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Study on montmorillonite/insulin/TiO_2 hybrid nanocomposite as a new oral drug-delivery system

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ABSTRACT

This study was conducted in two main stages. In the first stage, drug-loaded montmorillonite nanocomposites were prepared by intercalation of insulin into the montmorillonite layers in acidic deionized (DI) water. In the second stage, to increase the release of insulin from the prepared nanocomposites they were coated with TiO_2, an inorganic porous coating, by using titanium (IV) butoxide, as precursor. The prepared nanocomposites were characterized by FT-IR, XRD, FE-SEM, BET, DLS and Zeta potential analysis. After investigating the release behaviour of the nanocomposites by UV–Vis absorbance technique, the results revealed that incorporation of porous TiO_2 coating increased the drug entrapment noticeably, and decreased the amount of drug release, so that nanocomposites without and with TiO_2 coating released the drug after 60 min and 22 h in pH 7.4, respectively. These results could be used in converting the insulin utilization from injection to oral.

1. Introduction

Diabetes is a high prevalence and one of the most severe and lethal diseases in the world. The international diabetic federation reported that 366 million people were affected by diabetes in 2011 and estimated that by 2030 this number will raise up to 552 million [1]. Insulin (Ins) is commonly used to treat diabetes in order to give patients a better life condition. As a protein drug used to treat diabetes, insulin has been conventionally administered via subcutaneous injection [2]. This type of administration have many disadvantages such as the inconvenience of multiple injections, occasional hypoglycemia due to insulin overdose, local tissue necrosis, microbial contamination, and most importantly, poor patient compliance with injections [3–5]. To resolve these problems, in recent years scientists and researchers have replaced the other methods [3,6–10]. Among the proposed methods, the oral administration is considered the most convenient alternative to deliver insulin [11], but it faces important challenges. The low stability of insulin in the gastrointestinal tract and its low permeability across biological membranes in the intestine, are drawbacks to overcome [2]. Therefore, the encapsulation or intercalation of insulin into suitable matrices is considered as a good strategy to improve insulin oral bioavailability [12].

The disadvantage of using organic matrices in oral insulin delivery such as polymer-based nanocarriers and nanoparticles is degradation of the matrix and then releasing insulin in acidic and enzymatic medium of gastrointestinal tract [13–15]. This problem could be obviated by utilizing the inorganic carriers, because these carriers could be more stable in the gastrointestinal tract, compared to organic matrices.

Layered silicate clays are particularly interesting because of their geometric platelet shapes and natural abundance. Among them, montmorillonite (Mt) is well known due to its large surface area and a high cation exchange capacity [16]. Montmorillonite has layered structure with tunable interlayer distance that can be used for intercalation of a variety of drugs [17–19]. Montmorillonite has hydrophilic and hydrophobic regions that could interact with different functional groups of the insulin. Moreover, montmorillonite is low cost, nontoxic and stable in the low pH environment of the stomach, so it could be a good carrier for intercalation of insulin.

In our previous works, we introduced a new technique using a simple and cost-effective procedure in drug delivery systems [20,21]. In the present study, to develop this technique we used montmorillonite as carrier for controlled release of insulin. After preparation of Mt/Ins nanocomposites, they were coated by different amounts of titanium dioxide and in this way Mt/Ins/TiO_2 hybrid nanocomposites were

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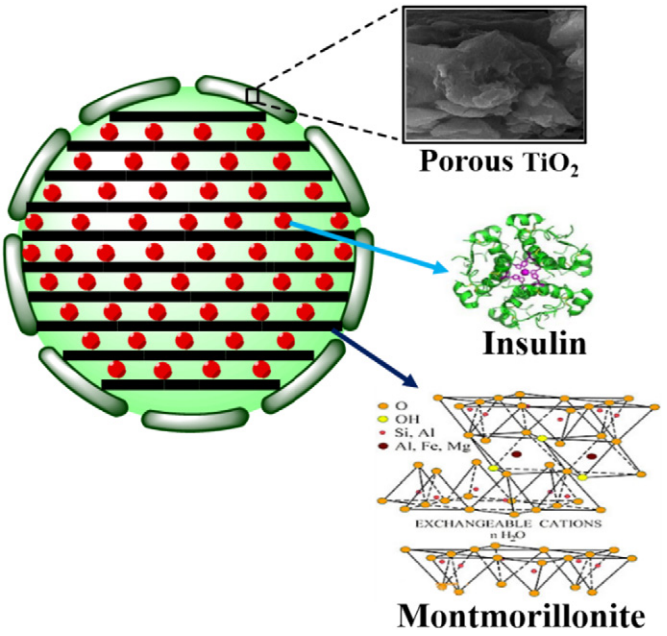

Scheme 1. Overall schematic of Mt/Ins/TiO_2 hybrid nanocomposite as a new oral drug delivery system.

prepared (Scheme 1). In vitro drug release of nanocomposites were studied by UV–Vis absorbance in pH 7.4 and the results showed that Mt/Ins nanocomposites released the whole insulin after 60 min, but the coated nanocomposites released about 70% after 10 h.

2. Experimental

2.1. Materials

Insulin (regular, human insulin, 1 vial of 10 mL, 100 units per mL) was obtained from Exir pharmaceutical Co. of Iran. Hydrochloridric acid (HCl, 37%), n-hexane, Na_2HPO_4, KH_2PO_4 and H_3PO_4 were obtained from Merck. Sodium montmorillonite (with cation-exchange capacity of 92 meq/100 g) and titanium (IV) tetra-n-butoxide (97%) were supplied from Sigma-Aldrich. All of reagents were used without any further purification. Human breast cancer cell line (MCF-7 cells) and human normal kidney cell line (Hek293T cells) were supplied from pasture institute (Tehran, Iran).

2.2. Characterization techniques

FT-IR spectrophotometer (Jasco 680plus) in the range of 400–4000 cm^{-1} was used to record the IR spectra. A diffractometer with Cu anode (Philips X'pert, PW3040, Netherland), scanning from 2° to 20° at 3°/min, was used for XRD analyses. The content of drug in buffer solution was quantified using UV–Vis absorbance (UV-2100, UNICO Instrument Corp.). The surface morphology of samples were observed by FESEM (TESCAN MIRA3) using an acceleration voltage of 15.0 kV. Adsorption-desorption isotherm of the selected nanocomposite was measured by NOVAWin2, version 2.2 (Quantachrome instruments). Surface area and mesoporosity of the nanocomposites were measured by the BET equation and BJH method, respectively [22,23]. The zeta potential and size distribution of samples were determined by Zeta sizer (Ver 6.00, Malvern Instrument Ltd., UK).

2.3. Preparation of Mt/Ins nanocomposites

For this purpose, initially 0.1 g montmorillonite was added to 50 mL deionized water and it was stirred for 2 h to be dispersed completely. Then, a calculated amount of insulin (10, 30, 50 wt% relative to the Mt) was added to the prepared suspension at pH 2 under vigorous stirring. Because insulin is difficult to dissolve in water, we decreased the pH of the solution with a diluted acidic solution until it reaches pH = 2. Also, at pH below 5.4, the charge of the hormone is positive and because Mt is a cation exchanger, the pH in which the intercalation takes place has a profound effect on the intercalation process, thus all the intercalation was performed without changing the pH (~2). After 3 days the products were filtered and rinsed several time with deionized water and dried under vacuum at 25 °C overnight to obtain montmorillonite/insulin (Mt/Ins) nanocomposites. The results of XRD analysis confirmed the intercalation of insulin between the montmorillonite layers. The information of prepared nanocomposites is summarized in Table 1.

2.4. Preparation of Mt/Ins/TiO_2 hybrid nanocomposites

In the first step, 0.1 g of the nanocomposite (Mt/Ins wt% = 50) was dispersed in 50 mL of dry *n*-hexane under vigorous stirring at 25 °C. Then given quantity of titanium tetra butoxide (30, 50, 100 wt%) dissolved in 5 mL of *n*-hexane and added dropwise to the mixture. After 1 h of stirring, to hydrolyse the titanium tetra butoxide the required amount of deionized water was added to the suspension. After 24 h, the resulting product was filtered by filter paper and washed few time with water and dried at room temperature. The information of synthesized hybrid nanocomposites is summarized in Table 2.

2.5. Drug release studies

In order to simulate the biological condition of body, the drug release of nanocomposites was investigated at the physiological temperature of 37 °C and pH 7.4. The content of Ins in buffer solution was quantified using UV–Vis absorbance (UV-2100, UNICO Instrument Corp.) at λ_{max} = 280 nm. For establishing environments similar to the gastric juice, intestine and blood, we used the buffer solutions with pH values of 1.2, 5.3 and 7.4, respectively. These buffer solutions were prepared according to the same procedure in our previous works [20,21].

2.6. Kinetic and mechanism of drug release

In this section, the prepared hybrid nanocomposites were studied kinetically using various important mathematical models to investigate the in vitro drug release behaviour [24]. The Korsmeyer–Peppas model has been described for different dissolution processes as the Eq. (1):

$$\frac{M_t}{M_\infty} = kt^n \quad (1)$$

In this equation, M_t/M_∞ is a fraction of drug released at time t, k is the release rate constant and n is the release exponent.

2.7. Cytotoxicity and cell viability assays

The in vitro cytotoxicity of montmorillonite/insulin/TiO_2 hybrid nanocomposite (B2) was investigated with MMT assay. For this purpose, MCF-7 and Hek293T cells were cultured in complete RPMI-1640 (Gibco, Scotland) medium supplemented with 10% fetal bovine serum and penicillin/streptomycin (100 units/mL). 200 μL of cell suspension (at concentration of 4×10^4 cells/well) was seeded into a 96-well U-

Table 1
Feed composition and drug content of Mt/Ins nanocomposites.

Entry	Abbreviation	Mt (g)	Ins (g)
Mt/Ins (10%)	A1	0.1	0.01
Mt/Ins (30%)	A2	0.1	0.03
Mt/Ins (50%)	A3	0.1	0.05