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Polyelectrolyte complex nanoparticles of amino poly(glycerol methacrylate)s and insulin

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ABSTRACT

Amino poly(glycerol methacrylate)s (PGOHMAs) were synthesized from linear or star-shaped poly(glycidyl methacrylate)s (PGMA)s via ring opening reactions with 1,2-ethanediamine, 1,4-butanediamine and diethylenetriamine, respectively. The resulting cationic polymers were employed to form polyelectrolyte complexes (PECs) with insulin. Parameters influencing complex formation were investigated by dynamic light scattering (DLS). PECs in the size range of 100–200 nm were obtained under optimal conditions, i.e., the pH value of PECs was 5.58–6.27, the concentration of NaCl was 0.02 mol/L, and insulin–polymer weight ratio was 0.8. The insulin association efficiency (AE) of current system increased with zeta potentials of PECs. Circular dichroism (CD) analysis corroborated that the structure of insulin in the PEC nanoparticles was preserved after lyophilization. Fourier transform infrared (FT-IR) and X-ray diffraction (XRD) experiments demonstrated that weak physical interactions between insulin and amino PGOHMAs play an important role in the formation of PECs. The release of insulin depends on both structure and architecture of amino PGOHMAs. These PECs would be potentially useful for mucosal administration.

1. Introduction

In the past two decades, polymeric nanoparticles have been investigated as carriers for hydrophilic macromolecular drugs such as peptides, proteins, vaccines, and DNAs to improve drug stability and permit drug administration through nonparenteral routes (Tobio et al., 1998). Self-assembly of natural or synthetic polyelectrolytes to form complexes with drug candidates has drawn increasing attention (Mao et al., 2001). Proteins interact strongly with both synthetic and natural polyelectrolytes. Polyanions or polycations could complex proteins below or above their isoelectric points. There are a wide variety of techniques available for producing nanoparticles including solvent evaporation, interfacial polymerization and emulsion polymerization methods. Most of these approaches, however, involve the use of organic solvents, heat or vigorous agitation, and some procedures which are potentially harmful to the structure and consequently biological activity of proteins (Guerrero et al., 1988; Kreuter, 1988). However, polymeric nanoparticles formed under mild processing conditions without heat process or organic solvents could maintain the stability of proteins (Gao et al., 2005, 2006). A number of studies have dealt with the formation of protein polyelectrolyte complexes (PPECs) in aqueous salt-free and salt-containing systems under different pH conditions (Izumi et al., 1994; Kokufuta, 1993).

Diabetes mellitus is by far the most common metabolic disorder. Insulin is the most effective and durable drug in the treatment of advanced stage diabetes (Dotsikas and Loukas, 2002). The administration of insulin in the form of bolus subcutaneous injection has been the basis of insulin therapy since its introduction. However, this feature is central to the problem of glycemic control since the pharmacokinetics of conventional insulin preparations given by this route makes it virtually impossible to replicate the normal pattern of nutrient related and basal insulin secretion (Binder et al., 1984; Galloway et al., 1981; Lauritzen et al., 1979). Numerous attempts to deliver insulin by application routes avoiding injections have been reported in the literature. Peroral, pulmonary, and nasal insulin administrations fall into this category, but enzymatic lability and inefficient transport across mucosae provide formidable challenges and lead to low and variable bioavailabilities (Pillai and Panchagnula, 2001). Intranasal drug

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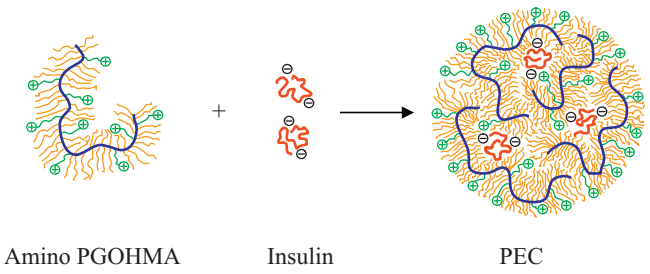

Fig. 1. Schematic formation of insulin-amino PGOHMA PEC.

delivery is a convenient and reliable method possessing many advantages, such as large absorptive surface area and high vascularity of nasal mucosa, where drugs absorbed from the nasal cavity pass directly into the systemic circulation, thereby avoiding first-pass liver metabolism (Chein and Chang, 1987). Nasal insulin absorption is low without co-administration of absorption enhancers which open the epithelial barrier and prolong the residence time in the nasal cavity (Dondeti et al., 1996; Morimoto et al., 1995; Verhoef and Merkus, 1994). Intranasal administration of cationic nanoparticles could enhance nasal absorption of insulin (Behl et al., 1998; Fernandez-Urrusuno et al., 1999; Illum et al., 1994). Svedhem et al. established surface sensitive analytical techniques to study the possible interaction with a charged interface (Edvardsson et al., 2009). Recently, they studied the structural rearrangements of polymeric insulin-loaded nanoparticles interacting with surface-supported model lipid membranes, and revealed that the low surface coverage of nanoparticles is probably due to structural rearrangements of the nanoparticles occurring at or just after the adsorption event. The driving force for such rearrangement is most likely dominated by electrostatic interactions between the components of the nanoparticles (polyelectrolyte and human insulin) and the fluid membrane (Frost et al., 2011).

PECs were resulted from strong electrostatic interactions between charged microdomains of at least two oppositely charged polyelectrolytes. Polymers like chitosan (Chit), alginate (Alg) and dextran sulfate (DS), as well as cyclodextrin derivatives as biocompatible, biodegradable and mucoadhesive materials have been used to form PECs (Gombotz and Wee, 1998; Huang et al., 2009; Ilium, 1998; Tiyaboonchai et al., 2003; Zhang et al., 2010). The criteria for acceptance of PEC systems for biological testing were hydrodynamic diameter statistically less than or equal to 200 nm (Carlesso et al., 2005; Panyam and Labhasetwar, 2003), with surface charge >|±30 mV| (Chern et al., 2004; Fatouros et al., 2005). However, the zeta potential of the PECs reported in the literature is usually below |±20 mV| (Mao et al., 2006; Zhang et al., 2010; Sarmento et al., 2006) and the partical size is over 300 nm (Mao et al., 2006).

Amino poly(glycerol methacrylate) (PGOHMA) is a well-known polymer in both industrial and biomedical applications because it is reactive, inexpensive, hydrophilic, biocompatible, and nontoxic (Horák, 2001). In our present study, 1,2-ethanediamine, 1,4-butanediamine and diethylenetriamine were used to modify poly(glycidyl methacrylate)s (PGMA)s to obtain cationic polymers. The purpose of this investigation is to evaluate the feasibility of polyelectrolyte complexes (PECs) formation between insulin and various amino PGOHMAs by self-assembly through electrostatic interaction and the formulation is shown in Fig. 1. Dynamic light scattering (DLS) was employed to study the properties of PECs of insulin and amino PGOHMAs. Various factors influencing the process were investigated in detail. Further studies with respect to possibly improved mucoadhesive properties are under progress.

2. Materials and methods

2.1. Materials

Glycidyl methacrylate (GMA), 2-bromoisobutyryl bromide, bipyridyl and Cu(I)Br were purchased from Shanghai Adamas Reagent Co., Ltd. (Shanghai, China). 1,4-Butanediamine were purchased from J&K Co., Ltd. (Beijing, China). 1,2-Ethanediamine, diethylenetriamine and acetonitrile were obtained from Tianjin Chemical Reagent Co. (Tianjin, China). Linear poly(glycidyl methacrylate) (L-PGMA) and eight-arm PGMA (S8-PGMA) were synthesized by atom transfer radical polymerization (ATRP) according to our previously reported methods (Gao et al., 2007). The PGMAs were then modified with different amines according to our previous report (Gao et al., 2010). Briefly, three kinds of aliphatic amines, 1,2-ethanediamine, 1,4-butanediamine and diethylenetriamine were added in excess to PGMA, respectively, to give PGOHMAs with different amines by ring-opening of epoxide groups. The obtained polymers were named as **L-E** and **S8-E** (E represents 1,2-ethanediamine), **L-B** and **S8-B** (B represents 1,4-butanediamine), **L-D** and **S8-D** (D represents diethylenetriamine). All other reagents were obtained from Tianjin Chemical Reagent Co. (Tianjin, China) and used as received, except for tetrahydrofuran (THF), which was dried over potassium hydroxide, distilled over sodium, and finally distilled over sodium benzophenone ketyl prior to use.

2.2. Characterization of amino PGOHMAs

All ^{1}H NMR spectra were recorded on a Bruker AV-400 spectrometer (400 MHz, Bruker, Freemont, CA). Samples were dissolved in deuterated chloroform, or deuterated water. Gel permeation chromatography (GPC) measurements were performed in water. Adequate molecular weight separation was achieved using three Waters Styragel columns (HT3, HT4, and HT5) in series at a flow rate of 1.0 mL/min at 35 °C. Calibration curves were obtained with nearly monodisperse polystyrene. The samples were measured with an elemental analysis instrument (elementar vario EL, GER) to determine the molar content of amino groups per gram of polymer.

In order to get the pK_a value of amino PGOHMAs, polymers were dissolved or suspended in double distilled water. Aqueous HCl solution (0.1 mol/L) was used to adjust the pH to 2. The polymer solution was stirred for 24 h to insure complete dissolution, followed by titration with aqueous NaOH solution (0.05 mol/L) from pH 2.0 to 12.0 with 3-min interval between two dosages for equilibrium. The ionization degree α of the amine groups in the copolymer was calculated as $\alpha = ([\text{basic}] - [OH^-] + [H^+])/C_{NH}$, where [basic], $[OH^-]$ and $[H^+]$ were the molarity of the added NaOH for titration, free hydroxide ion and hydrogen ion, respectively, and C_{NH} is the total molar concentration of the amino groups in molarity. The pH at $\alpha = 0.5$ is considered as the apparent dissociation constant pK_a of the PGMA derivatives.

2.3. Preparation of insulin nanocomplexes

Insulin solution (1 mg/mL) was prepared in two steps. The insulin powder was first dissolved in 87% (v/v) of 0.01 M HCl, followed by dilution with 13% (v/v) of 0.1 M tris-(hydroxymethyl) aminomethane solution (ionic strength $I = 0.01$, pH = 7.40). Polymer solutions of appropriate concentration were prepared by dissolving the dry polymer powders in 0.25% acetic acid solution under stirring followed by adjusting the pH to 5.5 with 1 M NaOH. Insulin and the polymers are negatively and positively charged, respectively, under this condition. The polymer solution was added to an equal volume of insulin solution in a glass vial under gentle

magnetic stirring. The mixture was then stirred for another 10 min at room temperature to obtain amino PGOHMA–insulin PECs.

2.4. Characterization of polymer–insulin complexes

2.4.1. Dynamic light scattering (DLS)

The size and zeta potential of the complexes were measured with a Zetasizer Nano ZS90 (Malvern Instruments, Southborough, MA). Scattering light was detected at 90° angle through a 50 μm pin hole at 25 °C. Scattering intensity, in the unit of kilo counts per second (kcps), was noted during the measurement.

2.4.1.1. Effect of the pH value of insulin solutions on the properties of complexes. Insulin solutions (1 mg/mL, C_{NaCl} = 0.02 mol/mL) of different pH values (pH = 7.0, 8.0 and 9.0) were added to equal volumes (1 mL) of various concentrations of **S8-D** solutions (pH 5.5, C_{NaCl} = 0.02 mol/mL) under gentle magnetic stirring.

2.4.1.2. Effect of ionic strength on the properties of complexes. S8-D solution (1 mg/mL, pH 5.5) was mixed with insulin solution (1 mg/mL, pH 8) with NaCl concentrations at 0, 0.02, 0.06, and 0.1 mol/L, respectively.

2.4.1.3. Insulin titration. S8-D solution (1 mg/mL, pH 5.5, C_{NaCl} = 0.02 mol/mL) was titrated with insulin solution (1 mg/mL, pH = 8, C_{NaCl} = 0.02 mol/mL) under mild stirring.

2.4.2. Lyophilization

Samples were frozen at −30 °C in culture dish. Freeze-drying was performed using the freeze dryer Beta 1 (Christ, Osterode, Germany). Samples were dried for 48 h at a working pressure of 0.07 mbar corresponding to a condenser temperature of −46 °C.

2.4.3. Insulin association efficiency (AE)

The PECs were prepared by mixing amino PGOHMA solution (1 mg/mL, pH 5.5, C_{NaCl} = 0.02 mol/mL) with insulin solution (1 mg/mL, pH = 8, C_{NaCl} = 0.02 mol/mL) under mild stirring. The amount of insulin associated with the nanoparticles was calculated by the difference between the total amount used to prepare the particles and the amount of insulin present in the aqueous phase after centrifugation. PECs were centrifuged at 15,000 rpm for 30 min at 4 °C. The amount of free insulin in the supernatant was measured by a method in which insulin was determined from the linear function of UV absorption band at 277 nm employing a calibration curve from 0.1 to 0.8 mg/mL of insulin at pH 7.4. The insulin AE was then calculated from the following equation:

$$\mathrm{AE} = \frac{\text{total amount of insulin} - \text{free insulin}}{\text{total amount of insulin}} \times 100\%$$

All samples were measured in triplicate and are reported as the mean value.

2.4.4. Fourier transform infrared (FT-IR) analysis

Amino PGOHMA–insulin complex was lyophilized for FT-IR (Bio-Rad FTS 6000) measurement. Samples were mixed with 300 mg of micronized KBr powder and compressed into discs at a force of 10 kN for 2 min using a manual tablet presser. Each spectrum was collected in the range of 4000–500 cm^{-1} at room temperature.

2.4.5. Far-UV circular dichroism analysis (Far-UV CD)

The freeze-dried sample and insulin were dissolved in hydrochloric acid solution (pH 2), respectively, as contrast. Potential changes in insulin structure after lyophilization were investigated using far-UV CD analysis. The CD spectra were obtained at room temperature on a Jasco J-715 Spectrophotometer (Japan). In the far-UV region CD spectra were recorded in a 0.01 cm cell from 250 to 200 nm, using a step size of 0.5 nm, a bandwidth of 1.5 nm, and an averaging time of 5 s, with the lamp housing purged with nitrogen to remove oxygen. For all spectra, an average of 5 scans was obtained.

Table 1
Characterization of amino PGOHMAs.

Polymer	Elemental N (%)[a]	Amination conversion (%)[a]	M_n (kDa)[b]	M_w/M[b]	p$K_{a,app}$[c]
L-E	10.89	77	15.6	1.21	9.1
L-B	11.47	95	17.8	1.34	7.9
L-D	13.13	77	18.3	1.25	6.8
S8-E	9.42	68	14.5	1.43	6.8
S8-B	9.79	76	15.7	1.24	7.6
S8-D	12.86	74	14.9	1.32	8.5

[a] Determined by elemental analysis.
[b] Determined by GPC.
[c] Determined by pH titration.

2.4.6. X-ray diffraction (XRD)

Crystallization properties of samples were determined by ARL X'TRA powder X-ray diffraction (XRD) system (Rigaku D/max 2500v/pc, Japan). 95% alcohol was used to remove residual salts. Samples were freeze-dried and pressed into films before measurement. X-ray generator was equipped with a rotating copper anode and nickel filter. All the complexes were scanned at 40 kV, 100 mA using Cu Kα_1 radiation (λ = 1.5406 Å) at the range of 3–50°.

2.4.7. Insulin in vitro release study

PECs (ins-**L-E**, ins-**S8-B**, ins-**S8-D**, ins-**S8-B**, ins represents insulin) were dispersed in 5 mL of phosphate buffered saline (PBS, pH = 6.8, 0.154 M NaCl) at 37 °C. At appropriate time intervals, a certain amount of release medium was taken and replaced by fresh medium. The insulin release was evaluated by measuring the free insulin by UV (as in Section 2.4.3) in the supernatant after centrifugation. The concentration of insulin released from the PECs was expressed as a percentage of the total insulin available and plotted as a function of time. The cumulative insulin release was calculated as:

$$\text{Cumulative insulin release}\,(\%) = \frac{M_t}{M_\infty}$$

where M_t is the amount of drug released from PECs at time t, and M_∞ is the amount of drug released from the PECs at time infinity. All the experiments were carried out in triplicate, and the data presented are the average of the three measurements.

3. Results and discussion

3.1. Polymer characterization

The polymers were characterized by elemental analysis, pH-titration, ^{1}H NMR, and FT-IR (Table 1, Figs. 2 and 4). FT-IR and NMR spectroscopy confirmed the transformation of epoxide to —OH and —NH addition. The amination conversion ranged between 74 and 95% as determined by elemental analysis. The steric hindrance of 8-PGOHMA caused a lower yield of amination. The aminated PGOHMA had an apparent pK_a comprised between 6.8 and 9.1, which was influenced by the chemical structure and the density of amino groups in the polymer.

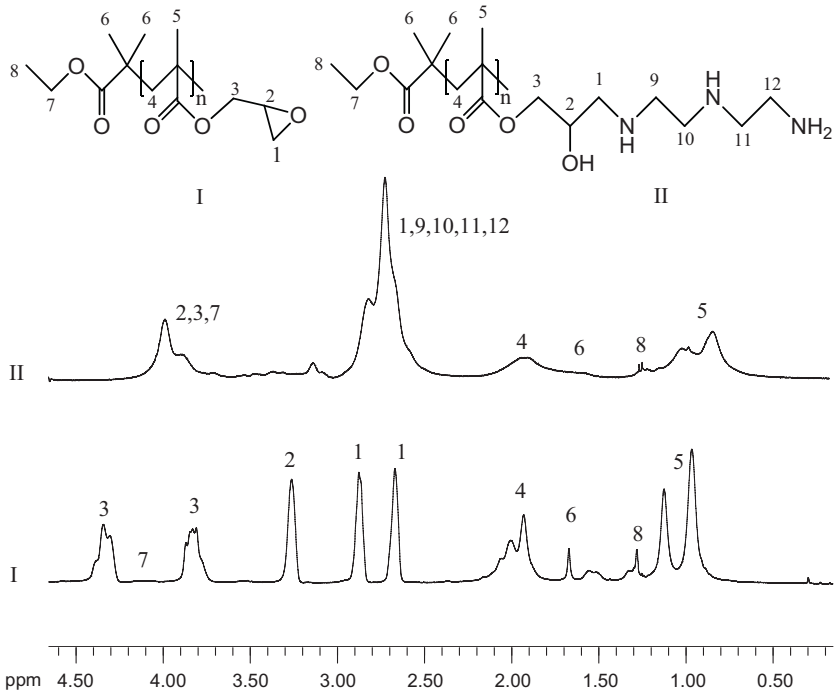

Fig. 2. ^{1}H NMR spectra of L-PGMA (I) in $CDCl_3$ and **L-D** (II) in D_2O (**L-D** is shown as a typical example).

3.2. *Effect of the pH value of insulin solution on the properties of PECs*

Fig. 3 shows the variation of PEC diameter as a function of the pH of insulin solution and the concentration of PGOHMA. The particle size of amino ins-**S8-D** PECs is dependent on both the pH of the insulin solution and the concentration of PGOHMA. The addition of electrolytes or change in pH affects the formation of PECs because complex formation between proteins and polyelectrolytes is primarily driven by coulombic interactions. The pHs of final systems were in the range of 5.58–5.72, 5.95–6.27, and 7.58–7.74 as the pHs of initial insulin solutions varied from 7 to 9, respectively. Larger particles were observed when the initial pH value of insulin was 9.0, compared with those prepared at pH 7.0 and 8.0. The amino groups in amino PGOHMA are less positively charged at higher pH. As a consequence, the interactions between amino PGOHMA and insulin are weakened. On the other hand, the complex is assumed a loop conformation in contrast to the compact ladder conformation at low pH (Mi et al., 1999). The desired particle sizes of insulin-PECs ranging from 100 to 200 nm could be formed when the amino PGOHMA concentration was in the range of 1.0–2.0 mg/mL, as well as the initial pH value of insulin solutions was 7 or 8.

3.3. *Effect of ionic strength of the medium on the properties of PECs*

The effect of salts on the course of PEC formation can hardly be predicted, because different processes may play an important role. Salts can cause secondary aggregation and flocculation, as well as a disintegration of the complexes (Dautzenberg and Rother, 2004). To elucidate the general tendencies of the influence of salts on PEC formation, we studied this process by DLS as a function of the ionic strength of the medium. In order to investigate the effect of salts on the amino PGOHMA-insulin association, the ionic strength of the solution was adjusted by adding sodium chloride. As can be seen in Fig. 4, a high level of aggregation was formed in deionized water and

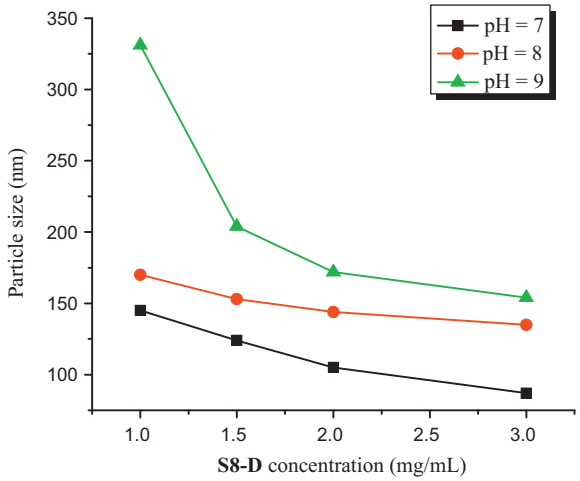

Fig. 3. Effect of initial pH of insulin solution and **S8-D** concentration on the particle size of the PEC.

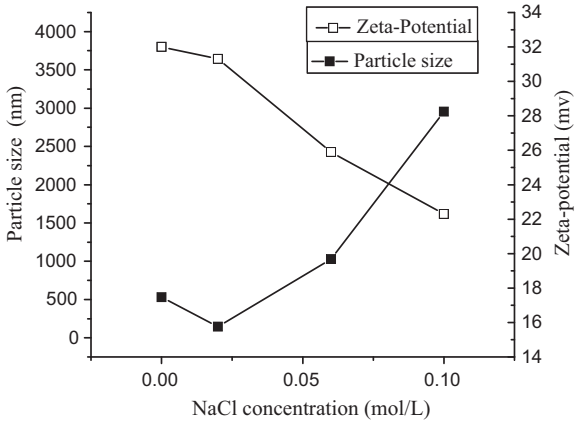

Fig. 4. Effect of NaCl concentration on the particle size and zeta-potential of the PEC.

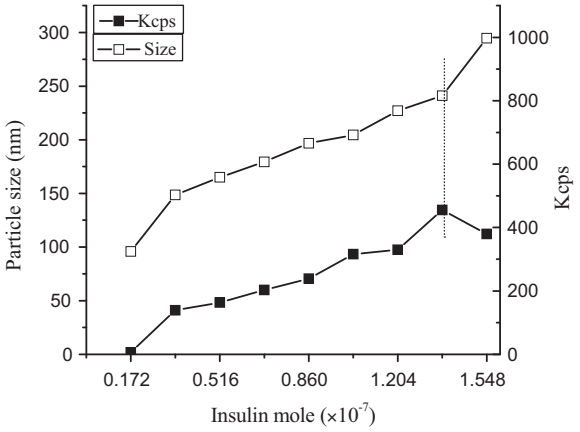

Fig. 5. Evolution of particle size and Kcps values of ins-**S8-D** complex versus insulin moles.

small amounts of salts decreased these levels drastically. This may be understood by the change of the polyelectrolyte conformation from a more or less stiff to a coiled structure due to the screening of the Coulomb repulsion along the charged chains of polymers (Dautzenberg et al., 1997). The particle size increased when the NaCl concentration was higher than 0.02 mol/L. That is because the repulsion between the shells is screened with the increase of ionic strength, then secondary aggregation could take place between the primary particles. The level of aggregation increased again. This explains the occurrence of a minimum in the ionic strength dependence.

3.4. Stoichiometric ratio of insulin and amino PGOHMAs in PECs

Polymer/insulin ratio influences both the degree of interactions and the properties of PECs. Herein, the stoichiometry was investigated by DLS. The optimal [polymer]/[insulin] charge ratio was calculated at the points which particle size began to increase dramatically and the Kcps values reached a plateau that indicates the endpoint of titration. During the initial stages of titration, the system pH was above the isoelectric point of insulin and the complex was weakly positively charged. Therefore, the size of PEC augmented with the increasing of insulin concentration after a critical point was reached, from where the particle size increased abruptly. It is noteworthy that the Kcps was inversely proportional to the quantity of insulin added. Larger PECs were formed at ≥optimal polymer/insulin ratio, whereas smaller complexes were obtained below this ratio.

The abrupt increase in particle size was accompanied by a slight Kcps decrease, implying either the redistribution of insulin in the polymer, or binding on the surface of polymer (Mao et al., 2006). Therefore, this critical point was selected to calculate the optimal ratio between insulin and amino PGOHMAs. The stoichiometric ratio observed for ins-**L-E** complex was 0.8 (w/w) (Fig. 5). The stoichiometric ratios observed for different combinations of components may be understood on the basis of a mass action law, assuming a different degree of dissociation between polyions and counter ions. PECs with excess polycation are more stable due to the repulsion of charged nanocomplexes in suspension.

3.5. Effect of molecular weight and structure of polymer on the properties of PECs

Zeta potentials and particle size of the PECs at optimal charge ratios were measured, and listed in Table 2. Both linear and star-shaped PGOHMA were tried to form complexes with insulin. No complexes could be observed from PGOHMAs indicating that the

Table 2
Characteristics of the PECs.

Polymer	Zeta-potential (mV)	Particle size (nm)	AE (%)
L-E	29.3	231.6	46.5
L-D	33.7	145.2	86.7
L-B	30.9	133.9	58.9
S8-E	32.1	150.4	51.6
S8-D	35.7	160.3	88.3
S8-B	32.5	143.1	70.1

introduction of positive charge on PGOHMA is the prerequisite of the formation of PECs. All of the formed PECs are positively charged without significant differences. The AE of PGOHMA with triamine is higher than that with diamine. The increased density of amino groups in the triamine-modified polymers could be probably attributed to the improved AE. The particle sizes of PECs were about 150 nm except that of ins-**L-E** complex was 231.6 nm. That may be due to the poor solubility of **L-E** in the final system with a pH of 5.95–6.27.

3.6. IR spectra analysis

FT-IR is a powerful technique to confirm the formation of PEC complex. FT-IR spectra of **L-B** as well as ins-**L-B** complex are recorded and shown in Fig. 6. The typical absorption peaks of insulin appear at 1655 and 1535 cm^{-1}. There was a significant difference between the physical mixture and the complex. The spectrum of the physical mixture shows the characteristic peaks of both insulin and **L-B**, in which the characteristic absorption peaks of insulin are still present at 1654 cm^{-1} and 1544 cm^{-1}. However, in the spectrum of their complex, the two characteristic absorption peaks of insulin were almost masked by that of **L-B**. The peak arised from C—O groups of polymers at 1163 cm^{-1} in the spectrum of **L-B** moves to 1078 cm^{-1} in the spectrum of the complex. Moreover, no new peak was observed in the mixture and complex. These observations suggest that some weak physical interactions between insulin and amino PGOHMA took place during the formation of the complex.

3.7. X-ray diffraction (XRD)

XRD was used to further demonstrate the interactions between insulin and amino PGOHMA. Fig. 7 shows the powder XRD patterns

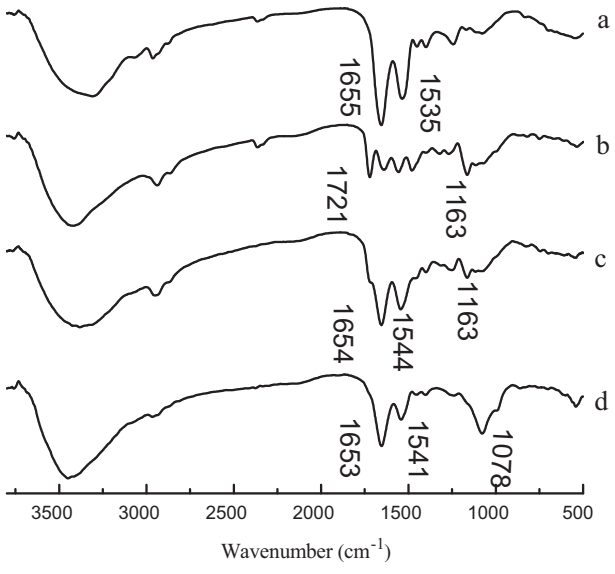

Fig. 6. Infrared spectra of (a) insulin, (b) **L-B**, (c) ins-**L-B** physical mixture and (d) ins-L-B complex.

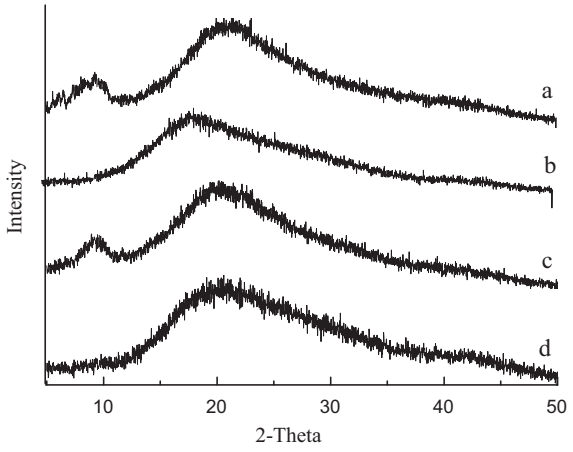

Fig. 7. X-ray diffraction patterns of (a) insulin, (b) **L-B**, (c) ins-**L-B** physical mixture, and (d) ins-**L-B** complex.

of insulin, **L-D**, their physical mixture, as well as their complex. The insulin powder diffraction pattern shown in Fig. 7(a) displayed partial sharp crystalline peaks, which are the characteristics of a macromolecule with some crystallinity. While **L-D** shown in Fig. 7(b) was amorphous lacking crystalline peaks. As seen in Fig. 7(c), some crystalline drug signal was still detectable in the physical mixtures of insulin and **L-D**. Compared with that of the physical mixture, the crystalline peaks had disappeared in the complex shown in Fig. 7(d). This suggested that insulin in the complex was either molecularly dispersed or amorphous.

3.8. Structural analysis of insulin

The secondary structure of protein molecules can be measured by CD spectroscopy. In order to investigate the feasibility of preparing PECs by lyophilization, the CD spectra of the original insulin and the insulin obtained from the dissolved ins-**L-D** complex were shown in Fig. 8. The spectrum of control insulin solution prepared freshly in the far UV region (200–250 nm) showed extrema at 209 and 222 nm corresponding to α-helix of insulin molecules. This spectrum is in close agreement with that of insulin obtained previously by other investigators (Ettinger and Timasheff, 1971; Wollmer et al., 1987; Wu and Yang, 1981). As shown in the spectrum, there are only minor differences which may be caused by the ionic interaction between insulin and polyelectrolyte applied in the

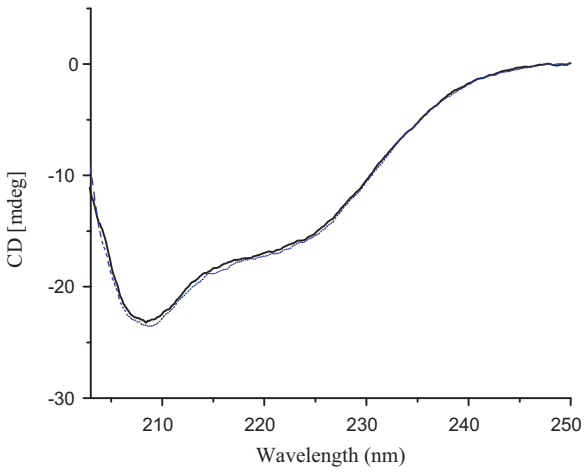

Fig. 8. Far-UV CD spectra of insulin (—) and insulin (– – –) obtained from the dissolved ins-**L-D** complex.

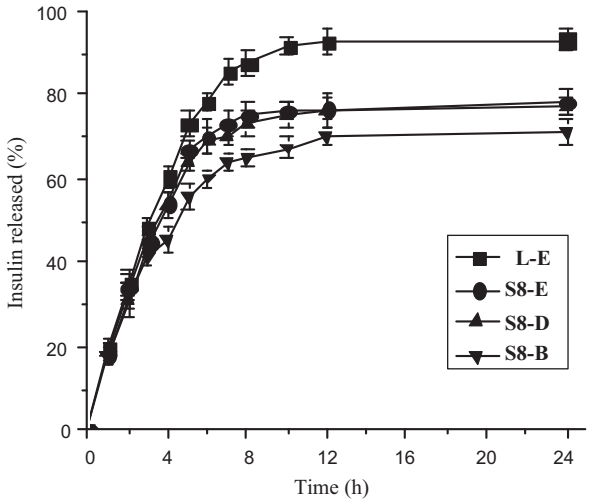

Fig. 9. The insulin in vitro release profile from PECs formed by different amion PGOHMA with insulin.

system in the complexes CD spectra (Sarmento et al., 2007). This suggests that the insulin-loaded **L-D** nanoparticles system can be lyophilized and recovered by dissolution, in a manner of preserving the structure and potentially maintaining the activity of insulin.

3.9. Insulin in vitro release study

In vitro drug release behaviors of PECs were studied at pH 6.8. As can be seen from Fig. 9, the insulin released from linear polymer **L-E** was about 90%. While the insulin released from the star-shaped polymer (**S8-D**, **S8-E** and **S8-B**) was about 75%. The strong interactions between insulin and 8-arm amino PHOHMAs, as well as the branching architecture may attribute to their lower release (Huang et al., 2009). In addition, the release of insulin from the ins-**S8-B** system was slower than that from **S8-D**, **S8-E**. Butyl side chain of **S8-B** is more hydrophobic and increases the stability of polymer aggregation, it took more time to release the cargo (Sun et al., 2011).

4. Conclusions

In this study, a series of amino PGOHMAs with different PGMA backbones (L or S8) were synthesized. The character of amino PGOHMAs and their molecular weights are controllable in polymerization process. The PEC formation process is influenced by a variety of parameters, including the system pH value, mass ratio of polymer/protein, polymer structure, and ionic strength. Amongst all of these parameters, the most important factor apparently was the system pH value. The nanoparticles with a size range from 100 to 200 nm and Zeta potentials about 30 mV could be produced and the AE could be obtained up to 86.7% under optimal conditions. The release of insulin was adjustable by variation of the structure of amino PGOHMA. These PECs systems show great promise as carriers for insulin and potentially other therapeutic polypeptides.

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