integration ratio between the methene proton (2H) (CH_2=CCH_3—) of HEMA and the methine proton (H) (—NHC*H*CO—) of PGA appearing at 5.46 ppm and 4.14 ppm (shown in Fig. 1A), respectively, by the following equation:

$$\text{HEMA coupling ratio (mol\%)} = \frac{I_a}{I_{h,h'}} \times 100 \quad (1)$$

where I_a and $I_{h,h'}$ are the integral values of the peaks at 5.46 ppm and 4.14 ppm, respectively. The HEMA coupling ratio of PGH was 8%.

HPC-AA was synthesized by esterification reaction of HPC and AA in the presence of EDC·HCl and DMAP as the coupling agent and catalyst, respectively, as depicted in Scheme 2(b). The hydroxyl groups of HPC reacted with AA to form HPC-AA with active double bond. ^{1}H NMR measurement was performed to determine whether the double bond group was introduced into HPC. As presented in Fig. 1B, HPC-AA exhibits new proton peaks at 6.44 ppm, 5.83 ppm (2H, CH_2=CH—), and 6.10 ppm (1H, CH_2=CH—) compared with pure HPC, which are attributed to the AA moieties introduced into HPC. From the ^{1}H NMR spectrum of HPC-AA, it is clear that HPC have been successfully modified with double bond. The coupling ratio of AA on HPC is calculated from the integral areas of the CH peak at 6.10 ppm from AA, the proton peaks of c, c′ over 3.1–4.4 ppm and the CH_3 peaks at 1.2 or 1.4 ppm from HPC by the following equation:

$$\text{AA coupling ratio (mol\%)} = \left\{ \frac{I_b}{[I_{c,c'} - (I_a + I_{a'})]} \right\} * \frac{7}{3} * 100 \quad (2)$$

where I_b, $I_{c,c'}$, I_a and $I_{a'}$ are the integral areas of the peaks at 6.10 ppm, 3.1–4.4 ppm, 1.2 ppm and 1.4 ppm, respectively. The $I_{c,c'}$ corresponds to all C*H*s and CH_2s on the glucose unit ($5CH + 1CH_2$) and on the hydroxypropyl substitutes ($1CH + 1CH_2$). Although the degree of hydroxypropyl substitution is unknown, they can be subtracted from $I_{c,c'}$ because each hydroxypropyl substitute consists of one CH, one CH_2 and one CH_3, and the latter gives signals at 1.2 or 1.4 ppm (I_a and $I_{a'}$, in Fig. 1B). Therefore, $I_{c,c'} - (I_a + I_{a'})$ corresponds to the seven protons on the glucose unit ($5CH + 1CH_2$) and Eq. (2) stands for the AA coupling ratio on HPC. From it the AA coupling ratio of HPC is calculated to be 9 mol%.

The microgels were prepared by free radical cross-linking between PGH and HPC-AA using APS as the initiator and TEMED as

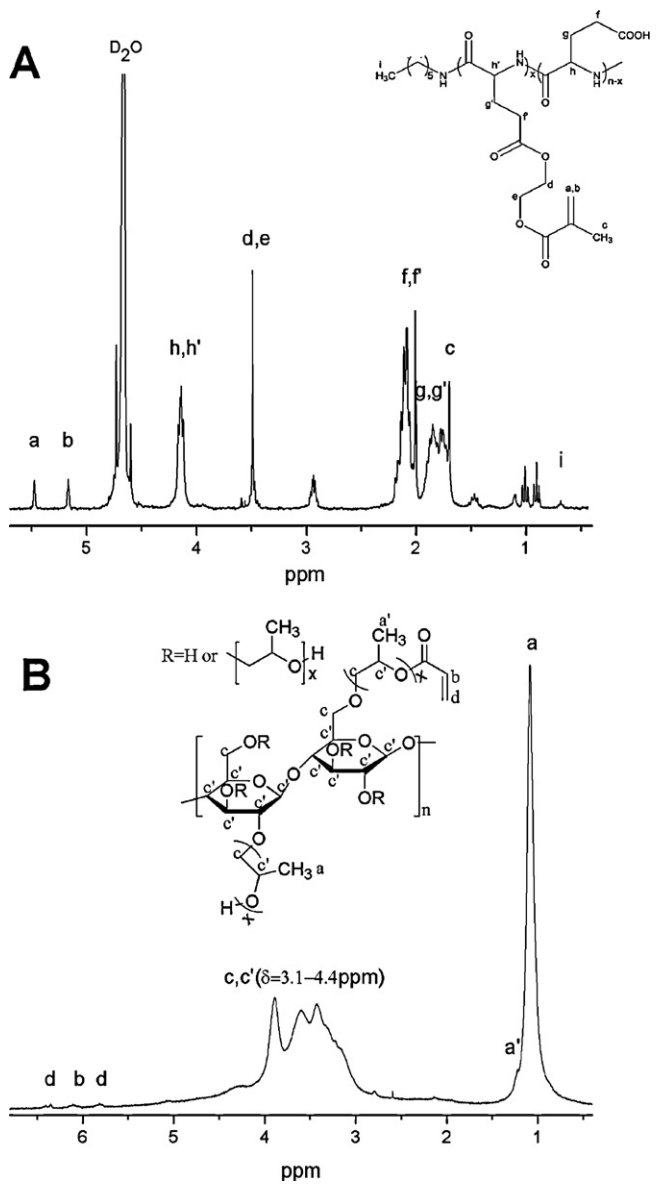


Fig. 1. ^{1}H NMR spectra of PGH (A) in D_2O and HPC-AA (B) in $CDCl_3$.

an accelerator according to the procedures described in Scheme 3. A series of HPC-AA and PGH with various weight ratios ranging from 30% to 70% were used to prepare the microgels, which were marked as MG3/7, MG5/5, and MG7/3, respectively.

The FT-IR spectra of HPC, HPC-AA, PGA, PGH and MG5/5 are shown in Fig. 2. Comparing the IR spectrum of HPC-AA with that of HPC, it can be seen clearly that there is a new absorption appearing at 1720 cm^{-1} (ν_{CO}), which can be assigned to the carbonyl group to confirm the formation of the acryloyl groups. The characteristic absorption of the amide group at 1656 cm^{-1} (amide I) and 1547 cm^{-1} (amide II) from the PGH and the hydroxyl from HPC at about 3459 cm^{-1} (ν_{OH}) of the microgels are also observed in the FT-IR spectrum of MG5/5. These results clearly confirm that the microgels are prepared successfully.

The contents of PGH (in weight percent) in the microgels are estimated from the elemental analysis results according to the following equation:

$$\text{PGH wt\%} = \frac{R}{14} * (129 * 90\% + 241 * 10\%) = \frac{R}{14} * 140 \quad (3)$$

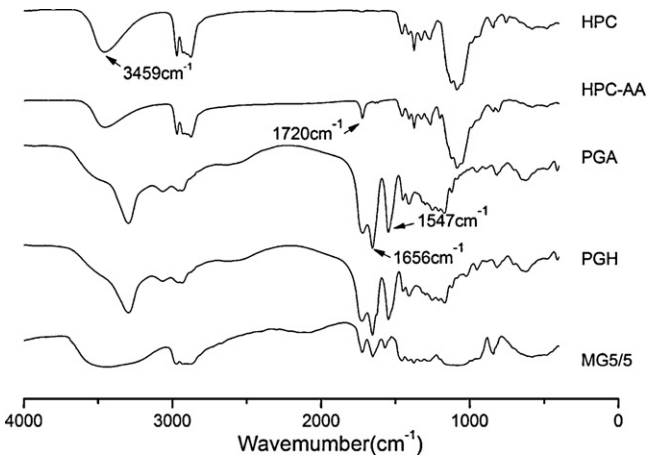


Fig. 2. FT-IR spectra of HPC, HPC-AA, PGA, PGH and MG 5/5.

where R is the weight percentage of nitrogen in the microgels determined by elemental analysis and 129*90% + 241*10% = 140 is the averaged molecular weight of the PGH units, assuming 10 wt% of HEMA coupling. As shown in Table 1, the PGH contents in the microgels increase in the order of the feeding ratios.

3.2. pH-triggered swelling of the microgel particles

Fig. 3 illustrates the pH-responsive swelling of the particles in solution as a function of pH at a constant temperature of 25 °C. As expected, the hydrodynamic radii of the microgel particles increase with an increase in pH value. This is ascribed to the transformation of PGA from the hydrophobic protonated form to the hydrophilic deprotonated form near its pKa. When the pH value is below the pKa of PGA, the size of microgels remains nearly constant due to the strong hydrogen bonding between the protonated PGA segments and HPC segments. When the pH value is above the pKa, the —COOH groups of PGA chains gradually dissociate, which cannot only weaken the hydrogen bonding between the HPC and PGA chains, but also induce the Coulombic repulsion among the ionized —COO^- groups, causing the gradual increase in the size of the microgels.

3.3. Effects of temperature and pH on the microgels

To investigate the hydrodynamic radius and their distribution of the obtained microgels, 0.1 mg/ml of the microgel solution at

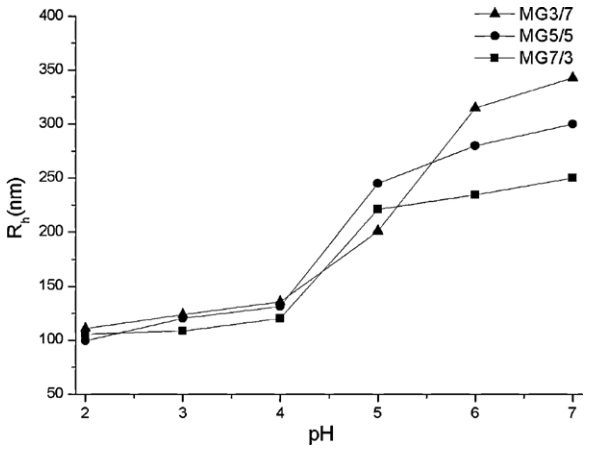


Fig. 3. Plot of hydrodynamic radius of the microgels as a function of pH value at 25 °C.

Table 1
Feed compositions of microgels.

Samples	Feed compositions							Measured PGH content[a] (wt%)
	HPC-AA (mg)	PGH (mg)	APS (mg)	TEMED (μl)	PB (ml)	Oil (ml)	PGH (wt%)	
MG3/7	15	35	5	5	5	50	70	73.9
MG5/5	25	25	5	5	5	50	50	50.5
MG7/3	35	15	5	5	5	50	30	29.8

[a] Estimated from the elemental analysis results.

pH 4.92 and 7.38 was tested by DLS, respectively. The measurement was carried out at 25, 35 and 60 °C, respectively. The effects of temperature and pH value on particle size are shown in Fig. 4. At a given pH value, no matter pH 4.92 or pH 7.38, the maximum particle size decreases with increasing temperature, obviously due to the temperature-dependence of the HPC segments. At a given temperature, the maximum particle size is always smaller at pH 4.92 than at 7.38 due to the pH-dependence of the ionization of PGA COOHs. As far as particle size distribution is concerned, the microgels exhibit a relatively narrow size distribution at a lower pH or at a higher temperature. Under these two conditions, smaller maximum or average particle size is observed. All results above indicate that the microgels have an excellent pH- and temperature sensitivity and can potentially be used to control the release of drug by changing the temperature and the pH values. SEM was also used to observe the morphology of the microgels. As shown in the inset of Fig. 4, the microgel exhibits well-defined and spherical morphology, consistent with the DLS measurement.

3.4. Phase transition behavior of microgels

The phase transition behavior of the microgels was characterized by monitoring the turbidity of the microgels as a function of temperature (Zhao et al., 2011). Fig. 5A shows transmittance percentage *versus* temperature of the microgel solutions at pH 6.81. It is clear that the LCST of the microgels increases with the weight percentage of PGH. The hydrogen bonding between the HPC chains and water molecules is a decisive factor to maintain the microgels in the solution state below LCST. The hydrogen bonds are destroyed above LCST and the hydrophobic interaction of the polymer chains becomes predominant, which leads to the collapse and phase separation. Because PGH itself is hydrophilic, its incorporation into the microgels would move the transition temperature to a higher temperature. Moreover, the transition temperature range becomes broader with the increase of PGH because of its hydrophilic nature.

Because the hydrophilicity of PGA can be modulated by pH variation, the phase transition behavior is expected to show pH-dependence. Fig. 5B illustrates the phase transition behavior of MG5/5 at pH 4.92, 5.29, and 6.81, respectively. The LCST is 42.8, 43.3 and 45.6 °C, respectively. This is because at a higher pH, PGA is more hydrophilic.

3.5. Insulin load and in vitro release

The above results show that the microgels system composed of crosslinked HPC-PGH exhibits both pH- and thermo-sensitivity, and thus, it is expected to protect a hydrophilic drug against the acidic environment of stomach and release it under the intestine condition.

Insulin was selected as a model drug to demonstrate the pH-regulated drug release. The insulin release behavior from the microgels was investigated *in vitro* in artificial gastric juice (pH = 1.2) and artificial intestinal liquid (pH = 6.8) at 37 °C, respectively. As shown in Fig. 6A, the release profiles of insulin from the microgels in artificial intestinal liquid (pH = 6.8) includes three stages. In the initial stage there is an obvious burst release (*ca.* 49–54%) in about 25 min. This burst release may be attributed to the release of insulin molecules located at the microgel surface. When the dialysis tube is placed into the artificial intestinal liquid, these surface drugs can be dissolved immediately, which leads to the burst release. In the second stage (sustained release, *ca.* 8–10%) in about 425 min the microgels serve as diffusion barriers and the drug is mainly released by the swelling-controlled diffusion mechanism, so the insulin molecules come out at a relatively slow speed. In the last stage little insulin is released and there seems to be an equilibrium between the microgels and the release medium. This is due to the reduced drug concentration difference between the microgels and the surrounding medium on one hand, and due to the interaction between the remaining insulin and the microgels network on the other hand, which prevents the release of the drugs.

It is also observed in Fig. 6A that the release rate is PGH-content dependent, *i.e.*, the insulin is released at a rate in the order of MG3/7 >MG5/5 > MG7/3. This is because at pH 6.8, PGA is highly ionized

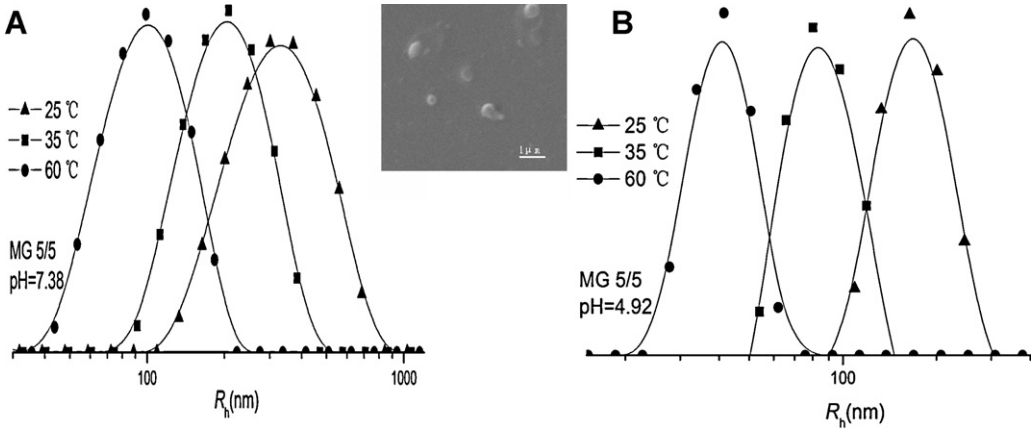


Fig. 4. Hydrodynamic radius distributions for MG5/5 in aqueous solution of pH 7.38 and pH 4.92 at 25, 35 and 60 °C, respectively. SEM image of MG5/5 is in the inset.

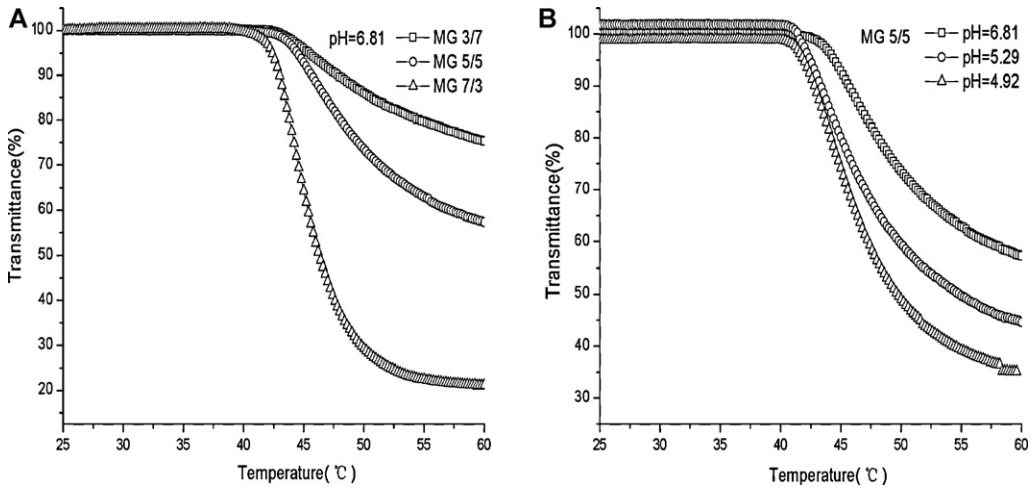


Fig. 5. Effect of temperature on the phase transition behavior of the microgels at pH 6.81 (A) and MG5/5 at different pH value (B).

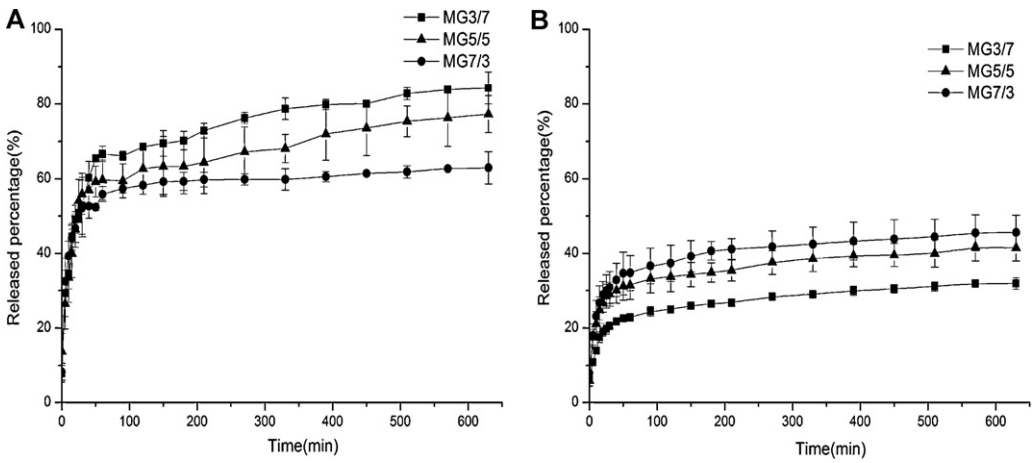


Fig. 6. Cumulative release of insulin from the microgels at pH 6.8 (A) and pH 1.2 (B) as a function of time at 37 °C.

and the microgels are in the swollen state. The more the PGH content, the more swollen the microgels and the fast the insulin release.

Fig. 6B shows the release profiles of insulin from the microgels in artificial gastric juice (pH = 1.2). Different from Fig. 6A, all samples show very slow release of insulin. This is because at pH 1.2, all carboxyl groups in PGA are in COOH state, strong H-bonding and hydrophobic interaction in the microgels result in shrinkage of the microgels. In other words, pH can trigger the release of insulin from the microgels. Specifically, little insulin can be released at pH 1.2 whereas it can be released rapidly at pH 6.8. Therefore, the microgel system of crosslinked HPC-PGH can serve as an oral delivery system for insulin.

It is noticed that the microgel composition shows different influences on release rate in Fig. 6B and A. As stated earlier, the release rate has the order of MG3/7 > MG5/5 > MG7/3 in Fig. 6A, but in Fig. 6B, this order is MG3/7 < MG5/5 < MG7/3. This completely opposite influence arises from the great changes of PGA from pH 6.8 to pH 1.2 as mentioned above, which indicates that the MG3/7 microgels might be the best candidate for oral insulin delivery among the three formulas examined.

4. Conclusion

A series of dual stimuli-responsive microgels composed of temperature-sensitive HPC and pH-sensitive PGH were prepared by free radical emulsion polymerization. The radius of the microgels reversibly increased with pH value increasing. The microgels showed more excellent pH sensitivity and a higher swelling ratio at a higher pH value. The *in vitro* release data of insulin from the microgels demonstrated that the drug release rate at pH 1.2 was much slower than that at pH 6.8 due to the protonation of the PGA part in an acidic condition. The results showed the possibility of using such pH- and temperature-sensitive microgels as intelligent oral drug delivery systems.

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