RESEARCH ARTICLE

Investigation of the stability of PEG stearate-coated Chitosan nanoparticles loaded with levothyroxine

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ABSTRACT

We report the formation and characterization of PEG stearate (PEG)-coated Chitosan (CS) nanoparticles. Chitosan nanoparticles were synthesized using tripolyphosphate (TPP) via the ionic crosslinking method. Preparation of PEG Stearate-grafted Chitosan is essential to improve the biocompatibility and water solubility of Chitosan. The size and morphologies of Chitosan nanoparticles were measured with transmission electron microscopy and scanning electron microscopy. Sizes of Chitosan nanoparticles were in the range of 150-200 nm. The particle size and zeta potential of PEG Stearate-coated Chitosan had been measured at 187.5 nm by Photon Correlation Spectroscopy (PCS). Drug entrapment efficiency (EE) was obtained to be 99%. The purpose of the present work was to develop a new nanoparticle system, consisting of polymeric nanoparticles coated with PEG Stearate. The modification procedure led to a reduction in the zeta potential values, varying from +43.3 mV for the uncoated particles to +20 mV for that of PEG Stearate-coated Chitosan. PEG Stearate coated nanoparticles were more stable due to their polymer coating layer which prevented aggregation of Chitosan nanoparticles. Consequently, it is possible that the PEG Stearate surrounds the particles reducing the attachment of enzymes and further degradation of the polymeric cores. Properties nanoparticles were affected by the preparation variables and the coating layer. Chitosan nanoparticles showed a smooth surface and globular shape. In this study, we explored the release behavior of levothyroxine was affected by the coating layer. Coating surface leads to a decrease in the burst release effect compared to uncoated nanoparticle due to gradual release of adsorbed levothyroxine from PEG coated Chitosan nanoparticles.

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INTRODUCTION

Chitosan is a neutral polysaccharide polymer that is widely applied in pharmaceutical products. This biopolymer is composed of β-2-amino-2-deoxy-D-glucopyranose (glucosamine units). Monomer units are combined by β-1,4-glycosidic linkages. Chitosan has unique properties such as biocompatibility, biodegradability, safety, and mucoadhesive. Pharmaceutical usage of chitosan is due to the amino groups on its chain. Chitosan application as a biomaterial to provide controlled release systems has received much attention [1-4]. Several studies have also increasingly focused on improving their stability in body fluids after administration by coating particles with hydrophilic polymers like PEG stearate derivatives [1].

Levothyroxine sodium, the same as many other hydrophobic drugs, is poorly transported through the intestinal epithelium. Levothyroxine is a drug with a narrow therapeutic index for which dosage must be titrated for each individual patient to achieve the necessary therapeutic effect [5]. Thyroid hormones affect protein, lipid and carbohydrate metabolism, influencing growth and development [6]. Diminished or absent thyroid function may result from functional deficiency, primary atrophy, the partial or complete absence of the

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thyroid gland, or the effects of surgery, radiation, or antithyroid agents [7]. Levothyroxine sodium may also be used for replacement or supplemental therapy in patients with secondary (pituitary) or tertiary (hypothalamic) hypothyroidism [5-8]. The development of more convenient extended-release methods for levothyroxine sodium delivery may encounter an innate challenge: the vehicle must also provide a controlled and defined dose within the narrow therapeutic window across the entire time period of drug release [6-7]. The main advantage of this study is to modify the surface of Chitosan nanoparticles for increasing intestinal absorption and decreasing nanoparticles uptake by immune system as a result of partial neutralization of strong negative or positive charge on nanocarrier surface [1, 8-11]. Moreover, surface modification of Chitosan nanoparticles by coating a hydrophilic polymer i.e. PEG stearate lead to enhancement of surface hydrophilicity of nanoparticles which results in an increment of nanoparticles transmucosal transport for drug delivery by nasal, oral and ocular way [8-13]. Controlled release efficiency and intestinal the wrapped drug destruction in the stomach and intestine and probable absorption of the drug through the intestinal mucosa are two different absorption models of drug [10-11].

MATERIALS AND METHODS

Materials

Chitosan was purchased from Sigma–Aldrich (USA) with a MW of $60*10^3$ and the degree of deacetylation was 93%. Levothyroxine sodium was obtained as a gift from the Iran Hormone Company. PEG stearate purchased from fluka, Tripolyphosphate (TPP), polysorbate 80 (Tween 80), acetic acid and other reagents were purchased from Merck.

Preparation of TPP-Chitosan nanoparticles

Chitosan solution was prepared by dissolving it in 1% acetic acid and polysorbate 80, as an emulsifier, (2% v/v) was mixed into the Chitosan solution. Levothyroxine, poorly soluble drug in water, was dissolved in DMSO (2:10) and then this oil phase was mixed with aqueous phase (Chitosan solution) by probe sonication applying 20 kHz frequency for 10 min. TPP solutions were dropped by spray gun into the Chitosan solution. Then, nanoparticles were washed with PEG-stearate solution then they were washed with distilled water repeatedly followed by vacuum drying for 12 h [4].

Drug content of nanoparticles

Drug loading was determined by ultracentrifugation of nanoparticles solution in 40000 rpm for 45 min, and then the amount of levothyroxine in the supernatant was measured using a spectrophotometer (Agilent 8453) at 225 nm. Loading efficiency (DL) % can be calculated as follows:

DL % = [(total amount of drug – free amount of drug) / total amount of drug]×100 [3].

In vitro release of levothyroxine from Chitosan nanoparticles

The experiment was conducted by a static horizontal Franz diffusion cell to evaluate the amount of releasing levothyroxine sodium. The receptor medium was 50 mL in volume and composed of an aqueous solution of physiological saline, phosphate buffer solution, which stirred by a magnetic stirrer at 100 rpm to homogenize the medium (pH=7.4). A 2 ml of Chitosan nanoparticles were separately loaded onto the donor compartment. 1 ml of the release medium was sampled using a syringe needle and the same volume of the fresh receptor medium was replaced at certain time intervals. The samples were analysed using a spectroscopic method as described previously [8].

Morphological characterization of Chitosan nanoparticles

Size and zeta potential were measured by photon correlation spectroscopy (PCS) (Nano ZS4700 nanoseries, Malvern Instruments, UK). To capture the transmission electron microscopy (TEM) (Zeiss-EM 10C- Germany) microphotographs, the nanoparticles samples stained with 2% (w/v) phosphotungstic acid were employed. The investigation of surface morphology of nanoparticles was performed by scanning electron microscopy (SEM, KYKY-EM3200, China).

Determination of entrapment efficiency and drug-loading capacity

The efficiency of drug entrapment was investigated by measuring the concentration of the drug in the supernatant of ultracentrifuged PEG-Chitosan solution. To achieve this purpose, PEG Stearate-Chitosan solution was ultra-centrifuged at 40,000 rpm for 45 minutes. The absorption of the upper solution was determined by UV spectrophotometer at 225 nm (Agilent 8453). In this way, the quantity of free drug could be

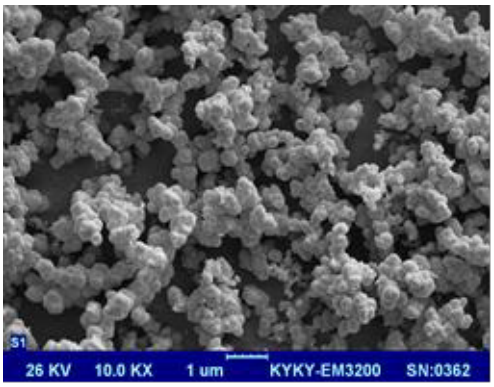

Fig. 1. SEM image of chitosan nanoparticles

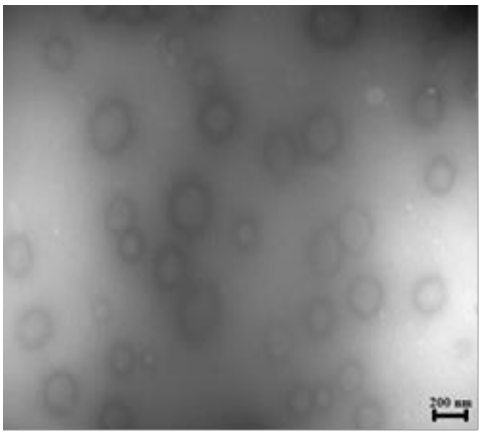

Fig. 2. TEM image of chitosan nanoparticles

obtained. The drug entrapment efficiency and drug-loading in the coated and uncoated chitosan NPs were calculated as follows: [12-13]

DL % = [(total amount of drug − free amount of drug) / total amount of drug] ×100

Stability of PEG 100-S-coated chitosan in simulated gastric media2.7.

A 1% (w/v) solution of particles was incubated in acid medium (0.063 M) and 0.320% w/v of pepsin to obtain a suspension at pH 1.2. Evaluation of the stability of coated and uncoated chitosan NPs, which were incubated in HCl medium for 1 h, was also performed. Subsequently, chitosan NPs were separated from the medium using centrifugation at 3000 × g for 5 min. The size of chitosan NPs in the upper solution was reexamined by Photon Correlation Spectroscopy (PCS). Relative turbidity is the ratio between the absorbance [Optical density (OD)] at λ = 225 nm after 1 h and before incubation to estimate the degradation of coated and uncoated nanoparticles in HCl (0.063 M) .[11,14-15]

RESULTS AND DISCUSSION

In this study TPP-Chitosan nanoparticles were fabricated by the virtue of ionic interaction between positively charged amino groups of chitosan and the negatively charged counter ions of TPP. It should be noted, TPP is a non-toxic and multivalent anion that can form cross-links [16-18].

SEM image of the samples containing drug loaded chitosan nanoparticle is shown in Fig. 1. The image showed spherical shape and smooth surface with a particle size in nanometric scale. The particle size of levothyroxine-loaded chitosan nanoparticles showed SEM images are in agreement with the results obtained by TEM (Fig. 2) and the result of PCS. All applied techniques are indicative of circular shape and no aggregation of the nanoparticles. The average diameter determined by both SEM and TEM was found to be around

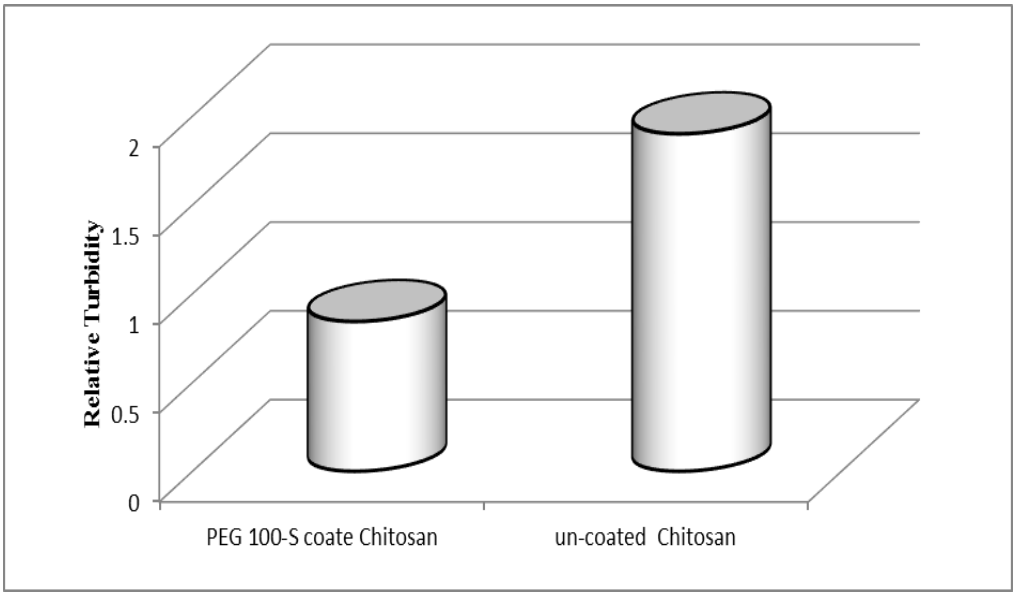

Fig. 3. Relative turbidity (Relative turbidity is the ratio between the absorbance [Optical density (OD)] at λ = 225 nm after 1 h and before incubation

200 nm [19-21]. The results of stability of NPs have been shown in Fig. 3. These results indicate that the gastric medium compromised the stability of the particles investigated in a different manner. Different behaviors were observed for uncoated and coated nanoparticles. Whereas the uncoated nanoparticles showed an increase in their particle size during the experiment, the PEG-stearate coated formulations underwent a small size increase. The clear size increase observed for the uncoated particles could be related to the known destabilizing effect that the acidic medium and enzymes have on lipid nanoparticles. It is possible that the PEG-S brush around the particles reduces the attachment of the enzymes and the further degradation of the triglycerides core [20-23]. A similar situation was observed for the lipid nanoparticles. Non-coated lipid nanoparticles displayed an instantaneous and massive aggregation following their incubation in gastric medium, whereas protective coatings such as PEG -stearate reduced this process. More specifically coated nanoparticles showed a slight size increase while the size of PEG-stearate coated nanoparticles remained unchanged. Moreover, this protective coating was found to prevent the molecular degradation of the triglyceride since less than 5% degradation was observed in gastric medium. In contrast, no information about molecular degradation is available for non-coated particles, as their immediate aggregation did not allow us to perform the corresponding assay. Consequently, the absence of degradation observed in all the polymer-coated nanoparticles suggests that neither acidity nor gastric protease was able to cleave the triglyceride ester bonds [22-23]. As we observed (Fig. 3) and also indicated in the literature uncoated chitosan NPs displayed an instantaneous aggregation following their incubation in gastric medium, whereas protective coatings such as PEG-stearate reduced this process [24-25]. In order to investigate whether pH is an important factor in the stability and degradation of the uncoated and PEG- stearate coated nanoparticles observed in the gastric medium, the stability of uncoated Chitosan NPs in an acidic medium (pH 1.2) was measured by estimating the relative turbidity at times 0 and 1 h post-incubation. Increase in relative turbidity demonstrates addition of levothyroxine concentration after incubation in acidic medium due to degradation of nanoparticles [22-24]. Uncoated Chitosan NPs showed an increase in relative turbidity during the experimental period, while a little change in relative turbidity was observed in the case of PEG-S Chitosan NPs (Fig. 3). The levothyroxine release of PEG- coated Chitosan NPs had been shown in Fig. 4. Following the initial burst, the systems provided a continuous and slow release of the levothyroxine.

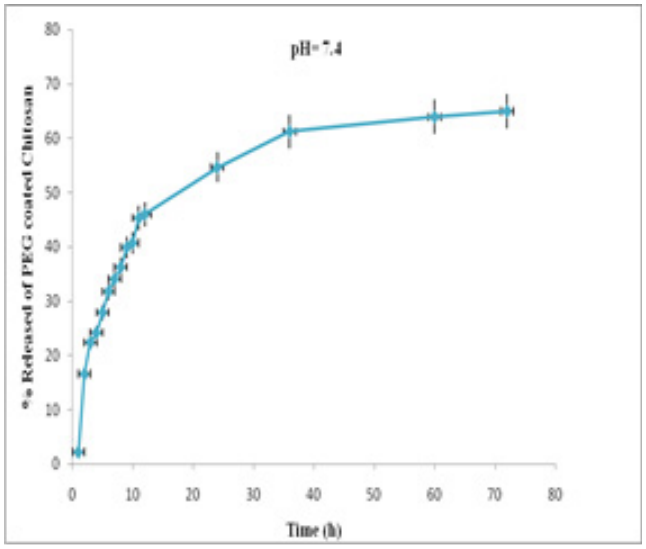

Fig. 4. Profiles Release of Levothyroxine from chitosan nanoparticles in phosphate buffer in pH=7.4

CONCLUSIONS

During the last decade, nanomedicine has provided a promising new field in medicine to apply nanoscale materials for drug delivery. Chitosan nanoparticles were prepared with tripolyphosphate (TPP) by ionic crosslinking method. The size and morphology of chitosan nanoparticles were determined by TEM and SEM that obtained in the range of 190-250 nm. Encapsulation efficiencies of levothyroxine were measured to be 85%. Increase in relative turbidity demonstrates the addition of levothyroxine concentration after incubation in acidic medium due to degradation of nanoparticles. Uncoated Chitosan NPs showed an increase in relative turbidity during the experiment period, while a little change in relative turbidity was observed in the case of PEG S- Chitosan NPs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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