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Synthesis, Characterisation of 5-Fu Loaded Chitosan Nanoparticles

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ABSTRACT

In the present study, 5-fluorouracil-loaded-Chitosan nanoparticles were prepared by ionic gelation technology. In the experiment chitosan dissolved in 1% acetic acid and TPP and 5 FU dissolved in the distilled water. TPP-Drug solution added to chitosan solution drop by drop under magnetic stirrer. Drug encapsulated on generated nanoparticles as a result of electrostatic interactions, The particle size, size distribution, surface morphology, and physical and chemical properties of the 5-FU-NPs were investigated by scanning electron microscopy (SEM), Dynamic light scattering (DLS) and Fourier transform infrared spectrometry (FTIR). The drug encapsulation efficiency (EE) of 5-FUNPs was investigated by LC-MSMS (Liquid Chromatography-Mass spectroscopy Mass spectroscopy). The drug loaded chitosan nanoparticles are roughly spherical in shape with a size distribution range of 300 to 350 nm in diameter. The zeta potential of the chitosan nanoparticles is 48.2 mV, The drug loading capacity of 5-FU loaded chitosan nanoparticles was 29.3%.

KEYWORDS : 5- Fluorouracil, Nanoparticles, Chitosan

INTRODUCTION

5-Fluorouracil (5-FU), a pyrimidine analogue that interferes with thymidylate synthesis, has a broad spectrum of activity against solid tumors, which is employed most extensively in clinical chemotherapy for the treatment of carcinomas of the colon or rectum, precancerous dermatoses and breast cancer (Seda *et al*., 2012).

The potential use of nanoparticles as drug carriers has been represented over the last few years as an important challenge, since nanoparticles have been designed to improve the pharmacological and therapeutic effects in terms of reducing their toxic side effects (Brannon-Peppas and Blanchette, 2004; Feng, 2004). Biodegradable polymer nanoparticles have attracted great interest in recent years for clinical administration of antitumor drugs (Paul and Sharma, 2000). Slow release and sustained drug action on lesions, Systemic side effects will be reduced, tumor targeting and a high capability to cross various physiological barriers are the main advantages of these biodegradable nanoparticles (Duarte *et al.,* 2006).

Chitosan is an abundant polysaccharide present in nature, which can be obtained by partial deacetylation of chitin in alkaline solution (Subramanian *et al.,* 2006). Chitosan is attracting more and more attention in the application as a drug delivery carrier due to its intrinsic nature, such as biodegradability, biocompatibility, nontoxicity, nonimmunogenic, noncarcinogenic, and antibacterial properties (Sun and Wan, 2007; Tiyaboonchai, 2003). It is a hydrophilic polymer with positive charge, which reveals a special characteristic to chitosan and it is an ideal drug delivery carrier for hydrophilic drugs such as 5-fluorouracil (Janes *et al*., 2001; Li *et al*., 2011a).

Like many other chemotherapeutic drugs, 5-FU also has limitations that include a short biological half-life due to rapid metabolism, toxic side effects on bone marrow and the gastrointestinal tract, and non-selective action against healthy cells. Thus, it has been suggested that chitosan nanoparticles might prevent the side effects induced by 5-FU (Su Li *et al*., 2008).

It's necessary to make some attempts to reduce its toxic and side effects and improve the selectivity. Hence, by considering all these points the present study was undertaken to synthesize and characterize 5- fluorouracil loaded chitosan nanoparticles

MATERIALS AND METHODS

Chitosan was purchased from Sigma-Aldrich (Medium Molecular Weight). The degree of deacetylation and molecular weight for the medium-molecular-weight chitosan (MWM chitosan) is 70–85% and 190–300 kDa based on viscosity, respectively. Sodium tripolyphosphate (TPP) (purity: 85%) and 5-fluorouracil (5-FU) (purity: 99.9%) were purchased from Sigma-Aldrich. All other reagents were in analytical grade.

Preparation of 5-FU loaded Chitosan nanoparticles

The preparation of 5-FU loaded chitosan nanoparticles (5-FUNP) is based on an ionic interaction between positively charged chitosan solution and negatively charged TPP solution. Chitosan dissolved in 1% aqueous acetic acid solution at a concentration of 3mg/mL, and TPP was dissolved in distilled water with a concentration of 2 mg/mL. 5-FU was dissolved directly in TPP solution at 1mg/dL concentrations before the synthesis of chitosan nanoparticles. Then, 4mL TPP solution (2 mg/mL) containing combination drugs was dropped into

10mL chitosan solution under magnetic stirring (1000 rpm) at room temperature. Nanoparticles were formed and suspension was kept stirring for 30 min for further crosslinking of nanoparticles. Finally, Chitosan nanoparticles were collected by centrifugation at 15,000 rpm and freeze-drying at -40°C for 24 h.

Charecterization of Nanoparticles

Scanning electron microscope (SEM) analysis

The Size and morphological characteristics of nanoparticles were observed under a scanning electron microscope (Quanta™250 FEG). One drop of dilute chitosan nanoparticles' solution and 5-FU encapsulated chitosan nanoparticles' solution was dropped on SEM sample stub and let air-dried and coated with gold film. Average size of unloaded and drug loaded nanoparticles were determined by using Image J software.

Fourier Transform Infrared (FTIR) Spectra Studies.

FTIR spectra of 5-FU loaded chitosan nanoparticles were recorded on KBr pellets with a FT-IR spectrophotometer (Thermo Scientific Nicolet iS10, USA)

Particle size and zeta potential measurement

Particle size distribution of 5-FU loaded chitosan nanoparticles was analyzed through Dynamic Light Scattering (DLS) analysis with Zetasizer Nano S (Malvern, UK). One ml of nanoparticle solution was taken in cuvet and placed in cuvet holder and reading was taken. The analysis was performed in triplicate at a temperature of 25◦C.

Evaluation of drug loading capacity

Encapsulation efficiencies of prepared chitosan nanoparticles were determined by LCMS-MS (MDS SCIEX Q-TRAP API 3200 mass spectrometer, CA, USA, equipped with an electro-spray ionization source)

RESULTS AND DISCUSSION

5-FU loaded chitosan nanoparticles

The preparation of 5-FU loaded chitosan nanoparticles was based on the ionic interaction of a positively charged chitosan solution and negatively charged TPP solution. The charge density of both Chitosan and TPP solution has a great effect on the ionic interaction.

5-FU loaded chitosan nanoparticles were prepared by ionic crosslinking method between chitosan and TPP solutions with 5-FU. This size of nanoparticles which may be due to the simultaneous electrostatic interaction attractions between TPP and 5-FU with chitosan. 5-FU encapsulated chitosan nanoparticles formed instantaneously when readily mixed TPP- 5-FU is added to chitosan solutions. The formation of nanoparticles depends dramatically on the concentration of free amino groups, which strengthens the electrostatic interactions between the nanoparticles and the drug, helping to reduce the particle sizes [Yong and Hon, 2009]. It is well known that 5-FU is negatively charged; since the pKa of the 5-FU is 8.0 and the pH of 5-FU solution is 8.4, 70% of 5-FU is ionized and negatively charged in the solution [Kondo and Araie, 1989]. Thus, an electrostatic attraction might exist between chitosan and the negatively charged drug while ionic gelation was performed between chitosan and TPP [Csaba *et al*., 2009].

Particle size analysis

Nanoparticles were successfully prepared in the nanometer range, SEM image showed an average size 5-FUNP was 320 nm. These nanoparticles were spherical in shape, with a narrow size distribution. Size of nanoparticles also confirmed by DLS (Dynamic light scattering).

It had been shown that parameters like molecular weight and deacetylation degree of chitosan dominantly affect chitosan nanoparticle size [Gan *et al*, 2005; Zhao and Wu, 2006; Csaba, 2009]. It is well known that particle size plays an important role on mucosal and epithelial tissue uptake of nanoparticles and on the alternation of pharmacokinetics by affecting the tissue distribution and clearance (Devalapally *et al.,* 2007).

Zeta potential and PDI measurements

Surface charge and there by the stability of the prepared nanoparticle system was determined by zeta potential measurements. Zeta potential value for the 5-FUNP was found to be 48.2 mV. This value lies in the stable range, indicating that the nanoparticles system was stable and possess negative surface charge.

DLS showed poly dispersity index (PDI) 0.271, which indicate highly uniform size 5-FUNP and also it showed average particle size diameter of nanoparticles189nm and 95.2 % of nanoparticles were below 320nm.

It has been reported that the value of zeta potential less than -40 mV or higher than +40 mV could be used to assure the good stability of nanoparticle suspensions. The positive values obtained for zeta potential indicated that the nanoparticle surface was positively charged. This may be due to the availability of free NH^{+3} groups on the polymer (Li *et al.,* 2011a). The values obtained for the prepared nanoparticles in the present study were above +40 mV, so it was stable.

FTIR analysis

Chitosan spectra showed the characteristic transmission bands of amide I band, N-H bending and C-N stretching were at 1654 cm^{-1}, 1592 cm^{-1} and 2874 cm^{-1} respectively. Spectra of the 5-FU showed transmission bands of amide I band, N-H bending, C-F stretch (Ar-F group) strong, ring overtone and C=O stretching were at 1650 cm^{-1}, 1502 cm^{-1},1244 cm^{-1}, 1896 cm^{-1} and 994 cm^{-1} respectively.

After ionic cross linking with mixture of TPP and chitosan, the characteristic peak at 1654.14 cm^{-1} in the Chitosan spectra shifted to 1546.00 cm^{-1}, while the peaks at 1592.59 cm^{-1} shifted to 1407.53 cm^{-1} due to the ionic interaction between positively charged chitosan and TPP solution. Characteristic peak at 1502 cm^{-1} in the 5-FU spectra disappeared in the 5-FUNP due to the ionic interaction between 5-FU and chitosan. Most of other peaks of 5-FU are not observed at the same position in the drug loaded nanoparticles, indicating the intense interaction between the drug and chitosan.

In the nanoparticles observed characteristic peak shift was due to the potential interaction of protonated amine/amide groups and negatively charged TPP cross-linking agent. The two possible mechanisms could be TPP interaction with the protonated amide and/or with the protonated amine from the residual chitosan (Rejinold *et al.,* 2010).

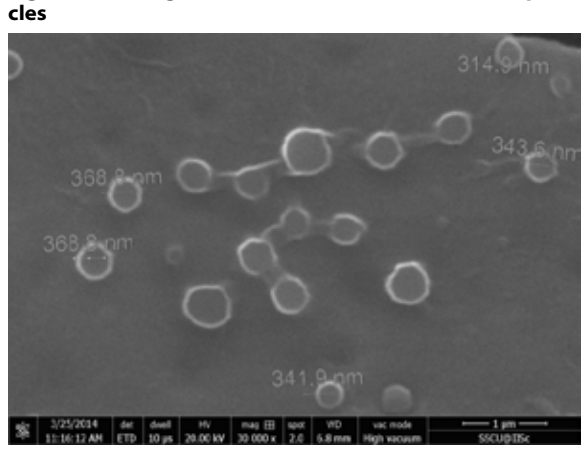
Drug loading capacity

During preparation of 5FU loaded chitosan nanoparticles, 100 mg of 5FU dissolved in 200 ml of chitosan and mixed with 80 ml of TPP, after lyophillization 180 mg of nanoparticles were yielded. By LC-MS/MS Studies, it was found that 5-FU Concentration in 5-FU loaded chitosan nanoparticles was 29300 ng/mL. 29.300 mg of 5-FU was present in every 100mg of nanoparticles. The drug loading capacity of 5-FU loaded chitosan nanoparticles was found to be 29.3%.

CONCLUSIONS

In the present work, 5- Fluorouracil loaded Chitosan nanoparticles were successfully prepared by ionic gelation. 5- Fluorouracil encapsulated chitosan nanoparticles would offer several advantages over conventional drug therapies and also expected to overcome side effects regarding to dosing and toxicity. However, further optimization studies like *in vitro* and *in vivo* toxicity, efficacy and also stabilization and targeting studies have to be performed.

Fig.1. SEM images of 5-FU loaded chitosan nanoparticles



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