Effects of *N*-trimethyl Chitosan Chloride as an Absorption Enhancer on Properties of Insulin Liquid Suppository In Vitro and In Vivo

Wen He,[1,2] Yumin Du,[2] Wenbing Dai,[1] Yan Wu,[1] Mian Zhang[1]

[1]*Department of Pharmacy of Renmin Hospital, Wuhan University, Wuhan 430060, Republic of China*
[2]*Department of Environmental Science, Wuhan University, Wuhan 430072, Republic of China*

Received 8 March 2005; accepted 28 May 2005
DOI 10.1002/app.22317
Published online in Wiley InterScience (www.interscience.wiley.com).

ABSTRACT: *N*-trimethyl chitosan chloride (TMC), with degree of quaternization (DQ) of 42.77% (TMC40) and 63.03% (TMC60), respectively, were synthesized and used as absorption enhancers in an insulin rectal liquid suppository from poloxamers. The effects of the TMCs on the liquid suppository were investigated. Compared with sodium salicylate (10%), which increased the gelation temperature and decreased the gel strength and bioadhensive force of the liquid suppository, both TMCs increased the three indices due to hydrogen bonding between the amino groups of TMCs and hydroxyl groups of poloxamers. The higher the concentration of TMCs was, the higher the three indices were. At the same concentration, the enhancing effect of TMC60 was higher than that of TMC40. Compared with those of 10% sodium salicylate, the enhancing effects of TMC40 with concentrations of 0.05% and 0.10% and TMC60 with concentration of 0.05% were weaker, while the other testing concentrations all showed better absorption enhancing ability with lower plasma glucose levels, $AUC_{0-4\ h}$ (the area below basal glucose level) and C_{nadir} (the plasma glucose levels at the nadir). Histological assessment was performed by observing irritation of the liquid suppository on the rectal tissues and observation rate of three types of gland changes in the rectal epithelium. Compared with the control (75.48 ± 16.76), TMC40 and TMC60 exhibited little change in observation rates of normal gland (72.10 ± 10.24 and 71.93 ± 9.88, respectively), while sodium salicylate showed significant lower observation rates of normal gland (51.28 ± 13.44). The insulin rectal liquid suppository with TMCs is more effective and safer than that with sodium salicylate.
 J Appl Polym Sci 99: 1140–1146, 2006

Key words: *N*-trimethyl chitosan chloride; absorption enhancer; insulin; liquid suppository

INTRODUCTION

Insulin, a macromolecular polypeptide, is difficult to be administered by gastrointestinal route because of inactivation by proteolytic digestion and poor absorption across mucosal membranes due to its hydrophilic nature and molecular mass. Therefore, it is still administered via the parenteral route in the clinical setting.

Some alternative dosage forms of insulin have been reported recently, such as infusion,[1] implant,[2] transdermal,[3] and mucosal delivery including mucosa of the small and large intestine,[4,5] nose,[6] lung,[7,8] and oral cavity.[9] The insulin rectal liquid suppository, which is a safer and more convenient dosage form, has also been developed.[10] Compared with the solid suppository, the thermoreversible liquid suppository is easier to be administered to the anus without much pain and is mucoadhensive to the rectal tissues without leakage and site change after the dose.

Using sodium salicylate as the absorption enhancer, the insulin liquid suppository could enhance the bioavailability of insulin in streptozotocin-treated rats. However, sodium salicylate was reported to be an irritatant to mucous membranes, which could lead to severe bloodshot in mucous membranes,[11] and Yun et al. proved that 10% (W/W) sodium salicylate was the tissue-damaging threshold level in the rectal liquid suppository system.[10] The notable shortcoming of sodium salicylate kept the insulin rectal liquid suppository from long-term clinical application.

N-trimethyl chitosan chloride (TMC), one of chitosan derivatives, has been investigated for permeation-enhancing properties and toxicity, using Caco-2 cells as a model for intestinal epithelium.[12–14] Results showed that TMC could considerably increase the permeation of neutral and cationic peptide analogs across Caco-2 intestinal epithelia. In experimental animals, TMC was shown to increase substantially the intestinal absorption and bioavailability of peptide analogs, with no indication of epithelial damage or cytotoxicity.[15–17] More importantly, TMC is also active at

Correspondence to: Y. Du (duyumin@whu.edu.cn).
Contract grant sponsor: The Department of Environment Science of Wuhan University.

neutral and basic pH values, similar to those found in intestine.[18] TMC can influence the mucosa by acting on the tight junctions reversibly,[19] by interaction of protonated TMC with anionic components of glycoprotein on the surface of the epithelial cells, and with fixed negative charges in the interior of the tight junctions. Therefore, the charge density of TMC, as determined by the degree of quaternization (DQ), is an important factor determining its potential use.[20]

In this study, thermoreversible insulin liquid suppositories were prepared with poloxamers (P407, P188) and TMC polymers with DQ of 40 and 60%, respectively. The quantitative evaluation of the effects of the two TMC polymers on properties of the liquid suppository in vitro and pharmacodynamic profiles of plasma glucose levels in vivo was carried out. The histological assessment of the rectal mucosa of rats was also conducted to judge the safety of the insulin liquid suppositories with TMCs as absorption enhancers in application to rectal administration.

EXPERIMENTAL

Materials and animals

Porcine insulin (27.7 IU/mg) was purchased from Jiangsu Wanbang Biochemistry Pharmaceutical Co. Ltd. (Jiangsu, China). Chitosan (Mw 210 kDa, DD $>$ 95%) from a shrimp shell was bought from Haipu Biotechnology Co. Ltd. (Qingdao, China). Poloxamer 407 and 188 were both supplied from BASF (Ludwigshafen, Germany). Sodium salicylate was of Chp 2000 grade. All the other chemicals were of analytical grade and used without further purification.

Male Sprague–Dawley rats weighing 280 $\pm$ 20 g were supplied from the Experimental Animal Breeding Center of Medical College of Wuhan University. All animal experiments complied with the rules set forth in the NHI Guide for the Care and Use of Laboratory Animals.

Synthesis and characterization of TMCs with different DQ

TMC with DQ of 42.77% (TMC40) and 63.03% (TMC60), respectively, were synthesized according to the methods reported by Sieval et al.[21] Briefly, sieved chitosan ($<$500 μm) was mixed with methyl-iodide in a basic solution of *N*-methylpyrrolidinone at 60°C for 75 min. The product was isolated by ethanol precipitation and subsequent centrifugation. After this first step, the obtained product underwent a second step of reductive methylation for 60 and 90 mins, yielding the final products, TMC iodide, having 42.77% and 63.03% DQ, respectively. Both products were precipitated by addition of ethanol, and isolated by centrifugation. The purification step of the final products included the exchange of the counterion iodide with chloride. The products were dissolved in 10% NaCl containing aqueous solutions, reprecipitated by ethanol, isolated by centrifugation, and thoroughly washed with ethanol and ether, then dried *in vacuo* at 40°C.

Both TMC40 and TMC60 were characterized by ^{1}H NMR [Fig. 1(a,b)]. The products were measured in D_2O at 80°C, using a 300-MHz spectrometer (Mercury Vx-300 Varian). The DQ of the synthesized TMC polymers were calculated with the following equation:[22] $DQ(\%) = [(\int TM/\int H) \times (1/9)] \times 100$, where $\int TM$ is the integral of the trimethyl amino group (quaternary amino) peak at 3.4 ppm and $\int H$ is the integral of the ^{1}H peaks from 5.0 to 6.0 ppm.

Preparation of liquid suppository

Aqueous solutions of insulin and the absorption enhancer, i.e., TMC40, TMC60, or sodium salicylate, were prepared by dissolving or dispersing in distilled water at room temperature and then cooled down to 4°C. Poloxamer 407 and 188 were then slowly added to the solutions with continuous agitation. The liquid suppositories were left at 4°C until clear solutions were obtained.

Ten different insulin liquid suppositories containing 100 IU/g insulin, respectively, were obtained with the compositions shown in the Table I.

Measurement of gelation temperature

A 30-mL transparent vial containing 20 mL of liquid suppository was placed in a thermostat water bath (DK-8B, Shanghai Accurate Experimental Instrument Co. Ltd., China). A digital thermosensor connected to a thermistor was immersed in the liquid suppository, which was heated at a constant rate of 0.1°C from 25.0°C, with constant stirring. Number 4-spindle of a rotational viscosimeter (NDJ-1, Shanghai Ande Instrument Co. Ltd., China) was revolving in the liquid suppository at initial rate of 12 rpm. The viscosity values at different temperature were recorded. When the abrupt change in the values appeared due to gelation, the temperature displayed on the thermistor was determined as the gelation temperature.

Measurement of gel strength and bioadhensive force

The methods were based on the processes reported by Choi et al.,[23] with some modification.

Briefly, liquid suppository (15 mL) was put in a 30-mL graduated cylinder (inside diameter of 2.2 cm) and gelled in a thermostat at 36.5°C. The apparatus for measuring gel strength (weight: 8.5 g) with a mess pan having 5 holes of 4.0 mm diameter each was then

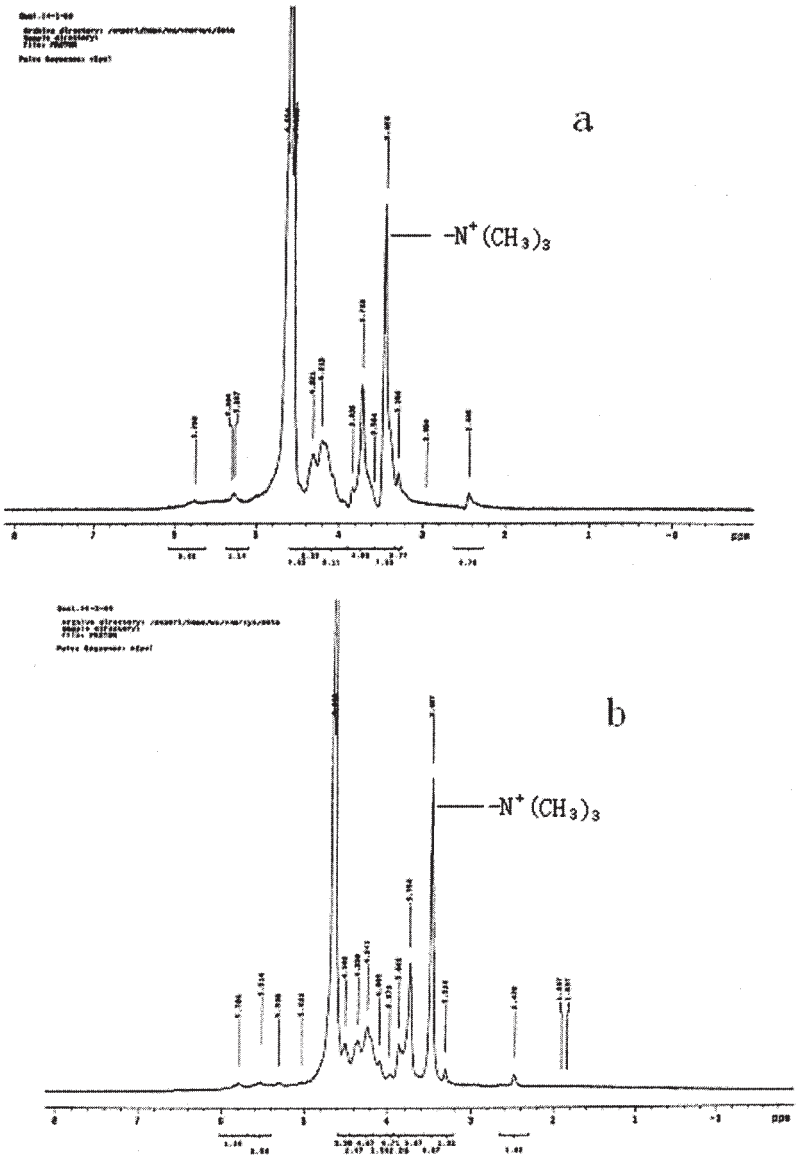

Figure 1 ^{1}H NMR spectra of (a) TMC40 (42.77% quaternized) and (b) TMC60 (63.03% quaternized), respectively. Peak assignment: $-N^+(CH3)3$: 3.4 ppm; –H proton : 5.0–6.0 ppm.

placed onto the liquid suppository (Fig. 2). The weights on top of the apparatus were constant at 240 g through preliminary tests. The gel strength was determined by the time (s) the apparatus took to sink 5 cm down through the liquid suppository.

The bioadhensive force of the liquid suppository was determined by using the measuring device in Figure 3. In brief, a section of tissue was cut from the fundus of rabbit rectum and secured with the mucosal side out onto each plastic vial (diameter of 0.8 cm) using a rubber band. The vials with the rectal tissues were stored at 36.5°C for 10 min. The liquid suppository (0.15 g) was added onto the rectal tissue on the lower vial, and the height of the vial was adjusted so that the liquid suppository could be placed between the mucosal tissues of both vials and kept balance

TABLE I
Compositions of Ten Different Insulin Liquid Suppository

Number	Insulin (%)	P407 (%)	P188 (%)	Sodium Salicylate (%)	TMC40 (%)	TMC60 (%)
A	0.36	15	20			
B	0.36	15	20	10		
C	0.36	15	20		0.05	
D	0.36	15	20		0.10	
E	0.36	15	20		0.50	
F	0.36	15	20		1.0	
G	0.36	15	20			0.05
H	0.36	15	20			0.10
I	0.36	15	20			0.50
J	0.36	15	20			1.0

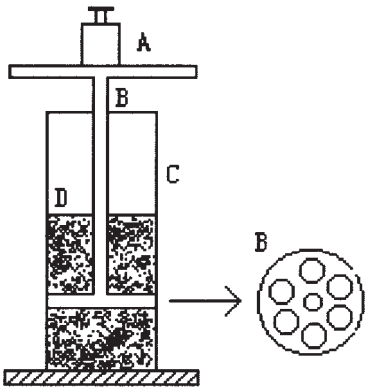

Figure 2 Gel strength-measuring device, (A) weights; (B) mess pan; (C) mess cylinder; (D) gel.

with the right side. Bioadhensive force, the detachment stress (dyn/cm^2), was determined from the minimal weights on the right side that detached the two vials. The rectal tissue pieces were changed for each measurement.

Stability of insulin in the liquid suppository

The stability of insulin during the preparation and gelation of liquid suppository at body temperature was investigated by detecting the insulin contents in liquid suppository after the preparation, the storage over 12 h at 37 ± 0.5°C, and over 3 months at 4°C. Insulin contents in liquid suppository were analyzed using a method from Chp 2000, by a high-performance chromatograph (Agilent-1100) equipped with a RP-C_{18} column (0.5 μm, 25 × 0.46 cm i.d.) and an ultraviolet spectrophotometric detector at 214 nm. The temperature of the column was 40°C. The mobile phase was 0.1*M* sodium phosphate monobasic adjusted to pH 3.0 with phosphoric acid and acetonitrile (73 : 27). The flow rate of eluent was 1.0 mL/min.

In vivo experiments

Alloxan-treated rats

Diabetes was induced by i.p. injection of a freshly prepared solution of alloxan (200 mg/kg) in saline. After 2–3 days, the induction effect of diabetes was assessed by the weight and blood glucose level of the rats.[24]

Administation and blood collecting

Fifty rats were divided into ten groups. The rats in each group were administered with the liquid suppository A-J containing 100 IU/g insulin, respectively. Before administration, the rats were fasted overnight, but allowed free access to water. Each rat, anesthetized in an ether-saturated chamber, was secured on a surgical board in the supine position, with a thread, followed by eliminating the feces from the anus with a stomach sonde needle. A polyethylene tube was inserted into the right femoral artery of the rat. Liquid suppositories (1 g/kg) were administered into the rectum 4 cm above the anus, through a stomach sonde needle fitted on a glass syringe.

Analysis of glucose in plasma

Blood samples were collected from the right femoral artery at designated time intervals during 4 h, *i.e.*, 0, 15, 30, 45, 60, 90, 120, 180, and 240 min after the dose, centrifuged to obtain plasma. Plasma samples were stored at −20°C until analysis of glucose using glucose-E kit.[4]

Histological test of rectal tissues

At 4 h after the dose, the rectum was isolated, rinsed with a saline solution, fixed in 10% neutral carbonate-buffered formaldehyde, embedded in paraffin using an embedding center, and cut into slices. The slices were stained with hematoxylin and eosin and observed under a light microscope (CK30/CK40 Olympus, Japan). The observation rate of the three types of gland changes in the rectal epithelium was evaluated and compared with those in fresh rectal epithelium without drug treatment as a control.[25]

RESULTS AND DISCUSSION

Effect of TMC on the physicochemical properties of the liquid suppository

According to Yun et al.,[10] the active material insulin did not affect the physicochemical properties of liquid suppository because of a negligible amount of insulin. Therefore, in this study, the effects of the absorption enhancers on the liquid suppository were monitored.

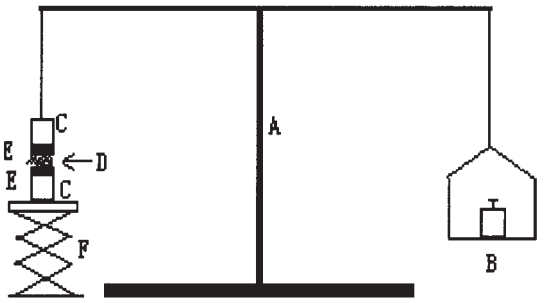

Figure 3 Bioadhesive force-measuring device, (A) modified balance; (B) weights; (C) plastic vial; (D) poloxamer gel; (E) rectal tissue; (F) height-adjustable pan.

TABLE II
Physicochemical Properties of the Insulin Liquid Suppositories With and Without Absorption Enhancers[a]

Liquid suppository	Absorption enhancer (%)	Gelation temperature (°C)	Gel strength (s)	Bioadhensive force ($\times 10^2$ dyne/cm^2)
A	—	29.4 ± 0.2	12.0 ± 0.5	101.7 ± 8.6
B	Sodium salicylate (10)	30.2 ± 0.2	9.1 ± 0.1	80.9 ± 4.4
C	TMC40 (0.05)	31.1 ± 0.5	40.0 ± 0.2	139.2 ± 3.2
D	TMC40 (0.10)	33.7 ± 0.3	86.2 ± 0.1	150.8 ± 4.5
E	TMC40 (0.50)	34.7 ± 0.4	107.3 ± 0.3	171.4 ± 2.5
F	TMC40 (1.0)	36.4 ± 0.3	148.5 ± 0.3	235.9 ± 3.9
G	TMC60 (0.05)	30.4 ± 0.3	35.7 ± 0.5	123.0 ± 4.7
H	TMC60 (0.10)	31.8 ± 0.2	80 ± 0.5	140.5 ± 5.2
I	TMC60 (0.50)	33.2 ± 0.4	92.2 ± 0.3	162.9 ± 8.8
	TMC60 (1.0)	35.1 ± 0.5	126 ± 0.2	200.8 ± 5.5

[a] Each value represents the mean ± S.D. ($n = 5$).

Various concentrations of TMC40 and TMC60 (0.05–1.0%) and sodium salicylate (10%) were added to an insulin liquid suppository base [insulin/P407/P188 (0.37/15/20%)], and the physicochemical properties such as gelation temperature, gel strength, and bioadhensive force of the liquid suppositories were evaluated (Table II).

The results showed that sodium salicylate (10%) increased the gelation temperature and decreased the gel strength and bioadhensive force because of weaker binding force of crosslinked reticular poloxamer gel resulting from sodium salicylate in the gel matrix.[10] Both TMC40 and TMC60, however, increased the three quality indices in vitro. The higher the concentration of TMCs was, the larger increase was seen. It is conceivable that strong hydrogen bonding between TMC polymers and poloxamers exists, which could increase the binding force of gel matrix. At the same concentration, the gelation temperature, gel strength, and bioadhensive force of the liquid suppositories enhanced by TMC40 were higher than those of the liquid suppositories enhanced by TMC60, indicating that hydrogen bonding of the former was stronger than the latter. It could be deduced that the intermolecular hydrogen bonding between TMCs and poloxamers dominantly existed between the free amino groups of TMC molecules and hydroxyl groups of poloxamer molecules. The lower DQ of TMC is, the more free amino groups in TMC molecules are, leading to stronger hydrogen bonding.

Previously, it was reported that the optimal liquid suppository should have the suitable range of gelation temperature (30–36°C), gel strength, and bioadhensive force to allow easy administration and to avoid leakage after the dose.[23] So, insulin liquid suppository F in Table I had the gel temperature, gel strength, and bioadhensive force unsuitable for practical use.

Stability of insulin in liquid suppository

The insulin contents after the preparation, stored over 12 h at 37 ± 0.5°C and over 3 months at 4°C, showed no significant changes compared with the input amount, suggesting that insulin was stable in the liquid suppository bases during preparation, gelation in the anus, and storage over 3 months at 4°C.

Pharmacodynamic tests

The induction of rat diabetes was evaluated by mainly plasma glucose level. After treatment with alloxan, rats showed more than threefold increase of plasma glucose level (112 ± 22 versus 382 ± 25 mg/dL), indicating that these model animals of diabetes were made successfully.

Plasma glucose level-time profiles and pharmacodynamic parameters are shown in Table III. The $AUC_{0-4\ h}$ (the area below basal glucose level calculated by the trapezoidal method over 4 h) and C_{nadir} (the plasma glucose levels at the nadir) of liquid suppository A did not significantly differ from those of liquid suppository A reported by Yun et al.,[10] while T_{nadir} (the time to reach the nadir) of liquid suppository A was shorter than that of previously reported

TABLE III
Pharmacodynamic Parameters of Insulin Delivered by the Ten Liquid Suppositories[a]

Liquid suppository	C_{nadir} (%)	T_{nadir} (h)	$AUC0_{0-4\ h}$ (% h)
A	72.06 ± 1.32	0.5 ± 50.20	314.33 ± 16.70
B	56.34 ± 3.87[b]	0.50 ± 0.15	277.92 ± 11.04[b]
C	64.28 ± 4.60[b,c]	0.75 ± 0.12	296.11 ± 7.67[b,c]
D	59.55 ± 5.01[b,c]	0.80 ± 0.12	283.73 ± 12.85[b,c]
E	42.65 ± 1.57[b,c]	0.95 ± 0.10	240.58 ± 5.90[b,c]
F	33.39 ± 2.94[b,c]	1.5 ± 0.20	198.66 ± 10.04[b,c]
G	60.14 ± 5.14[b,c]	0.70 ± 0.15	290.27 ± 9.17[b,c]
H	51.40 ± 4.4[b,c]	0.75 ± 0.16	250.12 ± 7.27[b,c]
I	38.10 ± 2.98[b,c]	0.80 ± 0.08	225.28 ± 8.82[b,c]
J	29.81 ± 1.76[b,c]	1.0 ± 0.13	179.54 ± 14.58[b,c]

[a] Each value represents the mean ± S.D. ($n = 5$).
[b] $P < 0.05$ compared with liquid suppository A.
[c] $P < 0.05$ compared with liquid suppository B.

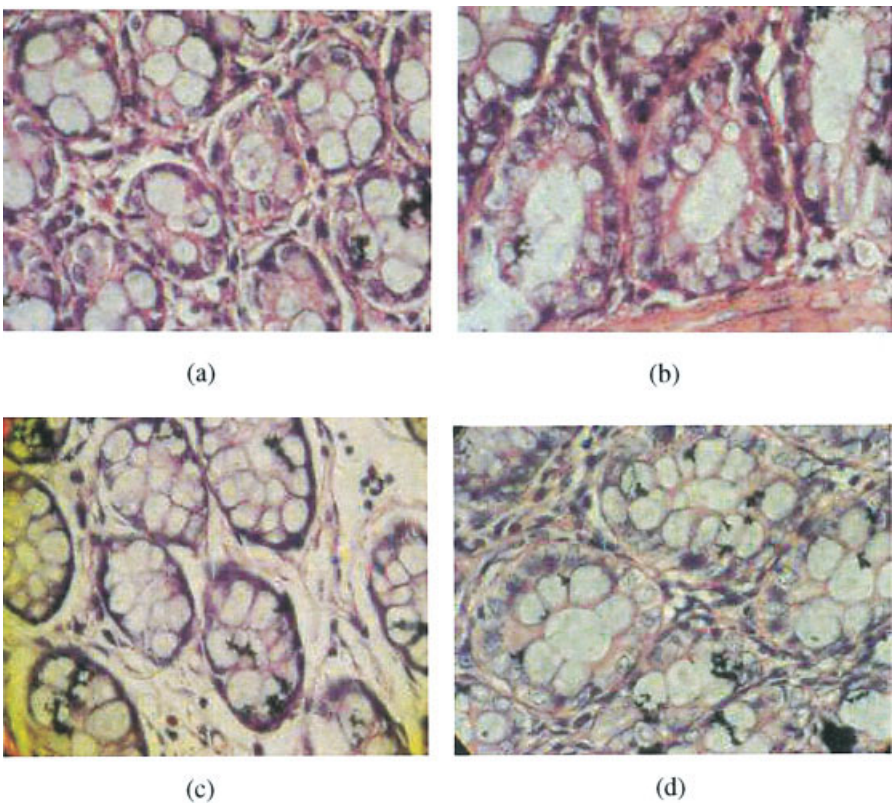

Figure 4 Morphology of rectal mucosa of streptozotocin-treated rats after the rectal administration of insulin liquid suppositories (×250): (a) liquid suppository A; (b) liquid suppository B; (c) liquid suppository F; (d) liquid suppository J. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

due to the absence of polycarbophile in our formulation.

Liquid suppository B with 10% sodium salicylate showed lower plasma glucose levels in rats, resulting from lower $AUC_{0-4\ h}$ and C_{nadir} compared with those of liquid suppository A without any absorption enhancers. This is in accordance with the results of Yun et al. For the same reason, T_{nadir} of liquid suppository B was also shorter than that of previously reported. Liquid suppository C-J enhanced by different concentrations of TMC40 and TMC60, respectively, all showed lower C_{nadir} and $AUC_{0-4\ h}$ while longer T_{nadir} in rats compared with liquid suppository A, indicating the two TMC polymers both had promising absorption enhancing ability and to some extent sustained release effect in insulin rectal liquid suppository systems. At the same concentration, the enhancing effect of TMC60 was higher than that of TMC40. The results were also coincident with those reported by other researchers.[20,26] Furthermore, at the same concentration, T_{nadir} of liquid suppositories enhanced by TMC40 was longer than that by TMC60, indicating more compact matrix in liquid suppository with TMC40 because of more hydrogen bonding between TMC40 and poloxamers.

Compared with sodium salicylate, the threshold concentration of TMCs generating absorption enhancement was much lower. But the enhancing effect of the two TMC polymers was still correlated with their concentrations in the formulation. From the Table III, it could be found that if the concentration of TMC40 was 0.05% and 0.10%, while the concentration of TMC60 was 0.05%, the absorption enhancing ability of TMC polymers was weaker than that of 10% sodium salicylate.

Histological assessment of rectal tissues

Histological assessment was performed by observing any irritation of insulin liquid suppository on the rectal tissues followed by evaluating the observation rate of the three types of gland changes in the rectal epithelium (Fig. 4).

Liquid suppository A without any absorption enhancers showed similar observation rates of normal gland to control (73.91 ± 20.54 versus 75.48 ± 16.76). However, liquid suppository B with 10% sodium salicylate exhibited significant lower observation rates of normal gland compared with control (51.28 ± 13.44 versus 75.48 ± 16.76). The result was in contrast to Yun et al.[10] One possible explanation is the absence of polycarbophil in our formulation. Polycarbophil could increase the viscosity of liquid suppository and act as a sustained release polymer, which could

TABLE IV
Observation Rate of the Three Types of Changes Observed in the Rectal Epithelium at 4 h after the Dose[a]

Liquid suppository	Classification (%)			
	Normal	Type I	Type II	Type III
Control[b]	75.48 ± 16.76	21.13 ± 6.15	4.37 ± 6.66	3.31 ± 3.83
A	73.91 ± 20.54	9.661 ± 4.02	10.25 ± 3.60	5.47 ± 8.64
B	51.28 ± 13.44[c]	8.58 ± 8.10	11.71 ± 6.92	4.57 ± 5.05
F	72.10 ± 10.24	9.42 ± 12.50	10 ± 5.73	5.34 ± 11.48
J	71.93 ± 9.88	9.40 ± 11.59	9.98 ± 4.15	5.32 ± 9.71

[a] Each value represents the mean ± S.D. ($n = 5$).
[b] Control means fresh rectal epithelium without drug administration.
[c] $P < 0.05$ compared with control.

decrease the irritation of sodium salicylate to some extent.

Previously, in a large number of cytotoxicity studies with Caco-2 cells and experimental animals, no deleterious effects of TMC polymers had been detected, indicating the safety of those chitosan derivatives.[19] Liquid suppository F (1.0% TMC40) and J (1.0% TMC60) both showed no changes in observation rates of normal gland compared with those of control (72.10 ± 10.24 and 71.93 ± 9.88, versus 75.48 ± 16.76) (Table IV). This indicates that TMCs are safer than sodium salicylate, to be applied in the insulin rectal liquid suppository for a long-term clinical use because of their inability to interact with cell membrane.

CONCLUSIONS

In this study, two TMC polymers with DQ of 40 and 60%, respectively, were synthesized and characterized successfully and used as absorption enhancers in the insulin rectal liquid suppository. Different from 10% sodium salicylate, the two TMC polymers could increase gel strength and bioadhensive force of the liquid suppository due to the strong hydrogen bonding formation between TMCs and poloxamers. Based on the results of pharmacodynamic profiles of insulin and histological assessment of the rectal tissues of rats after the dose, we conclude that TMC40 with concentration higher than 0.1% and TMC60 with concentration above 0.05% have better and safer enhancing effect than 10% sodium salicylate in the liquid suppository. The insulin rectal liquid suppository with TMCs as absorption enhancers could be a potential media for a safer and more effective rectal delivery system of insulin.

The authors thank the Measurement and Test Centre of Wuhan University for ^{1}H NMR and the Department of Examination of Renmin Hospital of Wuhan University for their help in the analysis of plasma glucose.

References

1. Goriya, Y.; Bahoric, A.; Marliss, E. B.; Zinman, B.; Albisser, A. M. Diabetologia 1980, 19, 452.
2. Burczak, K.; Gamian, E.; Kochman, A. Biomaterials 1996, 24, 2351.
3. Haffman, A.; Ziv, E. 1997, 4, 285.
4. Hosny, E.; Ahmady, O. E.; Shattawy, H. E.; Nabih, E. M.; Damancy, H. E.; Deen, S. G.; Kabbany, N. E. Drug Res 1994, 1, 611.
5. Asada, H.; Douen, T.; Waki, M.; Adachi, S.; Fujita, T.; Yamamoto, A.; Muranishi, S. J Pharm Sci 1995, 6, 682.
6. Kotze, A. F.; Thanou, M. M.; Verhoef, J. C.; Junginger, H. E. Release Bioact Mater 1998, 25, 479.
7. Chakraborti, R. N. J Ind Med Assoc 1999, 97, 493.
8. Martonen, T. B.; Musante, C. J; Segal, R. A. Respir Care 2000, 45, 712.
9. Laube, B. L.; Benedict, G. W.; Dobs, A. S. J Aerosol Med 1998, 11, 153.
10. Yun, M. O.; Choi, H. G.; Jung, J. H.; Kim, C. K. Int J Pharm 1999, 189, 137.
11. Richardson, J. L.; Illum, L.; Thomas, N. W. Pharm Res 1992, 7, 878.
12. Kotze, A. F.; Lue§en, H. L.; Leeuw, B. J. de.; Boer, A. Gde.; Verhoef, J. C.; Junginger, H. E. Pharm Res 1997, 4, 1197.
13. Kotze, A. F.; Thanou, M. M.; Lue§en, H. L.; Boer, A. G. de.; Verhoef, J. C.; Junginger, H. E. J Pharm Sci 1999, 88, 253.
14. Thanou, M. M.; Verhoef, J. C.; Romeiji, S. G.; Nagelkerke, H. L.; Boer, A. Gde.; Verhoef, J. C.; Junginger, H. E. Int J Pharm 1999, 185, 73.
15. Thanou, M. M.; Florea, B. I.; Langemeyer, M. W. E.; Verhoef, J. C.; Junginger, H. E. Pharm Res 2000, 17, 27.
16. Thanou, M. M.; Verhoef, J. C.; Marbach, P.; Junginger, H. E. J Pharm Sci 2000, 9, 951.
17. Thanou, M. M.; Verhoef, J. C.; Verheijden, J. H. M.; Junginger, H. E. Pharm Res 2001, 18, 823.
18. Thanou, M. M.; Verhoef, J. C.; Junginger, H. E. Adv Drug Deliv Rev 2001, 50, S91.
19. Junginger, H. E.; Verhoef, J. C. PSTT 1998, 1, 370.
20. Thanou, M. M.; Kotze, A. F.; Scharringhausen, T.; Lueβen, H. L.; Boer, A. G.;de.; Verhoef, J. C.; Junginger, H. E. J Controlled Release 2000, 64, 15.
21. Sieval, A. B.; Thanou, M. M.; Kotze, A. F.; Verhoef, J. C.; Brussee, J.; Junginger, H. E. Carbohydrate Polym 1998, 36, 157.
22. Hamman, J. H.; Kotze, A. F. Drug Dev Ind Pharm 2001, 27, 373.
23. Choi, H. G.; Jung, J. H.; Ryu, J. M.; Yoon, S. J.; Oh, Y. K.; Kim, C. K. Int J Pharm 1998, 165, 33.
24. Kim, A.; Oh, Y. K.; Kim, C. K. Int J Pharm 1999, 180, 75.
25. Reid, A. S.; Thomas, N. W.; Palin, K. J; Gould, P. L. Int J Pharm 1987, 40, 181.
26. Kotze, A. F.; Thanou, M. M.; Lue§en, H. L.; Boer, A. G. de.; Verhoef, J. C.; Junginger, H. E. Euro J Pharm and Biopharm 1999, 47, 269.