

# Self-Assembled Core-Shell Poly(Ethylene Glycol)-POSS Nanocarriers for Drug Delivery

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## ABSTRACT

*In this work, novel nanostructured core-shell poly (ethylene glycol) (PEG)-polyhedral oligosilsesquioxane (POSS) nanoparticles were used to encapsulate insulin as new drug delivery carriers. The morphologies, particle size and  $\zeta$  potential of the pure nanostructured core-shell PEG-POSS and the corresponding insulin-loaded PEG-POSS nanoparticles were investigated by transmission electron microscopy (TEM) and laser diffraction particle sizer. TEM analysis demonstrated that pure and insulin-loaded self-assembled PEG-POSS nanoparticles were of spherical shape with core-shell nanostructure, and were well-dispersed and uniform in size distribution. Insulin release test showed that insulin was well-protected inside PEG-POSS nanoparticles at gastric pH for 2 hrs, and was released at intestinal pH (pH 6 - 7) where the absorption and activation of the drug are necessary. We therefore believe that such nanostructured PEG-POSS nanoparticles could be useful as a potential carrier for insulin drug delivery systems.*

**Keywords:** Self-Assembly, Amphiphilic, Nanoparticles, POSS, Insulin, Oral Delivery

## 1. Introduction

In a recent year, nanotechnology has been utilized to develop new therapies and next generation nanosystems for “smart” drug delivery [1]. A variety of organic/inorganic nanomaterials and devices have been often used as delivery vehicle to enhance the therapeutic activity by prolonging drug half-life, improving solubility of hydrophobic drugs, reducing potential immunogenicity, and/or releasing drugs in a sustained or stimuli-triggered fashion. Insulin was known to treat diabetic. Current treatment methods involve regular injections of insulin, which can be both painful and inconvenient, thus leading to low patient compliance [2]. In order to overcome this problem, the oral route is considered to be the most convenient and comfortable means of drug administration for patients. However, oral administration of hydrophilic macromolecules such as peptide/protein drugs is encountered with many difficulties as the drugs have to confront various major barriers in the gastrointestinal (GI) tract. Firstly, peptide/protein drugs get denatured readily by low pH of gastric medium in the stomach. Secondly, different digestive enzymes in the stomach and small intestine may lead to degradation of peptide/protein drugs [3]. A new carrier system is therefore required to

protect peptide/protein drugs from the harsh environment in the GI tract.

We have recently developed the organic/inorganic hybrid materials, such as amphiphilic poly(ethylene glycol) (PEG)-polyhedral oligosilsesquioxane (POSS) and poly(vinyl alcohol) (PVA)-POSS hybrids incorporating POSS macromers onto chain-ends or polymer backbone as pendent groups, respectively [4-7]. Thanks to their amphiphilic properties (here, POSS is *hydrophobic*, PEG and PVA *hydrophilic*), it can be expected that those POSS-containing polymers can form the micelles by tailoring the composition ratio in polymers and solvent polarity, and this has important implications for drug delivery systems. For instance, we reported [8] that PVA-POSS hybrid showed unagglomerated nanoparticles within a diameter range of 60 - 90 nm, as confirmed by atomic force microscopy (AFM) and bio-transmission electron microscope (bio-TEM). The prepared nanoparticles were found to improve the control release of anti-cancer drug; *paclitaxel* as a model drug. However, there are few reports on the solution properties and micelles/nanoparticles formation of POSS-based polymeric materials for drug delivery system [9-11]. In this report, we prepare the hybrid core-shell nanostructured particles

composed of POSS as a hydrophobic inner core and PEG as a hydrophilic outer shell by using dialysis approach, and propose novel drug delivery carriers for protein drugs.

## 2. Experimental Section

### 2.1. Materials

Poly(ethylene glycol) (PEG) (molecular weight = 3.4 kDa, Aldrich) was purified by repeating twice the process of precipitation into *n*-hexane from chloroform solution. Isocyanatopropyltrimethylsilylcyclohexyl-polyhedral oligosilsesquioxane (POSS macromer) was purchased from Tomen Plastics Co., Japan. Dibutyl tin dilaurate (DBTDL; Aldrich, 95% purity) as a catalyst for urethane formation was used as received. Amphiphilic PEG3.4k-POSS telechelic studied herein was synthesized by direct urethane linkage between the diol end groups of PEG homopolymers and the monoisocyanate group of POSS macromers as catalyzed by DBTDL. <sup>1</sup>H-NMR results confirmed that the amphiphilic PEG3.4k-POSS telechelic was successfully prepared. Evidence for the formation of urethane linking groups comes from the emergence of a weak proton signal at about 4.26 ppm, accompanied by the disappearance of a proton signal of the -CH<sub>2</sub>-NCO group (3.15 ppm). That is, the level of incorporation of POSS macromers in the amphiphilic PEG3.4k-POSS telechelic could be determined quantitatively by the monitoring of the resonances for the cyclohexyl groups of POSS macromers. A degree of end functionalization was found to about 2.1, indicating quite consistent with the feed ratio. The detailed synthesis and characterization are described in our previous reports [4,12]. Toluene was dried over CaH<sub>2</sub> and then distilled under nitrogen prior to use. A sample of 100 mg of insulin, from bovine pancreas (activity: ≥25 USP units/mg, secondary activity: 2500 units) were purchased from Sigma-Aldrich. All chemicals were of analytical purity or higher quality and were used without further purification.

### 2.2. Preparation of Insulin-Loaded Hybrid PEG-POSS Particles

Insulin solution was prepared by dissolving 100 mg of insulin in 10 ml of 0.01 N HCl solution. Insulin encapsulation was carried out on the basis of self assembly process. Briefly, 40 mg of PEG3.4k-POSS was fully dissolved in 10 ml of THF. Then, 0.5, 0.9, 1.3 ml insulin solutions was added dropwise into the PEG3.4k-POSS solutions, respectively. The mixture was poured into dialysis tube (spectra/Por 6, MWCO: 3.5 kD) and dialyzed against distilled water at room temperature under magnetic stirring for 1 day. Afterwards, the distilled water was exchanged at least 3 times in order to remove the

THF and HCl residues. To determine the loading efficiency of insulin in hybrid PEG-POSS nanoparticles, the amount of free insulin in supernatants was assayed by UV-vis spectrophotometry. The drug loading efficiency was calculated using the equations listed below [13,14].

$$\begin{aligned} & \text{Loading Efficiency (\%)} \\ & = \frac{\text{total amount of insulin added} - \text{amount of free insulin}}{\text{total amount of insulin added}} \times 100\% \end{aligned} \quad (1)$$

### 2.3. Characterization

Particle size and ζ potential of pure and insulin-loaded PEG-POSS nanoparticles were measured depending on pH of the mixture by laser diffraction particle sizer (Nano-ZS, Malvern Instrument Ltd., UK). FI-IR analysis of the prepared nanoparticles and insulin was carried out using an IRPrestige-21 (Shimadzu Co., Japan) Transmission Electron Microscopy (TEM) images were taken out on a JEM-2100 LaB6 microscope (JEOL, Japan) operating at an accelerating voltage of 160 kV to observe the morphologies of the obtained pure and insulin-loaded PEG-POSS nanoparticles. The insulin-loaded PEG-POSS nanoparticles dispersed in water was directly dropped onto a carbon-coated copper grid (200-A mesh, Nissin EM Co., Ltd.), followed by drying at room temperature. Finally, the samples were dried in vacuum oven. The average particle size was determined from the TEM micrographs using an image analysis software (Image J, National Institutes of Health, Bethesda, U.S.).

### 2.4. pH Effects on Insulin Release from PEG-POSS Nanoparticles

The pH of the insulin-loaded nanoparticle suspensions was varied at the ranging from pH 2.5 to pH 7.4 to study the release behaviors of the insulin from PEG-POSS nanoparticles at room temperature. The insulin-loaded PEG-POSS nanoparticles treated with centrifugation 3000 rpm/15 min were placed into 10 ml of PBS buffer solution (pH 2.5) and incubated at 37°C for 2 hrs. 0.5 mL of the supernatants, which was isolated by centrifugation (2000 rpm/1 min), was taken by every twenty minutes for the measurement of the released amount of insulin from PEG-POSS nanoparticles and replaced by fresh medium. Afterwards, the same insulin-loaded PEG-POSS nanoparticles were again isolated by centrifugation (3500 rpm/15 min) and were transferred to 10 mL of phosphate buffer at pH 7.4 and incubated at 37°C for 3 hrs. The released amount of insulin from PEG-POSS nanoparticles were monitored by UV-vis spectrophotometry. A plot of cumulative release with time was reported, where

$$\text{Cumulative release (\%)} = \frac{A_t}{A_{MAX}} \times 100\% \quad (2)$$

where  $A_t$  is the absorbance of the characteristic peak at 275 nm ( $\epsilon = 5.53 \text{ mM}^{-1}\cdot\text{cm}^{-1}$ ) [15] at time  $t$ , and  $A_{MAX}$  is the maximum absorbance of this peak.

### 3. Results and Discussion

In our previous paper [4-7], we have reported that the synthesized organic/inorganic PEG-POSS and PVA-POSS hybrid materials were found to be amphiphilic, and thereby resulted in self-assembling into core-shell nanostructured micelles with hydrophobic inner-core (POSS moieties) and hydrophilic outer-shell (PEG or PVA moieties) in aqueous media. Therefore, hydrophobic drugs, such as insulin used in this work can be easily entrapped into the core. **Scheme 1** shows the schematic illustration of self-assembling process, which leads to self-aggregation into core-shell nanostructured particles (i.e., flower-like micelles) encapsulating insulin inside the hydrophobic core in aqueous solution. Here, it is worth mentioning that the formation of self-assembled nanostructures using POSS-PEG-POSS telechelic (ABA triblock copolymers) depends on two competing forces: the entropy loss due to loop formation of the central block in the micelle corona and the interfacial energy penalty that accompanies the transfer of insoluble block from the micelle corona to the solution [16].

#### 3.1. Characterization of Insulin-Loaded PEG-POSS Nanoparticles

**Figure 1** shows TEM images of pure and insulin-loaded self-assembled PEG-POSS nanoparticles. It can be clearly seen that insulin-loaded PEG-POSS nanoparticles were of spherical shape with core-shell nanostructure, and were well-dispersed and uniform in size distribution unlike pure PEG-POSS nanoparticles. The size of pure self-assembled PEG-POSS nanoparticles was about  $15.9 \pm 1.3 \text{ nm}$ , while the insulin-loaded self-assembled PEG-POSS nanoparticles (loading content  $\sim 0.9 \text{ mg}$ ) were  $330 \pm 80 \text{ nm}$ , as measured by TEM analysis, suggesting successful encapsulation of insulin molecules into a hydrophobic core. In addition, the loading efficiency (LE) of insulin in the insulin-loaded PEG-POSS nanoparticles with an added insulin contents of 5, 9, 13 mg was found to be 52.6%, 70.5% and 76.5%, indicating that PEG-POSS nanoparticles have good loading capability of hydrophobic drug, insulin [17,18]. In addition, FT-IR analysis demonstrated the existence of insulin in insulin-loaded PEG-POSS nanoparticles (**Figure 2**). The characteristic peaks at  $1651 \text{ cm}^{-1}$  and  $1531\text{-}1514 \text{ cm}^{-1}$  region were observed respectively, corresponding to C–N stretching and N–H bending modes of amide II region in

pristine insulin [19]. The Si–O–Si peak in the insulin-loaded PEG-POSS nanoparticles was shifted toward higher wavenumber, compared to pure PEG-POSS nanoparticles. This suggests that there are strong interactions between insulin and PEG-POSS segments [20].

#### 3.2. pH Effects on Insulin-Loaded PEG-POSS Nanoparticles

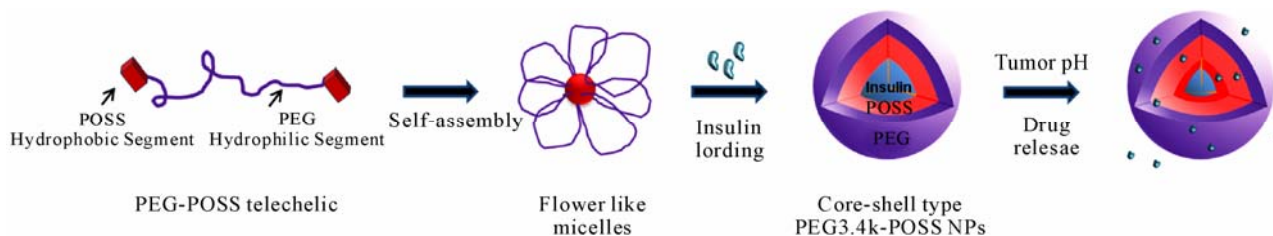
The  $\zeta$ -potential and size of the obtained hybrid nanoparticles was investigated at various pH and the results were shown in **Figure 3**. The  $\zeta$ -potential of pure PEG-POSS nanoparticles was close to constant zero  $\zeta$ -potential values as increasing pH, whereas the insulin-loaded PEG-POSS nanoparticles obviously exhibited increased negative charges, presumably due to ionized carboxyl groups in an encapsulated insulin (**Figure 3(a)**) [21]. Moreover, it is also expected that such negative charged nanoparticles may be less aggregated and show good dispersion-stabilities because of an electrostatic repulsion. Therefore, the resultant negatively charged insulin-loaded PEG-POSS nanoparticles exhibit well-dispersed nanoparticles, which can help them to be taken up by cells more easily than aggregated ones [8]. As expected, unlike pure PEG-POSS nanoparticles, the size of insulin-loaded PEG-POSS nanoparticles clearly increased as increasing pH (loading content  $\sim 0.9 \text{ mg}$ , **Figure 3(b)**).

#### 3.3. Insulin-Release Study

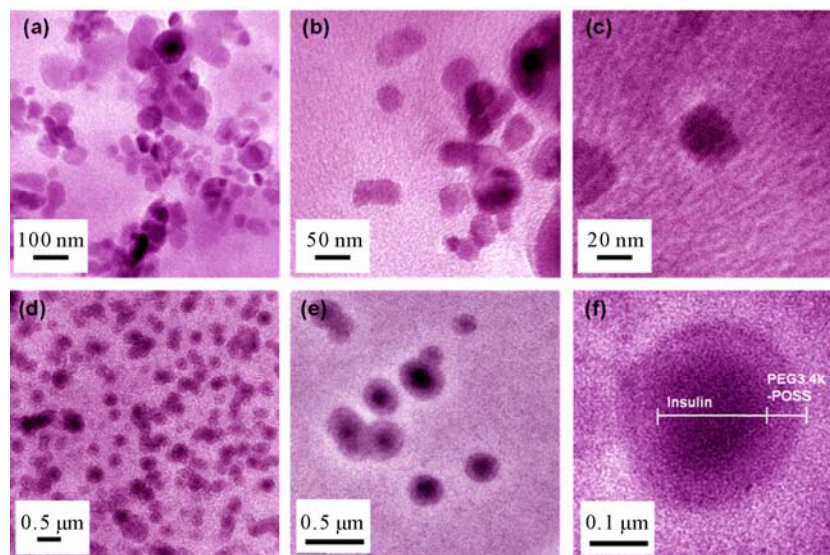
**Figure 4** shows insulin release behavior from insulin-loaded PEG-POSS nanoparticles at different pH values of 2.0 and 7.4. At pH = 2.0, even though a tiny release of insulin was observed, probably due to a weakly physical-adsorbed insulin, PEG-POSS nanoparticles appeared to have higher insulin retention capacity at low pH. Afterwards, following a pH change to 7.4, a dramatic release of insulin was observed during the first hour followed by a relatively sustained release, due to a fast dissociation of insulin from PEG-POSS nanoparticles at higher pH. This suggests that insulin was well-protected inside PEG-POSS nanoparticles at gastric pH for 2 hrs, and was released at intestinal pH (pH 6-7) where the absorption and activation of the drug are necessary. We believe that such PEG-POSS nanoparticles could be used as a potential carrier for insulin drug delivery systems. The release data have been further studied [22] by fitting the cumulative fraction release data,  $M_t/M_\infty$  to an empirical Equation (1). The drug release behavior according to diffusion controlled mechanism is usually governed by the following Equation (3),

$$M_t/M_\infty = kt^n \quad (3)$$

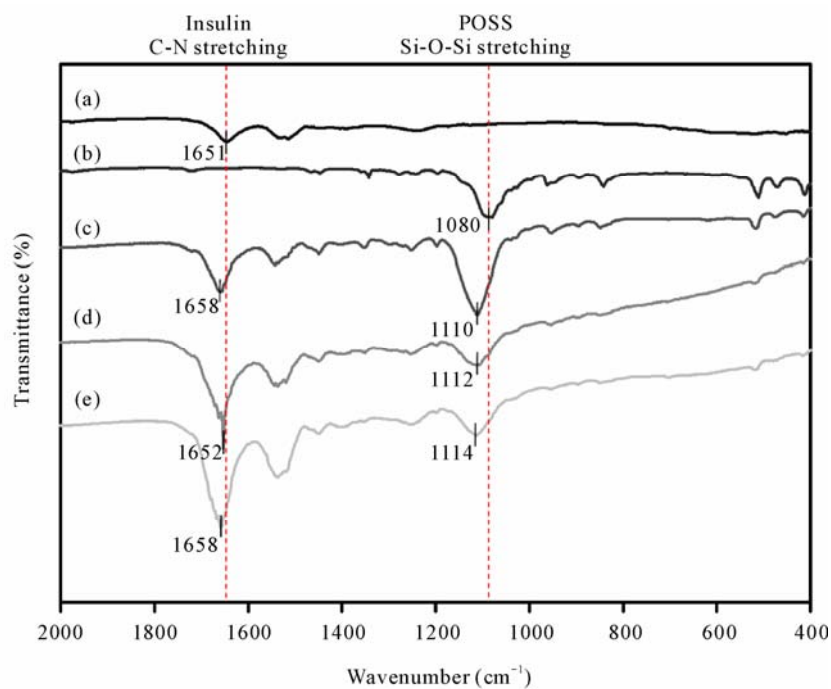
where  $M_\infty$  is the total amount of insulin in dosage form,



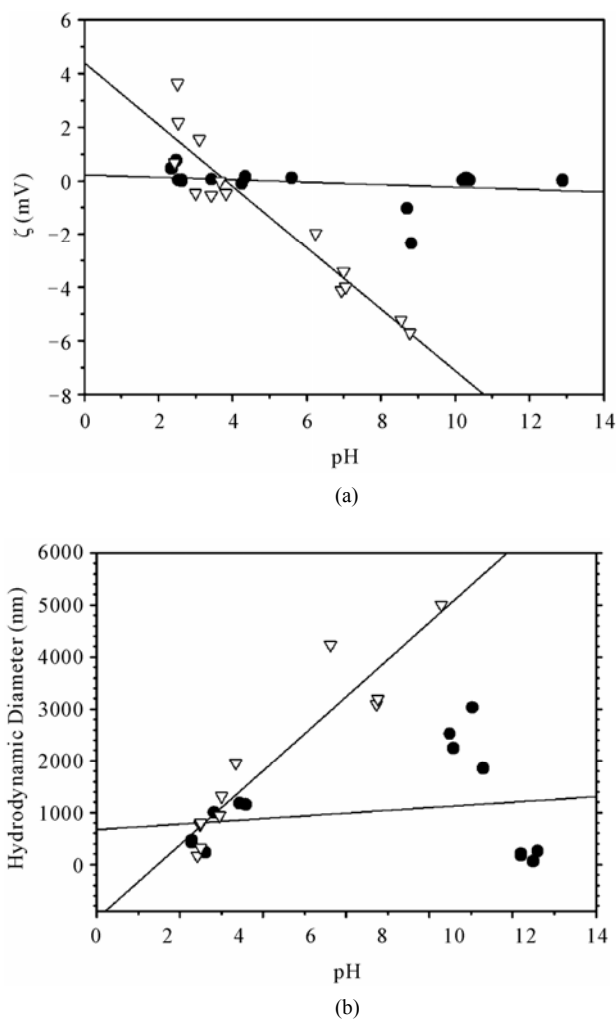
**Scheme 1. Schematic illustration of core-shell nanostructured PEG-POSS nanoparticles loaded with insulin.**



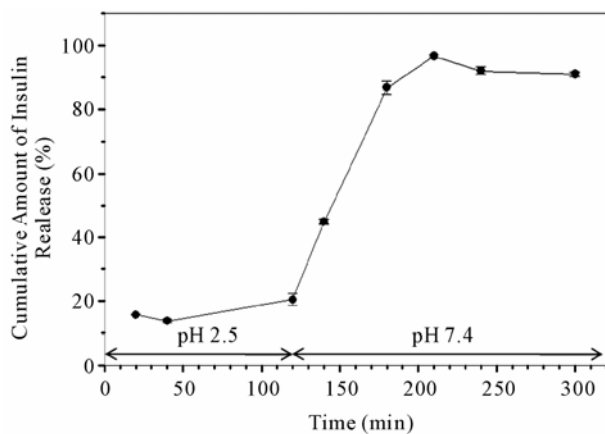
**Figure 1. TEM images of pure (a, b, c) and insulin-loaded (d, e, f) PEG-POSS nanoparticles at different magnifications.**



**Figure 2. FTIR spectra of (a) pristine insulin, (b) pure PEG-POSS nanoparticles, and insulin-loaded PEG-POSS nanoparticles with different amount of insulin of (c) 0.5, (d) 0.9 and (e) 1.3 mg.**



**Figure 3.** Zeta-potential (a) and particle size (b) of pure (●) and insulin-loaded (▽) PEG-POSS nanoparticles with respect to the pH.



**Figure 4.** Insulin release from insulin-loaded PEG-POSS nanoparticles produced with an insulin mass of 0.9 ml in gastric pH 2 simulated fluids for 2 hrs followed by additional 16 hrs in intestinal pH 7.4 simulated fluids at 37°C.

$M_t$  is the amount of insulin released at time  $t$ ,  $k$  is kinetic constant, and  $n$  is diffusion or release exponent constant. Using the least-squares procedure,  $n$  value was estimated to about 0.87, suggesting anomalous diffusion or non-fickian diffusion. This finding refers to combination of both diffusion and erosion controlled rate release. Accordingly, it is expected that insulin release from PEO-POSS nanostructured nanoparticles was pH-controlled, accompanying the swelling of insulin-loaded PEG-POSS nanoparticles.

#### 4. Conclusions

We have successfully prepared the pure and insulin-loaded nanostructured core-shell poly(ethylene glycol) (PEG)-polyhedral oligosilsesquioxane (POSS) nanoparticles via self-assembly process. TEM analysis demonstrated that pure and insulin-loaded self-assembled PEG-POSS nanoparticles were of spherical shape with core-shell nanostructure, and were well-dispersed and uniform in size distribution. Such PEG-POSS nanoparticles showed a good loading capability of hydrophobic drug, insulin. It was found that insulin was well-protected inside PEG-POSS nanoparticles at gastric pH for 2hrs, and was released at intestinal pH (pH 6-7) where the absorption and activation of the drug are necessary. As a result, insulin release from PEG-POSS nanoparticles was pH-dependent.

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