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Development and characterization of chitosan nanoparticles loaded with isoniazid for the treatment of Tuberculosis

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ABSTRACT

The objective of our study is to load first line antitubercular drug, isoniazid in chitosan Nanoparticles in order to enhance bioavailability and to reduce dose frequency. Chitosan was dissolved in acetic acid aqueous solution at various concentrations; Drug was dispersed in above Chitosan solution kept over magnetic stirrer at room temperature for a period of 30 minute. The Tripolyphosphate aqueous solution with various concentrations added drop wise to the above solution. Followed by sonication for 5 min. The resulting Chitosan nanoparticles suspension was centrifuged at 16,000 rpm for 30 min. After freeze drying the Nanoparticles were collected. SEM studies show that formulation No: 2 having optimum nanonised particles. Zeta potential shows good positive potentials. It shows good encapsulation efficiency. And good release profile follows first order release kinetics. From all these results it concludes that formulation No 2 is the best formulation and which is recommended for future studies like Nano dry powder preparation.

Keywords: Chitosan, Nanoparticles, TB, Isoniazid

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INTRODUCTION

Chitosan has been used as a nanoparticle material owing to its versatile biodegradability, biocompatibility, and natural origin. Drug-loaded nanoparticles have the potential to be used for pulmonary delivery of therapeutics for treating lung diseases. Delivery of therapeutic agents to the site of action for lung diseases may allow for efficient treatment of, tuberculosis. Isoniazid is an organic compound used as the first-line antituberculosis medication in the treatment of Tuberculosis.

A total of 196 (out of 212) countries and territories (hereafter “countries”) reported data; these countries collectively account for 99.6% of the world’s estimated TB cases.[1,2] Among countries which reported, at least 75% of the requested data were provided by 70–80% of countries for most sections of the data collection form. The topics for which reporting of data was much less complete were collaborative TB/HIV activities, treatment outcomes for patients with multidrug-resistant TB (MDR-TB), and public–public and public–private mix (PPM) [3,4].

Biodegradable nanoparticulate systems have received considerable attention as potential drug delivery vehicles. Chitosan (CS), a polysaccharide known to be a favorable pharmaceutical material because of its biocompatibility and biodegradability, forms an ideal hydrophilic carrier system^[5] Moreover, chitosan has been shown to be non-toxic and tissue compatible in a range of tests. Nanoparticles, which can be produced with a wide variety of polymers and nanotechnologies, have also been recently proposed as delivery systems for peptides and proteins through the pulmonary route [6]. In this respect, chitosan is a very attractive polysaccharide due to its reported low toxicity, biodegradability and mucoadhesivity.[7] In fact, chitosan has been demonstrated to induce low or absent toxicity in cell lines representative of the pulmonary route [8,9] .Our group has introduced the preparation of chitosan/tripolyphosphate (CS/TPP) nanoparticles by an extremely mild and rapid ionotropic gelation procedure between chitosan and its counter ion TPP which show an excellent capacity for protein association (as high as 90%), as well as an improvement of peptide absorption through several epithelia, such as the nasal, ocular and intestinal [10].Furthermore, recently reported the production of microspheres as carriers for protein-loaded chitosan nanoparticles to the lung, with the aim of improving their aerosolisation patterns.[11] These nanoparticle-loaded microspheres, obtained by spray-drying a suspension of nanoparticles in mannitol, exhibited adequate aerodynamic properties for lung delivery and demonstrated to be biocompatible with respiratory epithelial cell layers.[12] The spray-drying of liposomes has been reported to not compromise their stability and a work on the spray drying of solid lipid nanoparticles demonstrated that the presence of carbohydrates like mannitol, lactose and trehalose provided an increased stability to the spray-dried product, because the sugar layer around the particles prevented the lipids coalescence[13,14].Chitosan nanoparticles are obtained by the process of ionotropic gelation based on the interaction between the negative groups of the pentasodium tripolyphosphate (TPP) and the positively

charged amino groups of CS. This process has been used to prepare CS nanoparticles for the delivery of peptides and proteins [15].

The aim of this work was to develop a new formulation of Isoniazid based on chitosan nanoparticles for possible targeted delivery to the lungs.

MATERIALS AND METHODS

Preparation of chitosan nanoparticles

Chitosan nanoparticles were prepared according to the ionotropic gelation process. Blank nanoparticles were obtained upon the addition of a tripolyphosphate (TPP) aqueous solution to a Chitosan solution (3mg/ml) stirred at room temperature. The formation of nanoparticles was a result of the interaction between the negative groups of TPP and the positively charged amino groups of chitosan. The ratio of chitosan/TPP was established according to the preliminary studies. Isoniazid loaded nanoparticles were obtained according to the same procedure and the ratio of chitosan/TPP remained unchanged. Variable amounts of peptide were incorporated to the chitosan solution prior to the formation of nanoparticles in order to investigate the effect of the isoniazid concentration on the nanoparticle characteristics and In-vitro release profiles. Nanoparticles were collected by centrifugation at 16,000 rpm for a period of 30 minutes and supernatants were discarded. Followed by freeze drying, nanoparticles were collected.

Characterization of the nanoparticles

Scanning electron microscopy (SEM) was performed using a LEO 1530 (LEO Electron Microscopy Inc., Thornwood, NY) operating between 1 and 3 kV with a filament current of about 0.5 mA. Liquid samples were deposited on vitreous carbon stubs and dried at room temperature. They were coated with a palladium–platinum layer of about 4 nm using a Cressington sputter-coater 208HR with a rotary-planetary-tilt stage, equipped with a MTM-20 thickness controller. The size (Z-average mean) and zeta potential of the nanoparticles were analyzed by photon correlation spectroscopy and laser doppler anemometry, respectively, in triplicate using a Zetasizer 3000HS (Malvern Instruments, UK).

Evaluation of Isoniazid encapsulation

The Isoniazid loaded nanoparticles were separated from the aqueous suspension medium by centrifugation at 16,000 rpm and 25°C for 30 minute. The amount of free Isoniazid was measured in the clear supernatant by UV measured at a wavelength of 262 nm. The isoniazid loading capacity (LC) of the nanoparticles and their % loading capacity (%LC) were calculated.

Drug Excipient Compatibility Study Using FT-IR

FT-IR spectra were recorded with a FT-IR Shimadzu FTIR 8400S spectrometer in range 400–4000 cm^{-1} using a resolution of 4 cm^{-1} and 10, Weighed amount of drug (3mg) was mixed with 12 mg of potassium bromide. The mixture was taken and compressed under 10-ton pressure in a hydraulic press to form a transparent pellet. To evaluate the molecular states of micronized isoniazid and nano-isoniazid and also for the drug interaction study.

Mathematical modeling of isoniazid nanoparticles

In order to investigate the mode of release from the microcapsules, the release data were analyzed with the following mathematical models.

$$Q_t = K_0 t \text{ (Zero Order Kinetics)}$$

$$\text{Log} (Q_t / Q_0) = - K_1 t / 2.303 \text{ (First order Kinetics)}$$

$$Q_t = K_{KP} t^n \text{ (Korsmeyer and Peppas equation)}$$

$$Q_t = K_H t^{1/2} \text{ (Higuchi's equation)}$$

Where, Q_t is the percent of drug released at time 't', K_0 , K_1 , K_{KP} , K_H and K_{HC} are the coefficients of Zero order, First order, Korsmeyer-Peppas and Higuchi's equations.

RESULTS

FTIR Study

Many peaks of chitosan were observed which shows a broad-OH stretching absorption band between 3450 and 3100 cm^{-1} . Another major absorption band is between 1220 and 1020 cm^{-1} which represents the free amino group (-NH₂) at C2 position of glucosamine, a major peak present in chitosan. Peak at 1384 cm^{-1} represents the -C-O stretching of primary alcoholic group. Peak at 1184 cm^{-1} is due to ketone group present in the isoniazid.

Drug Entrapment Studies

Micro encapsulation efficiency of F1 formulation nanoparticles were found to be 67.76 %, F2 formulation Nanoparticles were found to be 90.90 %, F3 formulation nanoparticles were found to be 74.07 %. followed by F4 which accounts for 60.45, and F5 was 76.25 respectively. Ionic interaction between the polymer and drug may lead to increase entrapment of the drug in nanoparticles.

Particle Size and Morphological Characterization

Figure 1 and 2 shows that SEM study of Chitosan TPP Nanoparticle and Isoniazid-Chitosan Nanoparticles. Chitosan nanoparticles were prepared according to the procedure first

reported by Calvo et.al. (1997) based on the ionic gelation of CS and TPP anions. Nanoparticles as targeted carrier for Isoniazid were prepared by sodium TPP cross linking with using biocompatible and biodegradable polymer chitosan. The solubility of chitosan in acetic acid is important factor related to nanoparticles formation. Chitosan suspension was observed to be milky appearance & white color.

Isoniazid-Chitosan-Nano have average particle size diameter of 250 nm & 800 nm with positive zeta potential at maximum and minimum chitosan concentration. From the result it was observed that nanosize particles diameter, and zeta potential depends upon the chitosan concentration & isoniazid concentration.

In Vitro Release of Isoniazid from Nanoparticles

In vitro studies were carried out with optimized formulation for their in-vitro studies release pattern across cellophane membrane. Initial release of the drug is associated with those drug molecules dispersing close to the nanoparticle surface. The drug release many depend upon the chitosan concentration. Fig.: 3 shows that In F2 formulation showed drug release of 93.25% with in 24 hour give release pattern in controlled manner; F3 formulation showed drug release 74.07 % with in 24 hour give release pattern in controlled manner; F5 formulation showed drug release of 69.19 % with in 24 hour give release pattern in controlled manner. In first hour F1 formulation give burst release and then give sustained release as controlled manner. On comparison of the release profile of the three formulations, it was observed that release from formulation F3 was found to be slow and constant manner. From this observed data shows that increasing the concentration of chitosan decreases the cumulative percentage release. It was observed that cumulative drug diffusion is in the order $F2 > F3 > F5$ Formulation gives best release pattern than the other formulations.

DISCUSSION

Clinical efficiency is required for any novel drug delivery system. Chitosan- TPP Nanoparticles have a novel controlled targeted drug delivery which offer several potential benefits. Chitosan nanoparticles had shown an excellent capacity for the association of Isoniazid. It is an antitubercular drug, & was selected as drug candidate for present study because it possesses the requisite properties necessary for formulation of chitosan -TPP Nanoparticles drug delivery system, like rapid onset and relatively short duration of action. The aim of the present study was to develop Isoniazid loaded chitosan TPP Nanoparticles. Chitosan concentrations and drug/ polymer ratio in the nanoparticles influence the physiochemical characteristics such as zeta potential, Average nanosize diameter or percentage encapsulation efficiency of Isoniazid. Average Nano-size diameter, zeta potential, percentage encapsulation efficiency was found to be good for optimum formulation (F2). Previous studies on chitosan nanoparticles have reported encapsulation of several compounds, their in vitro release profiles and in vivo applications. In this study, we have encapsulated Isoniazid which has not been formulated in a drug delivery system yet. Moreover, this work can be considered as the first

step for further studies on the application of Isoniazid loaded-chitosan nanoparticles and a promising step for the possible targeted delivery to the Lungs.

Table 1: Mean particle size and zeta potential

Mean particle size and zeta potential of Chitosan- nanoparticles prepared using different concentrations of

CS solution concentration (% w/v)	Acetic acid	Mean particle size (nm)	Zeta potential (mV)
0.1	0.5 %	278+-05	34.38
0.2	0.5 %	283+-07	29.65
0.3	0.75%	281+-05	22.23
0.4	1%	520+-23	20.75
0.5	0.5 %	780+-20	11.14

Chitosan solution and acetic acid mixture volumes

Fig.1: SEM Study of Chitosan TPP Nanoparticles

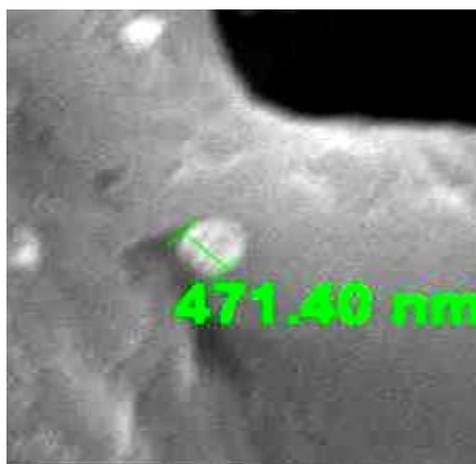
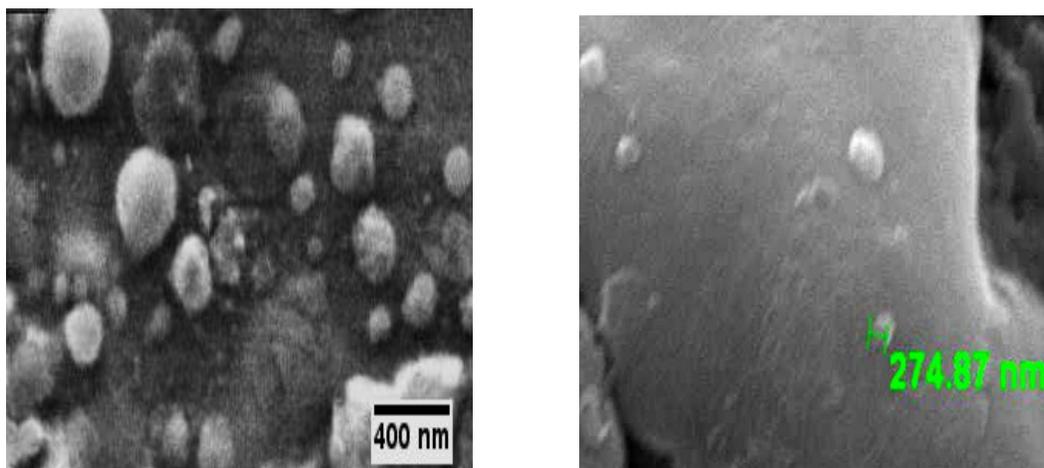


Fig.2 SEM Study of Isoniazid Chitosan Nanoparticles in F2 formulation

Fig.3 Release from Isoniazid Chitosan Nanoparticles of formulation (F2, F3&F5)

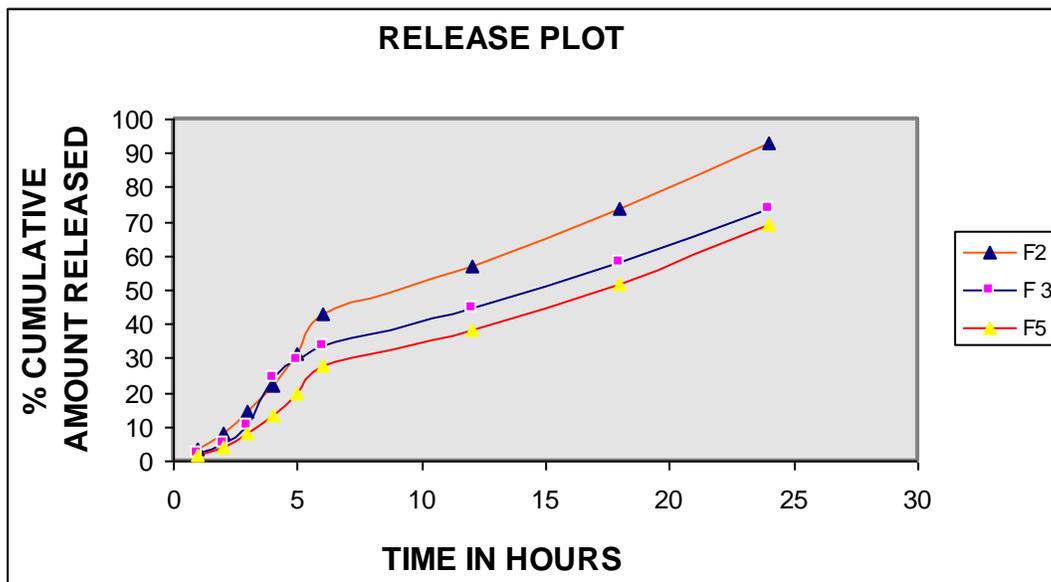
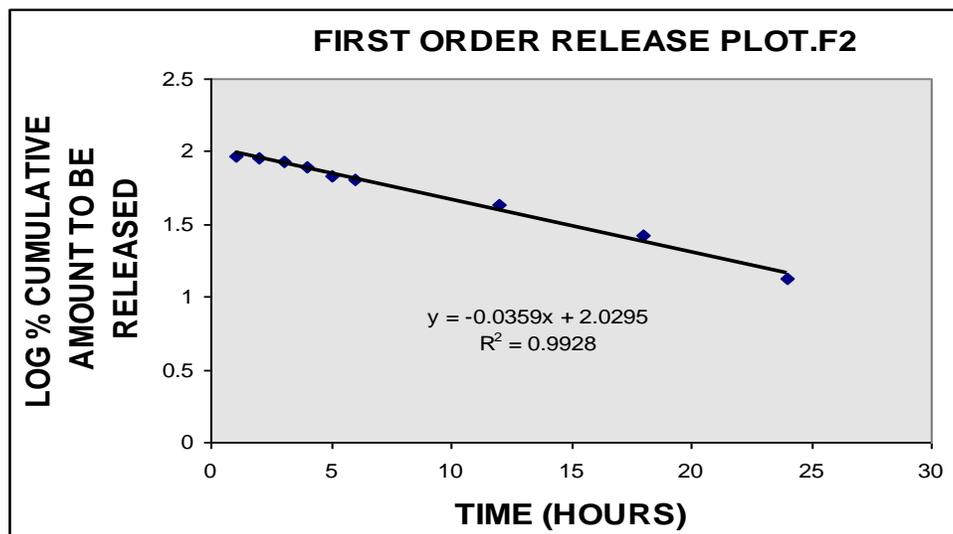


Figure: 4. First order plot of F2



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