

# Hydrophobic ion pairing of an insulin-sodium deoxycholate complex for oral delivery of insulin

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**Abstract:** Insulin was complexed with sodium deoxycholate to form an insulin-sodium deoxycholate complex (Ins-SD-Comp) using an hydrophobic ion pairing method in aqueous phase to enhance the liposolubility of insulin. In order to obtain the maximal complexation efficiency, the molar ratio of sodium deoxycholate to insulin was found. The zeta potential method was used to confirm the optimal ratio for formation of Ins-SD-Comp. The structural characteristics of Ins-SD-Comp were assessed using the Fourier transform infrared method. The apparent partition coefficient of insulin increased upon the formation of Ins-SD-Comp. Based on the preliminary study, Ins-SD-Comp was encapsulated into poly(lactide-co-glycolide) (PLGA) nanoparticles using an emulsion solvent diffusion method. The maximal encapsulation efficiency of Ins-SD-Comp into PLGA nanoparticles was  $93.6\% \pm 2.81\%$ , drug loading was about  $4.8\% \pm 0.32\%$ , and the mean diameter of the nanoparticles was  $278 \pm 13$  nm. Biological activity and in vivo results revealed that the bioactivity of insulin was not destroyed during the preparation process. Ins-SD-Comp-loaded PLGA nanoparticles have the potential to reduce serum glucose levels and increase the oral bioavailability of insulin.

**Keywords:** insulin complex, sodium deoxycholate, nanoparticles, zeta potential, oral bioavailability

## Introduction

Insulin is a 51-amino acid peptide that was discovered in 1921 by Branting and Best, together with MacLeod and Collip. It is excreted by pancreatic  $\beta$  cells, and has the ability to control glucose levels in the blood by facilitating glucose uptake in the cells of the organism. The most common therapy for diabetes mellitus is injection of insulin, but this is painful and inconvenient, resulting in poor patient compliance.<sup>1</sup> In recent decades, oral delivery of insulin has been widely investigated because it can improve patient compliance and transport insulin directly to the liver, which can prevent peripheral hyperinsulinemia.<sup>2</sup> In addition, orally delivered insulin undergoes hepatic bypass before entering the circulation, so has the potential to mimic the effects of insulin secreted by the pancreas in terms of inhibition of hepatic gluconeogenesis and hepatic glucose output.<sup>3-6</sup>

However, there are various obstacles concerning oral administration of insulin, including, but not limited to, poor bioavailability owing to its inherently poor absorption properties, poor absorption from the intestinal lumen due to the tight junctions in the epithelial lining,<sup>7</sup> and susceptibility to chemical and physiological degradation.<sup>8,9</sup>

In order to overcome the obstacles mentioned above and enhance the oral bioavailability of insulin, biodegradable polymeric nanoparticles, such as

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poly(lactic acid), poly(glycolic acid), and their copolymers, poly(lactide-co-glycolide) (PLGA) nanoparticles, have been widely studied by our group.<sup>10,11</sup> During encapsulation of hydrophilic insulin into the hydrophobic copolymer, the hydrophobic ion pairing technique was applied to enhance the hydrophobicity of insulin. It was reported that the hydrophobic ion pairing method can be used to enhance the hydrophobicity of protein/peptides.<sup>12–15</sup> This technique is simply performed by the interaction between ionic protein and the opposite ionic head group of fatty acid, surface-active agents or other amphiphilic molecules at suitable pH. In this study, sodium deoxycholate was chosen as the counterpart of insulin. As part of the enterohepatic circulation, sodium deoxycholate is produced in the liver and recirculated 10–20 times per day. It can be reabsorbed from the site of action in the intestinal lumen and returned to the liver via the portal vascular system after being released into the duodenum.<sup>16</sup> The selective reabsorption of sodium deoxycholate can potentially be used to improve the bioavailability of insulin. Moreover, it was reported that bile salts can disrupt tight junctions in the epithelial lining, allowing for paracellular and transcellular transport pathways.<sup>17,18</sup>

In this paper, a more liposoluble insulin-sodium deoxycholate complex (Ins-SD-Comp) was prepared using the hydrophobic ion pairing method. The lyophilized complex was then loaded into PLGA nanoparticles by the emulsion solvent diffusion method. Afterwards, the physicochemical characteristics of the PLGA nanoparticles, bioactivity of insulin in different forms, and hypoglycemic effect of orally administered Ins-SD-Comp PLGA nanoparticles were investigated.

## Materials and methods

### Materials

Porcine insulin (28.8 IU/mg) and streptozotocin were purchased from Sigma-Aldrich (St Louis, MO). Polyvinyl alcohol (PVA-403) was kindly supplied by Kuraray Co Ltd (Osaka, Japan). PLGA (75/25, molecular weight 20 kDa) and sodium deoxycholate were purchased from Wako Pure Chemical Industries Ltd (Osaka, Japan). Glucose GOD-PAD kits were purchased from Beijing BHKT Clinical Reagent Co Ltd (Beijing, China). All other reagents were of chemical grade.

### Animals

Male Wistar rats (weighing  $200 \pm 20$  g and aged 12–13 weeks) were provided by the pharmacological laboratory of Shenyang Pharmaceutical University. The study protocol

was reviewed and approved by the Institutional Animal Care and Use Committee, Shenyang Pharmaceutical University, People's Republic of China.

### Induction of diabetes

The rats were induced into diabetes by a tail vein injection of streptozotocin 65 mg/kg in a 10 mM citrate buffer at pH 4.5 as previously described.<sup>19</sup> Rats were considered diabetic when glycemia was higher than 16.7 mmol/dL 7 days after streptozotocin treatment.

### Preparation of Ins-SD-Comp

Ins-SD-Comp was prepared using the hydrophobic ion pairing method. Briefly, various amounts of sodium deoxycholate 1 mg/mL in purified water were added into 4.5 mg of insulin solution (pH 4.0, 4.0 mL) under magnetic stirring in dropwise manner. The solution immediately became cloudy and the precipitate was treated as the Ins-SD-Comp. The precipitative complex was easily collected by centrifugation at 14,000 rpm for 15 minutes while the native insulin still remained in the supernatant. The resulting complex was washed with distilled water three times and lyophilized into powder. The insulin complexation efficiency (CE [%]) was calculated by measuring the concentration of insulin in the supernatant using a high-pressure liquid chromatography (HPLC) method, and the bound insulin fraction was calculated based on the initial amount of insulin added. The insulin CE (%) was then calculated from the following equation:

$$\text{CE (\%)} = \frac{M_i - M_f}{M_i} \times 100\% \quad (1)$$

where  $M_i$  and  $M_f$  represent the initial amount of insulin added and the measured amount of free insulin in the supernatant, respectively. The HPLC mobile phase for insulin in this paper was composed of 0.1 mol/L  $\text{NaH}_2\text{PO}_4$  aqueous solution, 0.05 mol/L  $\text{Na}_2\text{SO}_4$  aqueous solution, and acetonitrile at a ratio of 35:35:30, and the final pH was adjusted to 3.0 with  $\text{H}_3\text{PO}_4$  solution. The detector wavelength, the column temperature, and the flow rate of the mobile phase were set at 214 nm, 35°C, and 1 mL/minute, respectively. The injection volume of the test samples was 20  $\mu\text{L}$ . The zeta potentials of Ins-SD-Comp in distilled water were evaluated in order to confirm further the optimal molar ratio of sodium deoxycholate to insulin for the charge-charge interaction, using a Zetasizer Nano-ZS90 (Malvern Instruments, Worcestershire, UK) at 25°C.

## Partitioning coefficient of Ins-SD-Comp

Two milliliters of sodium deoxycholate 1 mg/mL solution were added dropwise into 4.5 mg (4.0 mL) of insulin solution. The mixture was centrifuged at 14,000 rpm for 15 minutes to get the complex. Then 2.5 mL of 1-octanol and 2.5 mL of acidified water was added to the complex under magnetic stirring. After being allowed to equilibrate for 3 hours, the mixture was centrifuged for 15 minutes at 14,000 rpm. The concentrations of insulin in the aqueous phase and oil phase were measured by the HPLC method.

## FTIR analysis of Ins-SD-Comp

Fourier transform infrared spectrophotometry (FTIR spectrometer, Bruker IFS-55, Switzerland) was used to study the interaction between sodium deoxycholate and insulin. The infrared spectra of insulin, sodium deoxycholate, the Ins-SD-Comp, and the Ins-SD physical mixture were obtained by the KBr method.

## Preparation and characterization of Ins-SD-Comp-PLGA nanoparticles

Ins-SD-Comp-PLGA nanoparticles were prepared using the emulsion solvent diffusion method. Based on a preliminary study, 100 mg of PLGA and 5 mg of insulin-equivalent Ins-SD-Comp were dissolved in the organic phase of acetone-ethanol (4:1) mixture with a small quantity of 0.01 N HCl (speeding up solution). The oil phase was then added into 50 mL of aqueous solution containing 1% of PVA at 2 mL/minute using a peristaltic pump while being continuously stirred at 400 rpm with a propeller mixer. The solidified Ins-SD-Comp-PLGA nanoparticles were recovered by ultracentrifugation at 20,000 rpm for 10 minutes at 25°C and then washed with distilled water three times, followed by lyophilization. For the control group, Ins-SD-Comp was replaced by 5 mg of insulin while the other conditions remained unchanged. The concentration of insulin in the supernatant was analyzed by the HPLC method, and the entrapped insulin fraction was calculated based on the initial amount of insulin added. The particle size analysis and zeta potential measurements were performed using photon correlation spectroscopy with a Zetasizer Nano-ZS90 at 25°C.

The morphology of the formulated PLGA nanoparticles was observed by scanning electron microscopy, Jeol JSM 5600 LV (Tokyo, Japan), which required an ion coating with platinum by a sputter coater (JFC-1300, Jeol) for 40 seconds in a vacuum at a current intensity of 40 mA after preparing the sample on metallic studs with double-sided conductive tape.

## Cell growth assay

NIH 3T3 cells ( $1 \times 10,000/\text{mL}$ ) were suspended in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution and seeded into a 24-well tissue culture plate (Becton Dickinson, Franklin Lakes, NJ). Determined amounts of free insulin, insulin extracted from Ins-SD-Comp, or Ins-SD-Comp-PLGA nanoparticles were added to the cells, and then each culture was incubated for 24 hours in a humidified atmosphere of 5%  $\text{CO}_2$  in air at 37°C. Cell growth was then assessed by the [ $^3\text{H}$ ] thymidine ([methyl-1'-2'-3'-H thymidine (1.40 TBq/mmol)], Amersham, UK) incorporation assay. Radioactivity was counted with a scintillation counter (MicroBeta TRILUX, Pharmacia, Sweden).

## In vivo bioactivity evaluation

Prior to oral administration, the hypoglycemic effect of insulin in different forms was evaluated in normal rats by the subcutaneous method. Test solutions were prepared as follows:

For the control group, free insulin was dissolved in phosphate-buffered saline (pH 7.4).

For Ins-SD-Comp, the complex was first dissolved in dimethylsulfoxide (DMSO, 10 mg complex versus 0.5 mL DMSO) and then introduced into phosphate-buffered saline (pH 7.4). The theoretical amount of insulin in the complex (TAIC) was calculated by the following formula:

$$\text{TAIC} = (\text{TAI} - \text{TAS}) \quad (2)$$

where TAI and TAS represent the total amount of initial insulin and total amount of insulin in supernatant during preparation of the complex, respectively.

For the Ins-SD-Comp-PLGA nanoparticles, the theoretical amount of insulin in the supernatant was measured by the following method. One hundred milligrams of freeze-dried nanoparticles were dissolved in 10 mL of acetonitrile to dissolve the polymer and precipitate the insulin. The samples were centrifuged at 20,000 rpm for 15 minutes and the pellet was dissolved in DMSO (10 mg pellet versus 0.5 mL DMSO), and then diluted with phosphate-buffered saline (pH 7.4). The amount of insulin in the pellet was measured by the HPLC method as mentioned earlier. The theoretical amount of insulin in the supernatant (TAIS, pH 7.4) was calculated by the following formula, where TAP represents the total amount of insulin in 100 mg of PLGA nanoparticles.

$$\text{TAIS} = (\text{TAP} - \text{AIP}) \quad (3)$$

All of the three solutions were diluted to an insulin-equivalent concentration of 1 IU/mL (theoretically) with phosphate-buffered saline (pH 7.4) before administration.

The three kinds of solutions were administered subcutaneously to three groups of normal rats (six rats in each group) which had been fasted for 12 hours. Blood samples were collected from the retro-orbital plexus of the rats at baseline and one hour after insulin was administered subcutaneously at 1 IU/kg. Plasma was separated by centrifugation at 4000 rpm for 5 minutes and the glucose level was measured using the glucose oxidase method. The hypoglycemic response to insulin was characterized as follows: the initial plasma glucose (at baseline) was taken as 100% level and the plasma glucose at one hour was calculated as the percentage of the initial value.

## Investigation of hypoglycemic effect and relative bioavailability

Twenty-four male rats with streptozotocin-induced diabetes were fasted for 12 hours before administration of the study treatment. During the experiment, the animals had free access to water. The rats were randomized into four groups (six rats in each group). Aqueous free insulin 1 IU/kg was administered subcutaneously in the first group. Ins-SD-Comp-PLGA nanoparticles 20 IU/kg, free insulin 20 IU/kg, and saline were administered orally to the second group, the third group, and the fourth group, respectively. The methods for plasma separation and glucose level determination were the same as in the previous section. The hypoglycemic response to insulin was characterized as follows: the initial plasma glucose was taken as the 100% level and all the following concentration-time data were given accordingly as the percentage of the initial value. The areas above the plasma glucose level-time curves (AAC) were calculated using the trapezoidal rule. The specific value of AAC for the different groups to the AAC of subcutaneous injection group was used to calculate the relative pharmacological bioavailability (RB%) using the following equation:

$$RB\% = \frac{AAC_{Oral}}{AAC_{S.C}} \times \frac{Dose_{S.C}}{Dose_{Oral}} \times 100\% \quad (4)$$

## Results and discussion

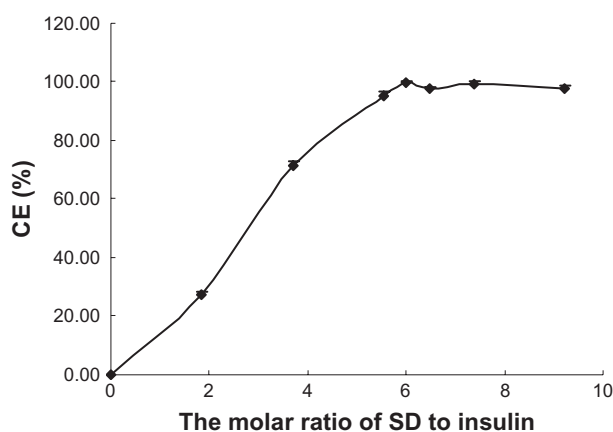
### Formation of Ins-SD-Comp

It is well known that insulin cannot be directly dissolved in hydrophobic organic solvents due to its hydrophilic nature. However, it is possible to alter its liposolubility using the hydrophobic ion pairing method. Sodium deoxycholate was

used because of its good biocompatibility, availability, and well known physiological activity. Further, sodium deoxycholate can enhance the apparent liposolubility of insulin in the form of Ins-SD-Comp. Figure 1 shows that as sodium deoxycholate was slowly added to the insulin solution, the CE (%) increased gradually until reaching a stoichiometric amount of 6:1. This can be explained as follows. Since the isoelectric point is about 5.35 to 5.45, it has a net positive charge of 6 (due to two histidine, one lysine, one arginine, and two N-terminal amino acid residues) at pH 4.0. Therefore, insulin could be complexed completely with sodium deoxycholate (negative charge of 1) under this condition at a molar ratio of 1:6. The CE (%) reached up to  $99.65\% \pm 0.46\%$ . In order to confirm the optimal molar ratio of sodium deoxycholate to insulin, the zeta potential of the complexes was measured. From Figure 2, it was found that the zeta potential of Ins-SD-Comp changed from  $+23.64 \pm 6.11$  through zero to a level of  $-9.23 \pm 3.94$  mV as the molar ratio of sodium deoxycholate to insulin changed from 1.85 to 9.23. As expected, the net surface charge on the Ins-SD-Comp was positive because of the surplus positive insulin when the molar ratio of sodium deoxycholate to insulin was below 6:1. As the molar ratio of sodium deoxycholate to insulin got to 6:1, the net surface charge on the Ins-SD-Comp became nearly zero due to the complete complexation of sodium deoxycholate and insulin. When the molar ratio of sodium deoxycholate to insulin was higher than 6:1, the zeta potential became negative, owing to the excess of sodium deoxycholate.

### Partitioning coefficient of Ins-SD-Comp

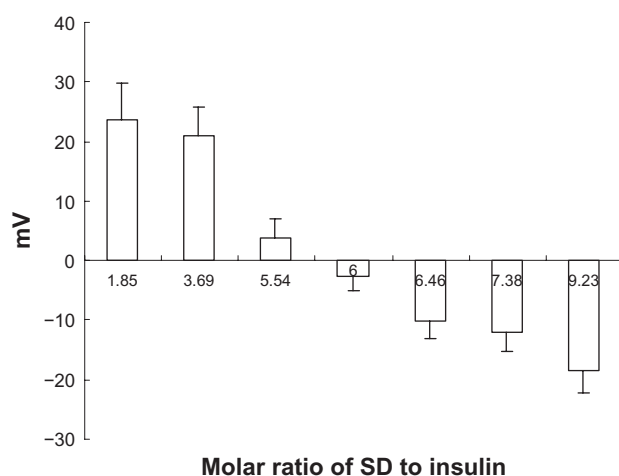
The partitioning coefficient in the 1-octanol/water system was measured in order to confirm the increase in lipophilicity of



**Figure 1** Insulin complexation efficiency versus molar ratio of sodium deoxycholate to insulin.

**Note:** Results are expressed as the mean  $\pm$  standard deviation,  $n = 3$ .

**Abbreviations:** CE, complexation efficiency; SD, sodium deoxycholate.



**Figure 2** Changes in zeta potential of the complex at different ratios of sodium deoxycholate to insulin.

**Note:** Results are expressed as the mean  $\pm$  standard deviation,  $n = 3$ .

**Abbreviation:** SD, sodium deoxycholate.

Ins-SD-Comp. We hypothesized that the insulin and sodium deoxycholate molecules formed a more lipophilic complex, and the aqueous solubility of the complex decreased due to enhanced lipophilicity. The concentrations of insulin and Ins-SD-Comp in the aqueous system were determined to be 0.8922 mg/mL and 0.4638 mg/mL, respectively. Concentrations of insulin and Ins-SD-Comp in 1-octanol were 0.0036 mg/mL and 0.4163 mg/mL, respectively. Partitioning of the complex into 1-octanol increased by two orders of magnitude compared with free insulin. The enhanced lipophilicity of the insulin was definitely due to the hydrophobic counterpart in the complex.<sup>20</sup>

## FTIR analysis of Ins-SD-Comp

FTIR was conducted in order to confirm the complexation behavior between insulin and sodium deoxycholate. We hypothesized that the anionic carboxyl group ( $\text{COO}^-$ ) of sodium deoxycholate may interact with the cationic amino group ( $\text{NH}_3^+$ ) of insulin and thereby form an ionic complex between the two compounds. As a result, some changes would be evident on the absorption bands of carboxyl groups and amide bonds in the infrared spectra.

Figure 3 shows the FTIR spectra of free insulin, sodium deoxycholate, Ins-SD-Comp, and the Ins-SD physical mixture. In the insulin spectrum shown in Figure 3A, four strong bands were observed, ie, 3398.9 (amide A), 1658.3 (amide I), 1516.4 (amide II), and 1240.6 (amide III)  $\text{cm}^{-1}$  bands.<sup>21</sup> Figure 3B shows three distinctive features at 2938.1, 2864.3, and 1561.2  $\text{cm}^{-1}$ , which are infrared absorption bands corresponding to the CH stretching vibration and the  $\text{COO}^-$  stretching vibration modes of sodium deoxycholate.<sup>22,23</sup>

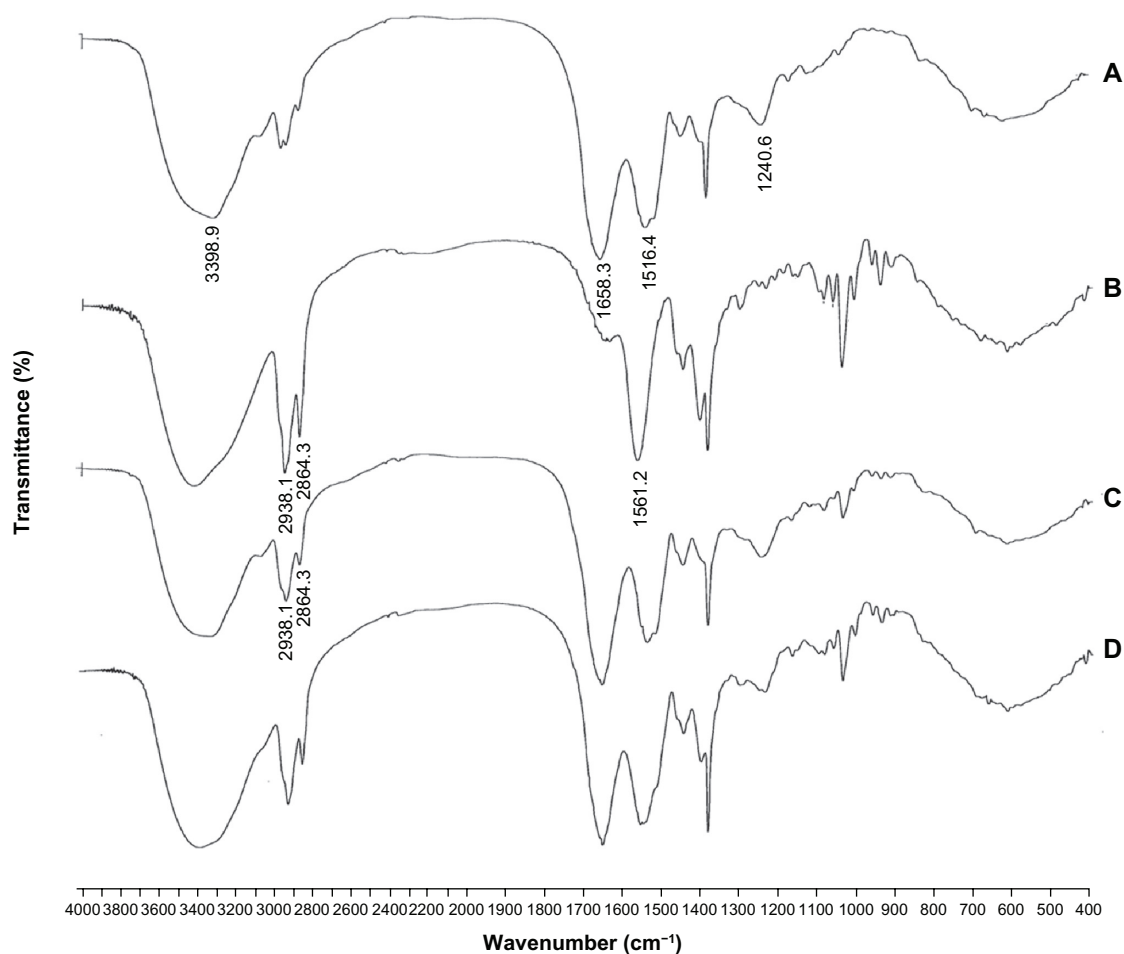
It was found that there was a significant difference between the infrared spectra of the insulin-sodium deoxycholate physical mixture and Ins-SD-Comp. The infrared spectra of the physical mixture (Figure 3D) were the simple superposition of the spectra of the two components. However, in the Figure 3C spectrum of Ins-SD-Comp, the stretching band of the carboxylic group at 1564.0  $\text{cm}^{-1}$  disappeared, while infrared absorption bands corresponding to CH stretching vibration at 2938.1 and 2864.3  $\text{cm}^{-1}$  could still be detected. This indicated that the carboxyl group of the anionic sodium deoxycholate interacted with the cationic amino group of insulin and formed an ionic complex between the two compounds.

## Preparation and characterization of Ins-SD-Comp-PLGA nanoparticles

As shown in Table 1, it was found that the entrapment efficiency and the drug loading of insulin increased in the Ins-SD-Comp form compared with the free insulin form, which can be explained as follows. Due to formation of Ins-SD-Comp, the apparent solubility of insulin in the organic phase increased, so it was much easier to encapsulate the complex into nanoparticles using the emulsion solvent diffusion method. The zeta potential of the Ins-SD-Comp-PLGA nanoparticles was a little higher than that of the insulin-loaded PLGA nanoparticles because of introduction of negative sodium deoxycholate. It is interesting that the particle size of Ins-SD-Comp-PLGA nanoparticles was a little larger than that of the insulin-loaded PLGA nanoparticles because of increasing entrapment of insulin. Further, the nanoparticles prepared by this method were nearly monodispersed and spherical, as shown in Figure 4.

## Biological activity of insulin

It was important to determine whether insulin still maintained its biological activity or not during the preparation process. Insulin acts as a growth factor (IGF), and can control glucose levels. NIH 3T3 mouse fibroblasts have few endogenous insulin receptors and are insensitive to insulin, but express IGF receptors and are responsive to IGF-1.<sup>24</sup> Both insulin and IGF regulate certain cellular functions via both overlapping receptor and postreceptor signaling pathways.<sup>25</sup> Therefore, we used these cells to test the biological activity of insulin extracted from Ins-SD-Comp and Ins-SD-Comp-PLGA nanoparticles compared with that of normal insulin. In control cells, [ $^3\text{H}$ ] thymidine uptake was  $9368.7 \pm 1231.9$  disintegrations per minute (Figure 5). The radioactivity of cells treated with extracted insulin had similar potency to



**Figure 3** Infrared spectra of (A) insulin, (B) sodium deoxycholate, (C) the insulin-sodium deoxycholate complex, and (D) the insulin-sodium deoxycholate physical mixture.

that of normal insulin. This shows that insulin retained its bioactivity during the preparation process.

### In vivo bioactivity evaluation

In order to confirm further whether insulin retained its bioactivity during the complexation and encapsulation process, the hypoglycemic effect of free insulin, insulin in the complex, and in the PLGA nanoparticles was evaluated by the subcutaneous method. It was reported that the noticeable insulin response to blood sugar appears 45–90 minutes after subcutaneous administration of insulin,<sup>26</sup> so plasma glucose levels in the rats were determined to confirm the bioactivity of insulin in different forms at 60 minutes after subcutaneous administration of the various samples.

As shown in Table 2, for free insulin, insulin in Ins-SD-Comp, and Ins-SD-Comp-PLGA-NP, subcutaneous administration in rats reduced plasma glucose levels to almost the same percentage for about 58%, 55% and 57%, respectively. However, there was no statistically significant difference between these values. This result clearly indicated that the preparation process did not decrease the bioactivity in vivo.

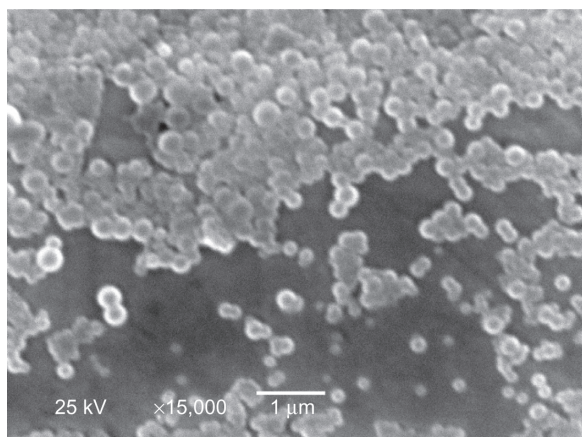
### Hypoglycemic effect and relative bioavailability studies

The serum glucose versus time curves of Ins-SD-Comp-PLGA nanoparticles are shown in Figure 6. It can be observed that oral administration of saline and free insulin did not

**Table 1** Characteristics of Ins-SD-Comp-PLGA nanoparticles and free insulin PLGA nanoparticles

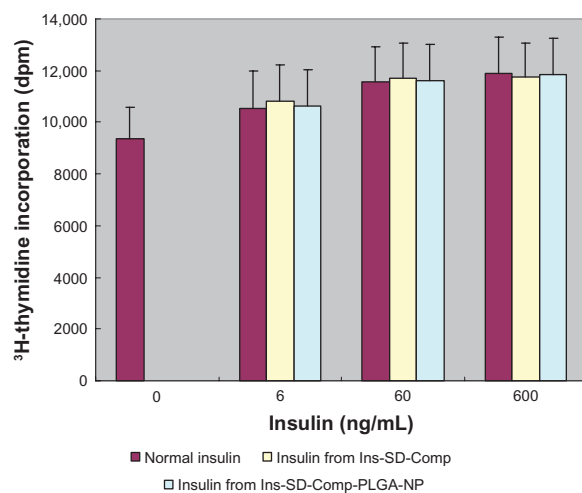
Samples	EE%	Drug loading (%)	Zeta potential (mV)	Particle size (nm)	Polydispersity index
Free insulin	42.3 ± 2.27	2.2% ± 0.21%	-29.4 ± 3.36	265 ± 10	0.10
Ins-SD-Comp	93.6 ± 2.81	4.8% ± 0.32%	-33.2 ± 3.18	278 ± 13	0.12

**Abbreviations:** EE, entrapment efficiency; Ins-SD-Comp, insulin-sodium deoxycholate complex; PLGA, poly(lactide-co-glycolide).



**Figure 4** Scanning electron microscopic morphology of insulin-sodium deoxycholate complex poly(lactide-co-glycolide) nanoparticles prepared under optimized conditions.

appreciably lower the blood glucose levels of the animals, demonstrating no insulin absorption. Conversely, oral administration of Ins-SD-Comp-PLGA nanoparticles produced significant plasma glucose reduction (percentage relative to the initial value). A *t*-test with a significance level of  $P < 0.01$  showed that there was a significant difference between the nanoparticles and the control group. This phenomenon illustrates that the Ins-SD-Comp-PLGA nanoparticles had an effective hypoglycemic effect. The relative bioavailability was about 11.7%, while the relative bioavailability of free insulin was less than 0.5%. The increased absorption of insulin could be due to several factors. Firstly, sodium deoxycholate is a biological compound that consists of an amphiphilic steroid nucleus with a hydrophobic  $\alpha$ -side and a hydrophilic  $\beta$ -side.



**Figure 5** Biological activity of normal insulin, insulin extracted from the complex, and insulin extracted from poly(lactide-co-glycolide) nanoparticles.

**Note:** Data are the mean  $\pm$  standard deviation,  $n = 6$ .

**Abbreviations:** Ins, insulin; SD, sodium deoxycholate; PLGA, poly(lactide-co-glycolide); NP, nanoparticles.

**Table 2** Bioactivity of various insulin samples

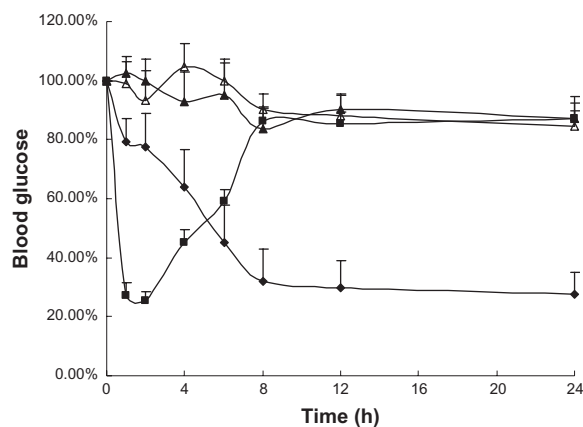
Samples	Plasma glucose level (% of initial)
Free insulin	58.2 $\pm$ 7.26
Insulin in Ins-SD-Comp	55.0 $\pm$ 6.69
Insulin in Ins-SD-Comp-PLGA NP	57.1 $\pm$ 6.17

**Note:** Data represent the mean  $\pm$  standard deviation,  $n = 6$  per group.

**Abbreviations:** Ins, insulin; SD, sodium deoxycholate; Comp, complex; NP, nanoparticles; PLGA, poly(lactide-co-glycolide).

It is synthesized in the liver and carried via the bile duct to the small intestine, and returned to the liver by absorption through the bile acid transporter.<sup>27</sup> Therefore, cycling of insulin in the enterohepatic circulation due to the presence of sodium deoxycholate could provide sustained release into the bloodstream. Secondly, due to the formation of Ins-SD-Comp, the apparent partitioning coefficient of insulin increased, which led to increased absorption of insulin. Furthermore, when encapsulated into PLGA nanoparticles, insulin can be partly protected from degradation by acid and enzymes in the gastrointestinal tract. In addition, the particle size of the complex was about 270 nm. Thus the complex may be taken up via Peyer's patches located in the mucosa of the small intestine before insulin is degraded.<sup>28</sup> The absorption mechanism will be studied further in our future work.

During our studies, fasting plasma glucose levels in the Ins-SD-Comp-PLGA nanoparticle group did not return to the initial level within 24 hours, probably as a result of reduced responses of counterregulatory hormones, such as glucagon, growth hormone, and cortisol.<sup>29</sup> This problem has also been encountered in other studies.<sup>30,31</sup>



**Figure 6** Changes (%) of plasma glucose level with time (hours) after administration of various samples.

**Notes:** Data represents the mean  $\pm$  standard deviation,  $n = 6$  per group (■) subcutaneous injection of 1 IU/kg insulin solution; oral administration of (◆) insulin-sodium deoxycholate complex poly(lactide-co-glycolide) nanoparticles (20 IU/kg), (▲) free insulin (20 IU/kg) and (Δ) saline.

## Conclusion

In this paper, hydrophilic insulin was hydrophobically modified with sodium deoxycholate and further encapsulated into PLGA nanoparticles, with high entrapment efficiency and high drug loading. The potential of Ins-SD-Comp-PLGA nanoparticles to improve the oral bioavailability of insulin was demonstrated, indicating that it is a promising candidate for oral insulin delivery.

## Disclosure

The authors report no conflicts of interest in this work.

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