Available online at www.sciencedirect.com
ScienceDirect
International Journal of Pharmaceutics 329 (2007) 182–191
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Distribution, transition, adhesion and release of insulin loaded nanoparticles in the gut of rats

Ming-Guang Li [a], Wan-Liang Lu [a], Jian-Cheng Wang [a], Xuan Zhang [a], Xue-Qing Wang [a], Ai-Ping Zheng [a], Qiang Zhang [a,b,*]

[a] *School of Pharmaceutical Sciences, Peking University, Xueyuan Road 38, Beijing 100083, China*
[b] *The State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Xueyuan Road 38, Beijing 100083, China*

Received 5 April 2006; received in revised form 27 July 2006; accepted 20 August 2006
Available online 1 September 2006

Abstract

The purpose of this work was to investigate distribution, transition, bioadhesion and release behaviors of insulin loaded pH-sensitive nanoparticles in the gut of rats, as well as the effects of viscosity agent on them. Insulin was labeled with fluorescein isothiocyanate (FITC). The FITC-insulin solution and FITC-insulin nanoparticle aqueous dispersions with or without hydropropylmethylcellulose (HPMC, 0.2%, 0.4%, or 0.8% (w/v)) were orally administered to rats, respectively. The amounts of FITC-insulin in both the lumen content and the intestinal mucosa were quantified by a spectrofluorimeter. The release profiles in the gut were plotted by the percentages of FITC-insulin released versus time. FITC-insulin nanoparticle aqueous dispersion showed similar stomach but lower intestine empty rates, and enhanced intestinal mucosa adhesion in comparison with FITC-insulin solution. Addition of the HPMC reduced the stomach and intestine empty rates, enhanced the adhesion of FITC-insulin to the intestine mucosa. The release of FITC-insulin from nanoparticles in the gut showed an S-shape profile, and addition of HPMC prolonged the release half-life from 0.77 to 1.51 h. It was concluded that the behaviors of pH-sensitive nanoparticles tested in gastrointestinal tract of rats and the addition of HPMC were favorable to the absorption of the drug loaded.

Keywords: Insulin; Nanoparticles; Fluorescein isothiocyanate; Hydropropylmethylcellulose; Gastrointestinal distribution

1. Introduction

Numerous studies have been carried out in attempts to deliver therapeutic proteins and polypeptides like insulin by oral route (Shen, 2003), but the availabilities were far from satisfying due to several problems, such as acid-catalyzed degradation in the stomach, proteolytic breakdown in the gastrointestinal tract, and absorption through the intestinal epithelium (Saffran et al., 1997). Many attempts to overcome these barriers have been reported (Hamman et al., 2005), but less attention was paid on another oral absorption barrier of proteins and polypeptides: the rapid clearance from the site of absorption in the gastrointestinal tract (Lee, 1991). Two approaches may help for enhancement of the mucosa adhesion to extend the contact time: (1) encapsulation of the drug into colloidal carriers like nanoparticles, and modification of the surface properties of the carriers and (2) changing the physical properties like viscosity of the formulations. Limited works have been reported about enhanced mucosa adhesion of drug loaded nanoparticles (Sakum et al., 1999; Arbos et al., 2003), alteration of gastrointestinal distribution of small solid particles (~1 mm) by increasing the viscosity of the dosages (Mesiha and Sidhom, 1995), or improved availability by co-administration of viscosity agents (Harris et al., 1986; Gupta and Robinson, 1995). No available literature reported the effects of viscosity on the gastrointestinal distribution, transition, and mucosa adhesion of nanoparticles.

Nanoparticle drug carriers for oral delivery of proteins and polypeptides have attracted extensive attention in recent years (Soppimath et al., 2001). Among many types of nanoparticles prepared with various materials and approaches, pH-sensitive nanoparticles had shown some advantages (Sahoo et al., 1998; General and Thünemann, 2001; Naa et al., 2004). For orally administrated pH-sensitive nanoparticles, it is necessary to

* Corresponding author at: The State Key Laboratory of Natural and Biomimetic Drugs, Peking University, Xueyuan Road 38, Beijing 100083, China. Tel.: +86 10 82802791; fax: +86 10 82802791.
E-mail address: zqdodo@bjmu.edu.cn (Q. Zhang).

0378-5173/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2006.08.040

clarify the following questions: (1) whether encapsulation into pH-sensitive nanoparticles affects the distribution, transition, and adhesion to the mucosa of the drug in the gastrointestinal tract; (2) whether the physical characteristics of the formulations like viscosity have any effects on the distribution, transition, adhesion to the mucosa, and release pattern of the drug from the nanoparticles in the gastrointestinal tract; (3) whether the nanoparticles retain their pH-sensitive property, and what is their release pattern in the gastrointestinal tract. However, none of the reports of pH-sensitive nanoparticles covered these contents.

As nanoparticles are often used for oral delivery of proteins and polypeptides, insulin, a most commonly used polypeptide, was chosen in the present study as the model drug. Insulin is used in clinical therapy for treatment of type I diabetes mellitus, an insulin-dependent disease. Its structure, stability, and physicochemical characteristics have been extensively studied (Owens et al., 2001). For the in vitro and in vivo studies, insulin was labeled with fluorescein isothiocyanate (FITC), a commonly used fluorescent probe (Kim et al., 2004). FITC-insulin loaded pH-sensitive nanoparticles were developed using a novel procedure developed by our group previously (Li et al., 2006). In the procedure, the complex co-acervation method was applied, with chitosan and Eudragit L100-55 as the matrix materials. High viscosity grade HPMC was chosen in the present study as a viscosity agent because the viscosity of HPMC solution remains the same when the pH value changed, and relatively high viscosity value can be reached at low concentration.

The propose of this report was to study the distribution, transition, mucosa adhesion, and release behaviors of a newly developed pH-sensitive nanoparticle drug carrier loaded with FITC labeled insulin in the gut, and also the effects of viscosity of the formulations on them. These investigations will be certainly helpful for us to further understand the absorption mechanism of the nanoparticle formulations.

2. Materials and methods

2.1. Materials

Crystalline porcine zinc insulin (27.8 IU/mg) was purchased from Xüzhou Biochemical Company (People's Republic of China). (methacrylic acid-*co*-methyl methacrylate) copolymer (Eudragit L100-55, Mw 250,000) was gift from Röhm (Darmstadt, Germany). Fluorescein isothiocyanate (FITC) and Sephadex® G-25 were purchased from Sigma (St. Louis, MO, USA). Hydroxypropylmethylcellulose (HPMC) was Methocel grades K15M (Dow Chemical Co., Midland, MI, USA). Chitosan (non-salt form) was purchased from Qingdao Haihui Company (People's Republic of China). The molecular weight of chitosan is about 100,000 Da and the deacetylation degree is 95%. All other reagents were of analytical grade, except those for HPLC assay, which were of HPLC grade. Sprague–Dawley (SD) rats were obtained from Animal Center of Peking University Health Science Center. All of the animal experiments adhered to the principles of care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Peking University Health Science Center.

2.2. Methods

2.2.1. Preparation, purification and characterization of FITC-insulin

Reaction and purification conditions were a modification of previous reports (Bromer et al., 1967; Hentz et al., 1997). Briefly, FITC was dissolved in acetone (1 mg in 200 μl) and added dropwise to a 2.0 ml solution containing the appropriate amount of pork insulin dissolved in 0.1 mol/l phosphate buffer solution (PBS, pH 7.1) containing 0.2 mmol EDTA. Upon addition of FITC, the reaction vial was protected from light and allowed to mix at room temperature for 2 h. The mixture was then placed on a gel filtration column (20 cm × 1.5 cm) with a bed volume of 25 ml containing G25 Sephadex which pre-equilibrated with phosphate buffer (pH 7.1). After the appropriate fractions were collected, FITC-insulin were precipitated through adjustment of the pH to 4.5 with HCl and store at 4 °C for 24 h. The precipitation were centrifuged (5,000 × *g*, 15 min) and washed with a few milliliters of cold 0.01 mol/l ammonium acetate at pH 4.5 and then lyophilized to get crude FITC-insulin.

2.2.2. Preparation and purification of FITC-insulin-loaded nanoparticles

The pH-sensitive nanoparticles were prepared using the procedure developed previously by our group (Li et al., 2006). Briefly, an appropriate amount of FITC-insulin (2 mg) was dissolved in 0.4 ml hydrochloric acid (pH 2.5), and mixed with 4.0 ml of 0.2% (w/v) chitosan solution (pH 5.8). The above solution was then injected into 24.0 ml of 0.2% (w/v) Eudragit L100-55 solution (pH 5.8). During injection, the mixture was stirred at 500 rpm. The opalescent dispersion was immediately formed, and the obtained dispersion was filtered by paper filter.

Raw insulin nanoparticle dispersion was purified by discontinuous sucrose density gradient centrifugation, as reported previously (Mao et al., 2001). Briefly, 5 ml of 80% (w/v) sucrose, 5 ml of 50% (w/v) sucrose, and 5 ml of 35% (w/v) sucrose were gently put into one centrifuge tube, respectively. Finally, 20 ml of raw insulin nanoparticle dispersion was layered on the top of the sucrose solutions in the tube. The tube was then centrifuged at 100,000 × *g* for 30 min at 4 °C. After centrifugation, insulin nanoparticles were concentrated in the middle layer (50% sucrose). The middle layer was then collected, and dialyzed using dialysis tubing (14,000 Da) for 24 h in the dark against physiological saline (0.9% NaCl). The saline was changed every 8 h for three times. After dialysis, insulin nanoparticle dispersion in the tubing was collected, and stored at 4 °C.

2.2.3. Physiochemical characteristics and entrapment efficiency of the nanoparticles

Particle size and zeta potential of FITC-insulin nanoparticles were measured with Zetasizer (ZEN3600, Malvern Instruments, England). FITC-insulin entrapment efficiency in nanoparticles was measured as follows: a volume of 10 ml raw FITC-insulin nanoparticle dispersion was ultra centrifuged at 120,000 × *g*

for 30 min. The FITC-insulin in the supernatant was measured by fluorescence using a spectrofluorimeter with $\lambda_{excitation}$ 494 nm and $\lambda_{emission}$ 517 nm (RF-5301 PC, Shimadzu, Japan). Standard curve was prepared from zero to 1.2 μg/ml, the formula was expressed as: $A = 699.94C + 42.73$, $R^2 = 0.9991$, where A is the intensity of florescence and C is the concentration of FITC-insulin (μg/ml). FITC-insulin entrapment efficiency was expressed as the following equation: $EE\% = (W_t - W_s)/W_t$, where EE% is the entrapment efficiency, W_t the total weight of insulin added initially and W_s is the weight of insulin in the supernatants.

Simulated gastric fluid (pH 2.0, without enzymes) and simulated intestinal fluid (pH 6.8, without enzymes) were used as the release media and the ultracentrifuge method was adopted in the in vitro release study (Magenheim et al., 1993; Dai et al., 2004). Purified FITC-insulin nanoparticles were diluted with deionized water to reach a final concentration of 1.0 mg/ml FITC-insulin. Each release tube was filled with 3.0 ml of the release medium, and then 0.3 ml of appropriately diluted FITC-insulin nanoparticle dispersion was added. The release samples were shaken at a speed of 100 times per minute in an incubator (THZ-82B, Jintan Medical Equipment Co., Jangsu, China) at 37 °C in the dark. Eight tubes at one time were taken out from the shaking incubator (one tube for each Group) at 0.5, 1, 2, 4, and 6 h, and were ultra centrifuged at 120,000 × g for 30 min. The supernatants were deserted and the pellets were digested using 0.1 mol/l NaOH. The solutions were centrifuged at 16,000 × g for 30 min, and the FITC-insulin amounts in the supernatants were defined as the unreleased parts.

2.2.4. Gastrointestinal distribution, adhesion, and in vivo release

Male Wistar rats, average weight 225 ± 13 g were fasted overnight but allowing for free access to water. Rats were fed with 2 ml aqueous dispersion of the test formulations, containing 0.5 mg FITC-insulin (about 2.2 mg/kg body weight). The animals were sacrificed by cervical dislocation at 0.5, 1, 2, 4 and 6 h after administration. The abdominal cavity was opened and the gastrointestinal tract removed. Then, the gut was divided into four anatomical regions: stomach (Sto), small intestine (cut into six portions and termed as follows: I1, I2, I3, I4, I5, and I6), caecum (Ce) and colon (Co). Each segment was opened lengthwise and rinsed with either 10 ml (intestinal portions) or 40 ml (stomach, caecum and colon) of physiological saline (0.9% NaCl) in order to recover the non-adhered fraction. These rinsing liquids were centrifuged at 120,000 × g for 30 min, and FITC-insulin amounts in the supernatants were quantified and termed as free FITC-insulin. The pellets were digested in 1 ml 3 mol/l NaOH for 24 h, and centrifuged at 120,000 × g for 30 min. The FITC-insulin amounts in the supernatants were assayed and termed as unreleased FITC-insulin. The free and unreleased FITC-insulin was added together to get the total amounts of FITC-insulin in the lumen content, in order to estimate the gastrointestinal distribution and transition parameters.

Furthermore, each rinsed mucosa segment was cut into five portions (intestinal) or 10 portions (stomach, caecum and colon), and each portion was digested in 1 ml 3 mol/l NaOH for 24 h.

Table 1
The characteristics of FITC-insulin nanoparticles

Characteristics	FITC-insulin nanoparticles
Size (nm)	196.7 ± 5.70
Zeta potential (mV)	−29.51 ± 2.08
Entrapment efficiency (%)	72.57 ± 5.50
Drug loading (%, w/w)	3.10 ± 0.28

The data are expressed as the mean ± S.D., and each experiment was repeated for triplicates.

The samples were then centrifuged at 120,000 × g for 30 min. Aliquots (0.5 ml) of the obtained supernatants were diluted with water (2.5 ml) and assayed for FITC-insulin content by spectrofluorimetry. These data enabled us to estimate the fraction of FITC-insulin adhered to the mucosa.

2.3. Data analysis

2.3.1. Transit parameters

The transition of FITC-insulin through the gastrointestinal tract was evaluated using a modification of the kinetic model designed by (Akiyama et al., 1995). It was assumed that nanoparticles were emptied, from the stomach to the intestine and from intestine to the caecum, following a zero-order kinetic. The gastric emptying rate (k_{ge}) was defined as the terminal elimination rate of the particles from the stomach to the intestine, while the intestinal emptying rate (k_{ie}) was that from the intestine to the caecum. Each rate constant was calculated with the pharmacokinetics software WinNonlin 1.0.

2.3.2. Bioadhesion parameters

For each nanoparticle formulation, the total amounts of FITC-insulin adhered to the intestinal tract was plotted versus time, and from these curves, the parameters of bioadhesion (Q_{max}, AUC_{adh}, k_{adh} and MTR_{adh}) were estimated (Arbos et al., 2002). Q_{max} was defined as the maximal amount of nanoparticles adhered to the intestine surface and is related to the capability of the material to develop adhesive interactions. The k_{adh} was the terminal elimination rate of the adhered fraction with

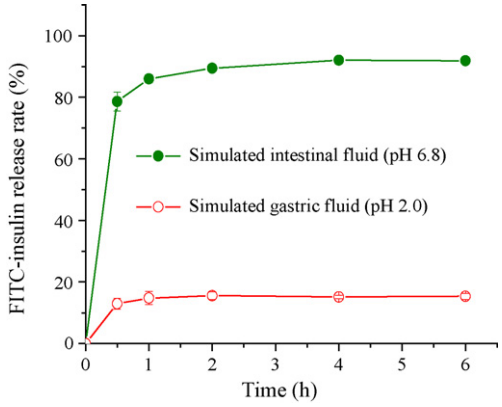

Fig. 1. In vitro release profiles of FITC-insulin nanoparticles in simulated intestinal fluid (pH 6.8) and simulated gastric fluid (pH 2.0), respectively. The data are expressed as the mean ± S.E.M, and each experiment was repeated for triplicates.

the gastrointestinal mucosa and calculated using the WinNonlin 1.0 software. AUC_{adh} (the area under the curve) of bioadhesion was evaluated by means of the trapezoidal rule up to t_z, which denoted the last sampling point, and permitted to quantify the intensity of the bioadhesive phenomenon. Finally, MTR_{adh} was the mean residence time of the adhered fraction of FITC-insulin to the mucosa and evaluated the relative duration of the adhesive interactions. MRT (h) = $AUMC_{adh}$ (μg h)/AUC_{adh} (μg h), where $AUMC_{adh}$ (area under the first moment curve) is approximated by the trapezoidal rule and extrapolated to infinity. However, AUC_{adh} was accepted if atleast 80% of its value was incorporated by AUC_{adh} $(0 - t_z)$.

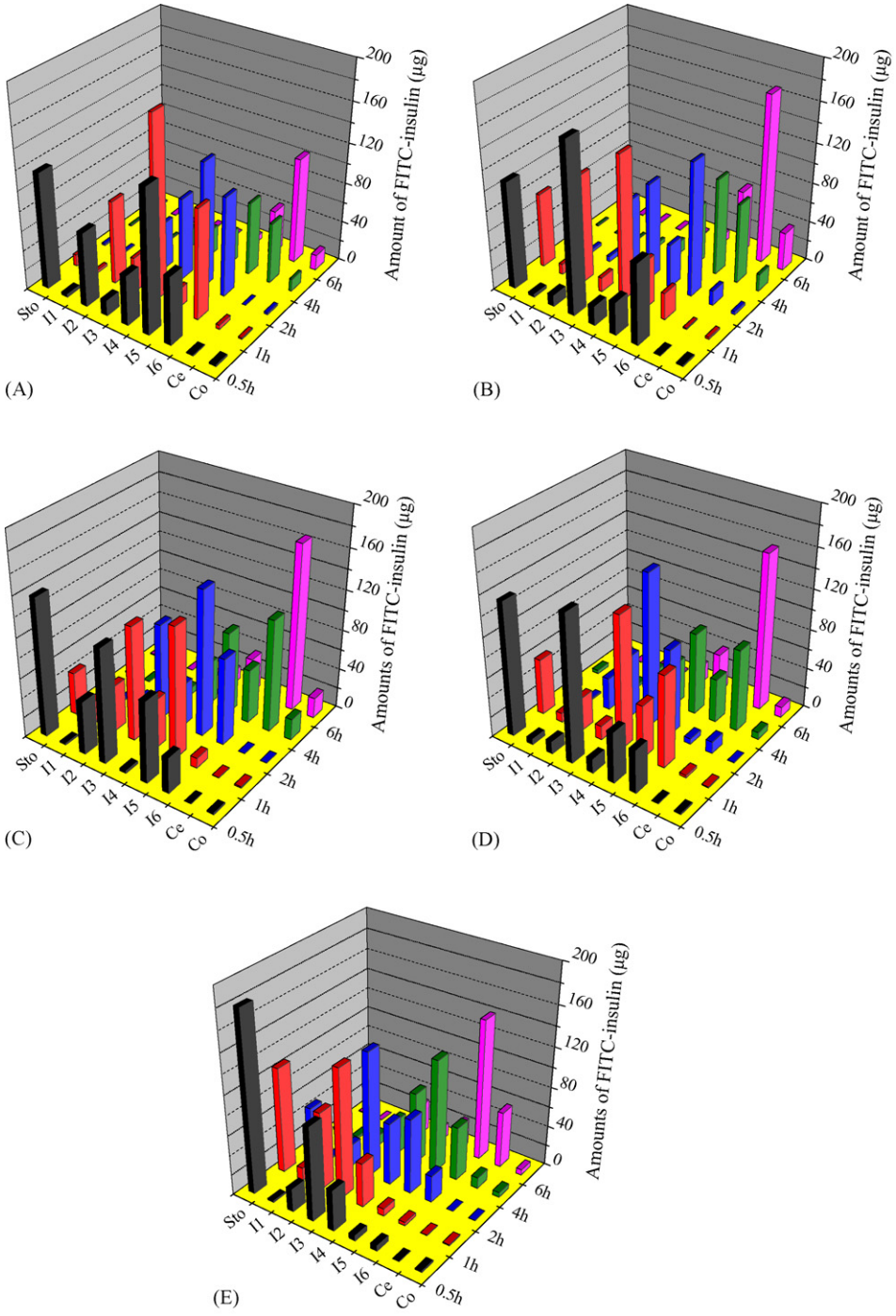

Fig. 2. Distribution of FITC-insulin in gastrointestinal tract (non-adhered fraction in the lumen), after the oral administration of 2 ml aqueous solution or nanoparticle dispersion containing 0.5 mg FITC-insulin. The gut was divided into four anatomical regions: stomach (Sto), small intestine (cut into six portions and termed as follows: I1, I2, I3, I4, I5, and I6), caecum (Ce) and colon (Co). Each experiment was repeated for triplicates. The standard deviations were left out in these plots for the sake of clarity. (A) FITC-insulin pH 7.4 PBS solution; (B) FITC-insulin nanoparticle dispersion; (C) FITC-insulin nanoparticle dispersion with 0.2% (w/v) HPMC; (D) FITC-insulin nanoparticle dispersion with 0.4% (w/v) HPMC; (E) FITC-insulin nanoparticle dispersion with 0.8% (w/v) HPMC.

2.3.3. The modeling of in vivo release profiles

FITC-insulin release rates from the pH-sensitive nanoparticles in the gut were calculated as follows: release rate (%) = $(W_i - W_u)/W_i \times 100$, where W_i is the amount of FITC-insulin administrated initially, and W_u is the unreleased FITC-insulin within the nanoparticles at a given time point. The release profiles of the FITC-insulin nanoparticles in the gut were plotted using the release rates versus time. The data of release profiles were modeled using the modified empirical equation for fitting sigmoidal curves, as described by (Cerny et al., 1981). This equation was illustrated by $y = (A_1 - A_2)/(1 + \exp((x - x_0)\,dx)) + A_2$, where x is the time, x_0 denotes the time at 50% release rate and dx represents time constant (initial value for operation takes 1); y denotes the percentage of FITC-insulin released; A_1 (initial value = 0 for operation) is the operation constant 1 associated with percentages of FITC-insulin released versus time profile, and A_2 (initial value = 100), the operation constant 2.

2.3.4. Statistical analysis

Parameters were analyzed to determine statistical significance. One-way analysis of variance (ANOVA) was used to determine significance among groups, after which post hoc tests with the Bonferroni correction were used for comparison between individual groups. A value of $p < 0.05$ was considered to be significant.

3. Results

3.1. Physiochemical characteristics of FITC-insulin nanoparticles

The size, zeta potential, entrapment efficiency and drug loading of FITC-insulin nanoparticles are listed in Table 1. The nanoparticles had a mean size of about 200 nm, and a negative surface charge of −30 mV. The entrapment efficiency was slightly higher than 70% and drug loading were about 3%. These data were quite close to those of nanoparticles prepared previously by our group using insulin instead of FITC-insulin as the model drug, indicating that the labeling of FITC did not obviously affect the characteristics of the nanoparticles.

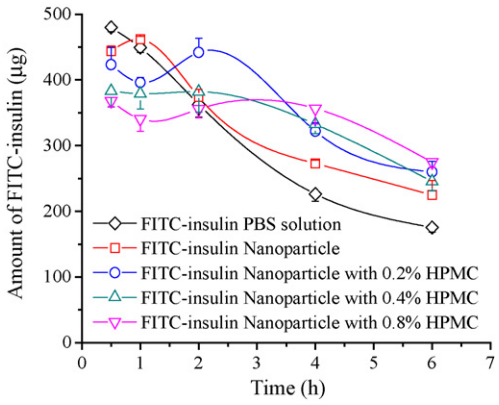

Fig. 3. The total amounts of FITC-insulin in the lumen content of the gastrointestinal tract after the oral administration of 2 ml aqueous solution or nanoparticle dispersion containing 0.5 mg FITC-insulin. Each experiment was repeated for triplicates.

The in vitro release profiles of FITC-insulin from the nanoparticles are illustrated in Fig. 1. It was found that the FITC-insulin nanoparticles showed a pH-sensitive property in the release media. Less than 10% of FITC-insulin released from the nanoparticles in simulated gastric fluid after 6 h, but in the simulated intestinal fluid, almost 90% of FITC-insulin released. It was also suggested that HPMC (0.2%, 0.4% or 0.8% (w/v)) had no obvious effect on the release profiles of the FITC-insulin nanoparticles in both release media (data not shown).

3.2. Distribution of FITC-insulin in the gastrointestinal tract

Fig. 2 illustrates the lumen contents of FITC-insulin (non-bioadhesion fraction including released and unreleased FITC-insulin) in the stomach, intestinal segments and caecum at 0.5, 1, 2, 4 and 6 h after the oral administration of the different formulations containing 0.5 mg FITC-insulin. For all the five formulations, the FITC-insulin remained in the stomach at 0.5 h was in the follow order: FITC-insulin (23.25%) < FITC-insulin nanoparticle (29.47%) < FITC-insulin with 0.2% (w/v) HPMC (37.52%) < FITC-insulin with 0.4% (w/v) HPMC (39.00%) < FITC-insulin with 0.8% (w/v) HPMC

Table 2
Kinetic parameters (gastric and intestinal emptying rates, coded as k_{ge} and k_{ie}) of FITC-insulin solution (pH 7.4 PBS) and FITC-insulin nanoparticle aqueous dispersion with various concentrations of HPMC in the gut

Formulation	k_{ge} (h^{-1})	k_{ie} (h^{-1})
FITC-insulin PBS solution (pH 7.4)	1.22 ± 0.07	0.89 ± 0.09
FITC-insulin nanoparticle dispersion with water	1.14 ± 0.05	0.56 ± 0.11[a]
FITC-insulin nanoparticles dispersion with 0.2% HPMC	1.12 ± 0.09	0.33 ± 0.06[a,b]
FITC-insulin nanoparticles dispersion with 0.4% HPMC	1.07 ± 0.06[a]	0.20 ± 0.05[a,b,c]
FITC-insulin nanoparticles dispersion with 0.8% HPMC	0.9 ± 0.11[a,b,c,d]	0.18 ± 0.04[a,b,c]

The data are expressed as the mean ± S.D., and each experiment was repeated for triplicates.
[a] $p < 0.05$ vs. FITC-insulin solution.
[b] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion.
[c] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.2% HPMC.
[d] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.4% HPMC.

(54.10%). The results indicated that both nanoparticles themselves and addition of HPMC increased the remaining time of FITC-insulin in the stomach.

Fig. 3 shows the evolution of total FITC-insulin in the lumen contents versus time. For FITC-insulin PBS solution, it was found that the amounts of FITC-insulin decreased rapidly to about 40% of the dose administrated initially up to 6 h. It seemed that encapsulation into nanoparticles slowed the elimination of FITC-insulin from the gastrointestinal tract. Addition of HPMC slowed the process further, but no obvious difference was observed among three nanoparticle formulations with various HPMC concentrations.

3.3. Transition of FITC-insulin in the gastrointestinal tract

The gastric (k_{ge}) and intestinal (k_{ie}) emptying rates of FITC-insulin or FITC-insulin nanoparticles are listed in Table 2. In comparison with FITC-insulin solution, FITC-insulin

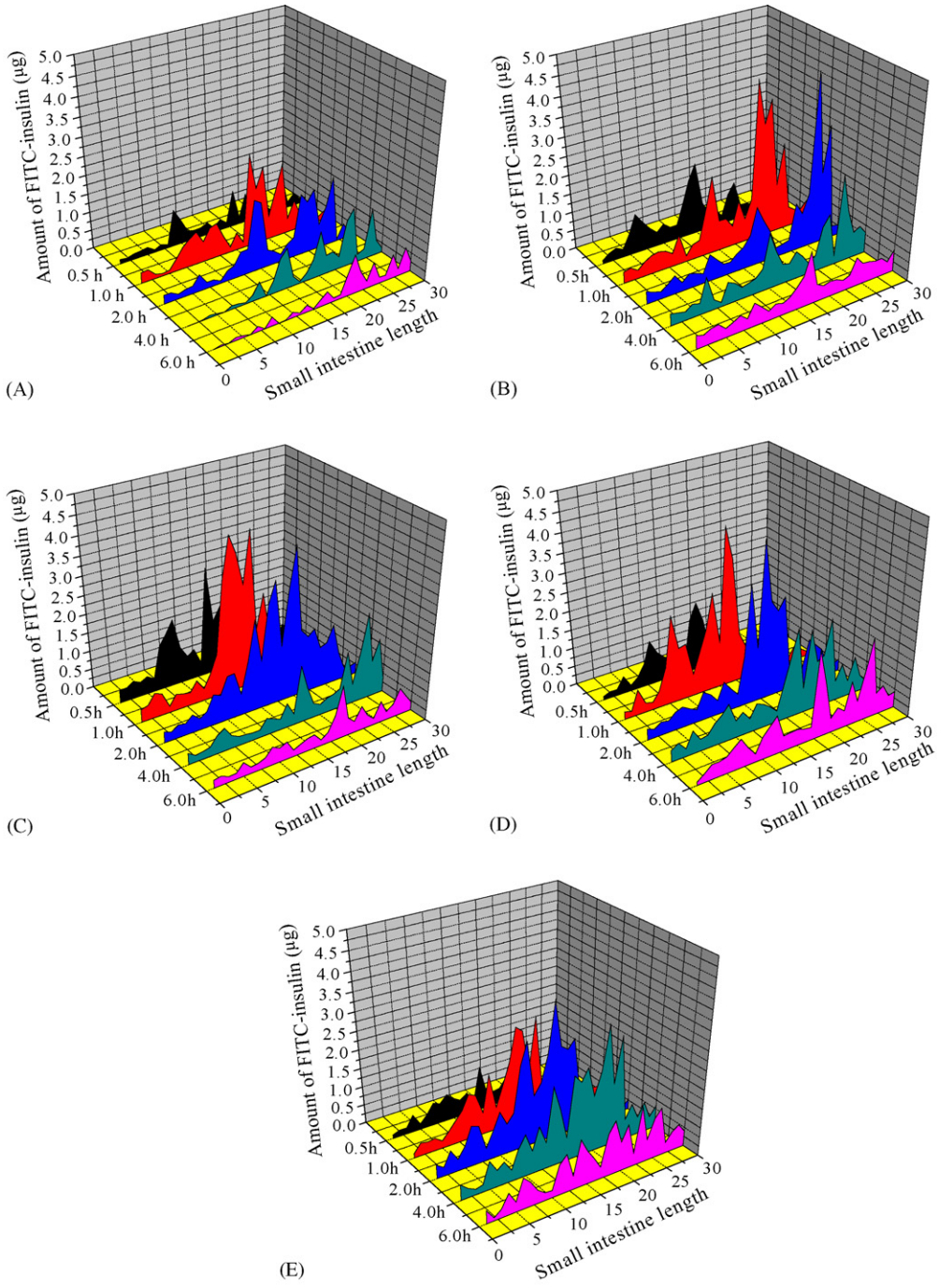

Fig. 4. Adhesion of FITC-insulin to the small intestine mucosa after the oral administration of 2 ml aqueous solution or nanoparticle dispersion containing 0.5 mg FITC-insulin. The small intestine was divided into 30 parts. Each experiment was repeated for triplicates. The standard deviations were left out in these plots for the sake of clarity. (A) FITC-insulin pH 7.4 PBS solution; (B) FITC-insulin nanoparticle dispersion; (C) FITC-insulin nanoparticle dispersion with 0.2% (w/v) HPMC; (D) FITC-insulin nanoparticle dispersion with 0.4% (w/v) HPMC; (E) FITC-insulin nanoparticle dispersion with 0.8% (w/v) HPMC.

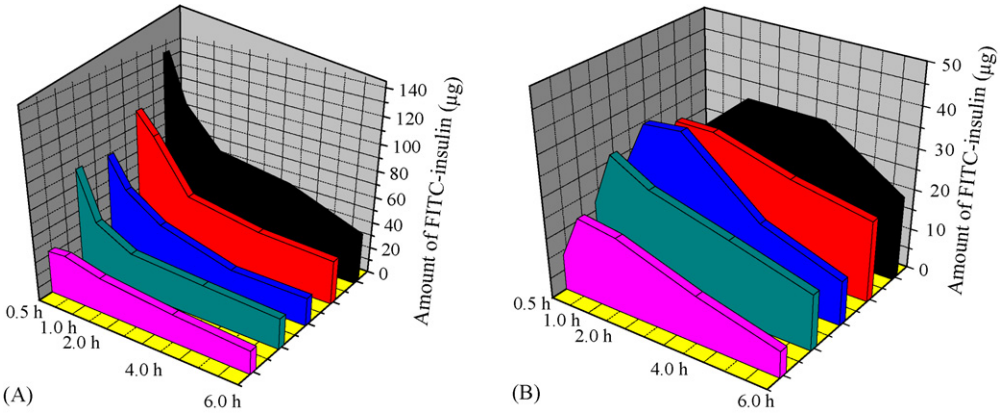

Fig. 5. The total amounts of FITC-insulin adhered to: (A) the gastrointestinal mucosa, and (B) the intestinal mucosa at various time after the oral administration of 2 ml aqueous solution or nanoparticle dispersion containing 0.5 mg FITC-insulin. The standard deviations were left out in these plots for the sake of clarity. Each experiment was repeated for triplicates.

nanoparticle dispersion had nearly the same k_{ge} but reduced k_{ie}. Addition of HPMC (0.2%, w/v) reduced both, with k_{ie} more significantly affected. Increasing the concentration of HPMC decreased the values of k_{ge} and k_{ie} further.

3.4. Bioadhesion of FITC-insulin in the gastrointestinal tract

Fig. 4 shows the amounts of FITC-insulin adhered to the intestine mucosa at various time for the five formulations, respectively. For all the formulations, only weak peaks detected at 0.5 h, and the adhesion peaks appeared after 1 h. It was found that the FITC-insulin showed weak adhesion ability when administrated in pH 7.4 PBS, with the highest peak less than 3.0 μg. Encapsulation of FITC-insulin in nanoparticle seemed help greatly for mucosa adhesion, indicated by the appearance of high but narrow peaks at 1 and 2 h (about 5.0 μg), covering 15–20% of the total length of the intestine. When HPMC was added in the nanoparticle dispersion, the adhesion peaks turned to be wider, covering longer length of the intestine (30–50% of the total length). But the increase of HPMC concentration seemed not increase the adhesion amounts of FITC-insulin.

It is shown in Fig. 5 that the evolution of total amounts of FITC-insulin adhered to the gastrointestinal and intestinal tract versus time. The total amounts of FITC-insulin adhered at 0.5 h were higher obviously for all the nanoparticle formulations with or without HPMC, in comparison with FITC-insulin PBS solution. The total amounts decreased rapidly at the early time after administration except the FITC-insulin PBS solution. Compared with the amounts adhered to the total gastrointestinal tract, the amounts adhered to the intestine part were much lower, reached peaks at around 1–2 h and decreased slowly at the following time. The amounts of FITC-insulin adhered to the intestinal mucosa were higher obviously for all nanoparticle formulations in comparison with FITC-insulin PBS solution, and formulations with higher concentration of HPMC seemed to have higher amounts of bioadhesion.

Table 3 summarizes the parameters used to quantify the in vivo bioadhesive characteristics of the different formulations tested. The area under concentration–time curve (AUC) of FITC-insulin nanoparticle dispersion was significantly ($p < 0.05$) higher than FITC-insulin solution. Adding of HPMC also obviously increased the AUC, but no significance was found among different concentrations of HPMC. Although the combination of FITC-insulin into nanoparticles and adding of HPMC in the dispersion prolonged the MRT of FITC-insulin, in comparison with FITC-insulin solution, the Q_{max} decreased slightly with the increase of the of HPMC concentration.

Table 3
Area under the concentration–time curve of FITC-insulin adhered to the small intestine mucosa

Formulation	AUC (μg h)	MRT (h)	k_{adh} (h^{-1})	Q_{max} (μg)
FITC-insulin solution	65.19 ± 8.15	2.67 ± 0.04	0.34[a]	17.05 ± 3.73
FITC-insulin nanoparticle dispersion	105.15 ± 3.64[b]	2.81 ± 0.03[b]	0.27[a,b]	26.83 ± 2.65[b]
FITC-insulin nanoparticle dispersion with 0.2% HPMC	143.14 ± 9.64[b,c]	2.97 ± 0.05[b,c]	0.26 ± 0.01[b]	36.59 ± 6.73[b,c]
FITC-insulin nanoparticle dispersion with 0.4% HPMC	140.34 ± 12.71[b,c]	3.03 ± 0.08[b,c]	0.24 ± 0.02[b,c]	29.61 ± 4.53[b]
FITC-insulin nanoparticle dispersion with 0.8% HPMC	158.69 ± 11.33[b,c]	3.17 ± 0.07[b,c,d]	0.19 ± 0.02[b,c,d,e]	32.93 ± 3.25[b,c]

The data are expressed as the mean ± mean ± S.D., and each experiment was repeated for triplicates.
[a] S.D. <0.01.
[b] $p < 0.05$ vs. FITC-insulin solution.
[c] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion.
[d] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.2% HPMC.
[e] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.4% HPMC.

Table 4
The predicted release parameters of FITC-insulin nanoparticles in the gut

Formulation	A_1	A_2	dx	x_0
FITC-insulin nanoparticle dispersion	0	92.48 ± 3.70	1.32 ± 0.30	0.77 ± 0.07
FITC-insulin nanoparticle dispersion with 0.2% HPMC	0	92.13 ± 4.19	1.14 ± 0.29[a]	0.98 ± 0.10[a]
FITC-insulin nanoparticle dispersion with 0.4% HPMC	0	91.33 ± 7.10	0.81 ± 0.27[a,b]	1.14 ± 0.21[a,b]
FITC-insulin nanoparticle dispersion with 0.8% HPMC	0	91.31 ± 0.20	0.84 ± 0.12[a,b,]	1.51 ± 0.10[a,b,c]

The in vivo release profiles were modeled using the Sigmoidal equation.
[a] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion.
[b] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.2% HPMC.
[c] $p < 0.05$ vs. FITC-insulin nanoparticle dispersion with 0.4% HPMC.

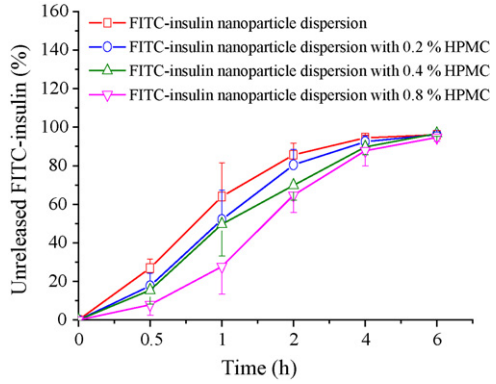

Fig. 6. Release profiles of FITC-insulin nanoparticles in the gastrointestinal tract (lumen content) after the oral administration of 2 ml aqueous dispersion containing 0.5 mg FITC-insulin. The data are expressed as the mean ± S.E.M., and each experiment was repeated for triplicates.

3.5. Release of FITC-insulin from nanoparticles in the gut

Fig. 6 demonstrates the release profiles of the FITC-insulin nanoparticles in the gut of rats after the oral administration of the different formulations containing 0.5 mg FITC-insulin. With the concentration of HPMC increased from 0% to 0.8% (w/v), the release profiles were right-shifted, indicating the decrease of the percentages of FITC-insulin released at the same time point.

The sigmoidal equation was commonly used for modulation of S-shaped profiles. The parameter x_0 represents the release half-life in the present study. Table 4 lists the modulated parameters for the release profiles. The values of x_0 increased significantly ($p < 0.05$) (from 0.77 to 1.51) with the elevation of the HPMC concentration. These results indicated that addition of HPMC reduced the release rates of FITC-insulin from the nanoparticles in the GI tract.

4. Discussion

FITC-insulin pH-sensitive nanoparticles can be easily prepared by a complex co-acervation method using chitosan and Eudragit L100-55 as the matrix materials. In vitro release study indicated that the FITC-insulin nanoparticles showed a pH-sensitive property, which was also consistent with the previous study of insulin nanoparticles. Thus, the in vivo results of FITC-insulin nanoparticles may be rationally extended to insulin nanoparticles.

As reported previously (Gupta and Robinson, 1995), the volumes of the formulations also had some influence on the distribution in the gastrointestinal tract, and relatively large volume may give a better distribution than small volume. The physicochemical properties of small volume dispersion are more easily affected by the secretion and absorption of the gut than that of large volume. For this reason, a 2 ml of volume was used in all studies, containing about 16.5 mg of nanoparticles.

It has been reported that once past the pylorus, a dosage form has little, if any, chance of distributing itself (Davis et al., 1986, 1987). In the present study, however, it seemed that this only happened at the early time after the dosages administrated (0.5–1 h). Two hours after administration, the FITC-insulin concentrated at the lower parts of the intestine, with the concentration peaks moving downwards, reaching the caecum at ∼6 h. This phenomenon may be explained as follows: after the nanoparticle formulations entered the small intestine, the formulations transit easily with large volume because of the movement of the intestine. But after 1–2 h, most of the water was absorbed from the formulations, and the total volume became much smaller. The movement of intestine would not affect a great part of the formulation. This postulation may be validated in our experiments. When the abdominal cavity of the rats opened and the intestine move at early time (0.5–1 h) after administration, parts of the intestine were full of liquid. But at late time (after 2 h), almost no obvious liquid existed in the intestine.

Addition of HPMC as a viscosity agent seemed result in a wider and smoother distribution of the dosages in the intestine. Similar results were reported previously using small particles (∼1 mm) (Gupta and Robinson, 1995). It is postulated that the increasing of viscosity made it more difficult for the nanoparticle formulations to transit in large volume along with the movement of the intestine.

The rapid clearance of protein drugs from the site of absorption was considered to be one of the barriers for insulin absorption (Lee, 1991). For this reason, increasing the retention time of insulin on the mucosa may result in a better bioavailability. Incorporating the drug into colloidal carriers like nanoparticles may help for this purpose. It has been proposed that mucoadhesion of colloidal carriers is one of the important properties for improving the bioavailability of poor absorptive drugs (Kreuter, 1991; Couvreur and Puisieux, 1993). It seemed that the results

in the present study confirmed this postulation, as nanoparticles showed significant ($p < 0.05$) increased adhesion amounts of FITC-insulin than PBS solution.

Another convenient approach for increasing the retention time of drugs on the mucosa may be changing the physical characteristics like viscosity of the formulations, and had been proved to be effective in limited studies (Harris et al., 1986; Gupta and Robinson, 1995). To investigate the effects of viscosity agent on the adhesion of FITC-insulin on the intestinal mucosa, three concentrations of HPMC (0.2%, 0.4%, and 0.8% (w/v)) were used in the present study. The results indicate that HPMC can increase the adhered amount and the adhesion time of FITC-insulin, but no obvious effect-concentration correlation was found, as the AUC was not increased significantly ($p > 0.05$) when the concentration increase from 0.2% to 0.8% (w/v). We postulated that the relatively higher viscosity of the formulations might reduce the mobility of the nanoparticles, and thus hinder the interaction of nanoparticles with the mucosa.

The addition of HPMC did not affect the in vitro release profiles significantly. This may be due to that in the release tubes the nanoparticle dispersion was diluted by the release media, and the concentration of HPMC was lower than 0.1% (w/v). Unlike the in vitro study, the in vivo release of FITC-insulin nanoparticles showed an S-shape profile. With the addition of HPMC, the release profiles were right-shifted, indicating the release of FITC-insulin from the nanoparticles was delayed in these formulations. This phenomenon may be mainly caused by the reduction of stomach empty rates when HPMC was added. As proved by numerous works, proteins and polypeptides may be absorbed with higher efficiency at certain location in the intestinal tract (Lambkin and Pinilla, 2002), the alteration of release profiles by HPMC may provide a more ideal release pattern.

5. Conclusion

The pH-sensitive nanoparticle dispersions tested in our studies showed similar stomach empty rate but lower intestine transit rate in comparison with FITC-insulin pH 7.4 PBS solution, and they increased the amounts of FITC-insulin adhered to the gastrointestinal mucosa. Addition of HPMC at 0.2%, 0.4%, or 0.8% (w/v), reduced both the stomach and intestine empty rates, and enhanced the gastrointestinal mucosa adhesion of FITC-insulin in comparison with the nanoparticle dispersions alone. Increasing the concentration of HPMC did not increase the amount of the FTIC-insulin adhered to the gastrointestinal mucosa. Moreover, all of three formulations with HPMC displayed wider and smoother distribution of FITC-insulin both in the lumen content and the intestinal mucosa. The pH-sensitive nanoparticles alone displayed an S-shape release pattern with the half-life less than one hour in the gut of rats. Addition of HPMC into the formulations prolonged the release half-life. It was concluded from our investigations that the distribution, transition, bioadhesion and release behaviors of the pH-sensitive nanoparticles in gastrointestinal tract of rats, as well as the addition of HPMC, seems to be favorable to the absorption of the drug loaded.

Acknowledgement

This study was funded by the National Natural Science Foundation of China (Grant no. 30430760).

References

Akiyama, Y., Nagahara, N., Kashihara, T., Hirai, S., Toguchi, H., 1995. In vitro and in vivo evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using polyglycerol esters of fatty acids and a poly(acrylic acid) derivative. Pharm. Res. 12, 397–405.

Arbos, P., Arangoa, M.A., Campanero, M.A., Irache, J.M., 2002. Quantification of the bioadhesive properties of protein-coated PVM/MA nanoparticles. Int. J. Pharm. 242, 129–236.

Arbos, P., Campanero, M.A., Arangoa, M.A., Renedo, M.J., Irache, J.M., 2003. Influence of the surface characteristics of PVM/MA nanoparticles on their bioadhesive properties. J. Control. Rel. 89, 19–31.

Bromer, W.W., Sheehan, S.K., Rerns, A.W., Arquilla, E.R., 1967. Preparation and properties of fluoresceinthiocarbamyl insulin. Biochemistry 6, 2378–2388.

Cerny, L.C., Stasiw, D.M., Zuk, W., 1981. The logistic curve for the fitting of sigmoidal data. Physiol. Chem. Phys. 13, 221–230.

Couvreur, P., Puisieux, F., 1993. Nano- and micro-particles for the delivery of polypeptides and proteins. Adv. Drug Deliv. Rev. 10, 141–162.

Dai, J.D., Nagai, T., Wang, X.Q., Zhang, T., Meng, M., Zhang, Q., 2004. pH-sensitive nanoparticles for improving the oral bioavailability of cyclosporine A. Int. J. Pharm. 280, 229–240.

Davis, S.S., Hardy, J.G., Fara, J., 1986. Transit of pharmaceutical dosage forms through the small intestine. Gut 27, 886–892.

Davis, S.S., Khosla, R., Wilson, C.G., Washington, M., 1987. Gastrointestinal transit of a controlled release pellet formulation of tiaprofenic acid and the effect of food. Int. J. Pharm. 35, 253–258.

General, S., Thünemann, A.F., 2001. pH-sensitive nanoparticles of poly (amino-acid) dodecanoate complexes. Int. J. Pharm. 230, 11–24.

Gupta, P.K., Robinson, J.R., 1995. Effect of volume and viscosity of coadministeried fluid on gastrointestinal distribution of small particles. Int. J. Pharm. 125, 185–193.

Hamman, J.H., Enslin, G.M., Kotze, A.F., 2005. Oral delivery of peptide drugs—barriers and developments. Biodrugs 19, 165–177.

Harris, A., Nilson, I., Wagner, Z., Alkner, U., 1986. Intranasal administration of peptides: nasal deposition, biological response, and absorption of desmopressin. J. Pharm. Sci. 75, 1085–1086.

Hentz, N.G., Richardson, J.M., Sportsman, J.R., Daijo, J., Sittampalam, G.S., 1997. Synthesis and characterization of insulin-fluorescein derivatives for bioanalytical applications. Anal. Chem. 69, 4994–5000.

Kim, P.S.G., Petersen, N.O., Sham, T.K., Hu, Y.F., 2004. Soft X-ray excited optical luminescence (XEOL) studies of fluorescein isothiocyanate (FITC) and FITC-labeled proteins. Chem. Phys. Lett. 392, 44–49.

Kreuter, J., 1991. Peroral administration of nanoparticles. Adv. Drug Deliv. Rev. 7, 71–86.

Lambkin, I., Pinilla, C., 2002. Targeting approaches to oral drug delivery. Expert Opin. Biol. Ther. 2, 67–73.

Lee, V.H.L., 1991. Trends in peptide and protein drug delivery. BioPharm 3, 22–25.

Li, M.G., Lu, W.L., Wang, J.C., Zhang, X., Zhang, H., Wang, X.Q., Wu, C.S., Zhang, Q., 2006. Preparation and characterization of insulin nanoparticles employing chitosan and poly(methacrylic acid and methacrylate) copolymer. J. Nanosci. Nanotechnol. 6, 2874–2886.

Magenheim, B., Levy, M.Y., Benita, S., 1993. A new in vitro technique for the evaluation of drug release profile from colloidal carriers-ultrafiltration technique at low pressure. Int. J. Pharm. 94, 115–123.

Mao, H.Q., Roy, K., Troung-Le, V.L., Janes, K.A., Lin, K.Y., Wang, Y.J., August, T., Leong, K.W., 2001. Chitosan-DNA nanoparticles as gene carriers: synthesis, characterization and transfection efficiency. J. Control. Rel. 70, 399–421.

Mesiha, M., Sidhom, M., 1995. Increased oral absorption enhancement of insulin by medium viscosity hydroxypropyl cellulose. Int. J. Pharm. 114, 137–140.

Naa, K., Lee, K.H., Bae, Y.H., 2004. pH-sensitivity and pH-dependent interior structural change of self-assembled hydrogel nanoparticles of pullulanacetate/oligo-sulfonamide conjugate. J. Control. Rel. 97, 513–525.

Owens, D.R., Zinman, B., Bolli, G.B., 2001. Insulins: today and beyond. Lancet 358, 739–746.

Saffran, M., Pansky, B., Budd, G.C., Williams, F.E., 1997. Insulin and the gastrointestinal tract. J. Control. Rel. 49, 89–98.

Sahoo, S.K., De, T.K., Ghosh, P.K., Maitra, A., 1998. pH- and thermo-sensitive hydrogel nanoparticles. J. Colloid Interf. Sci. 206, 61–368.

Sakum, S., Sudo, R., Suzuki, N., Kikuchi, H., Akashi, M., Hayashi, M., 1999. Mucoadhesion of polystyrene nanoparticles having surface hydrophilic polymeric chains in the gastrointestinal tract. Int. J. Pharm. 177, 161–172.

Shen, W.C., 2003. Oral peptide and protein delivery: unfulfilled promises? Drug Discov. Today 8, 607–608.

Soppimath, K.S., Aminabhavi, T.M., Kulkarni, A.R., Rudzinski, W.E., 2001. Biodegradable polymeric nanoparticles as drug delivery devices. J. Control. Rel. 70, 1–20.