

Development And Characterization of Inhaled Chitosan Nanoparticles Loaded with Isoniazid

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Abstract: The objective of our study is to load first line anti-tubercular drug, Isoniazid in chitosan Nanoparticles in order to enhance bioavailability and to reduce dose frequency. The chitosan nanoparticles containing the drug Isoniazid were prepared by the method of spontaneous emulsification. Chitosan gel containing drug is cross linked with Glutaraldehyde and nanoparticle suspension obtained was centrifuged at 5000 rpm. It was then evaluated for Drug loading, swelling index, Mucoadhesive force, Zeta potential, DLS studies, DSC studies, SEM studies, In vitro Drug release, Pharmacokinetic Studies and Stability studies. Formulation 1(F1) shows maximum Drug Loading, Swelling index and mucoadhesive force. The positive zeta value was obtained for all formulations due to positive charge of polymer used in preparation of dispersion. The DLS plot of Formulations shows that Average particle diameter are in the range of 661.8-823.8nm. The SEM study revealed that the micrographs of cross linked chitosan nanoparticles have smooth surface. The thermogram of the formulations showed the shifting of endotherm. This indicates the possible change in the release kinetics and bioavailability of the drug. In vitro drug releases was found to be maximum for formulation F6. Pharmacokinetic evaluation shows all the formulation shows first order rate release profile and release mechanism from nanoparticles is diffusion controlled. Stability studies indicated that the developed chitosan nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.

Keywords: Isoniazid, Chitosan, Tuberculosis, Inhalation

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1. INTRODUCTION

Tuberculosis is a major health problem throughout the world, infecting more than 8 million individuals each year. Oral therapy using the currently employed Anti Tubercular drugs (ATDs) is very effective, but is still associated with a number of significant drawbacks. More than 80% of TB cases are of pulmonary TB alone and high drug doses are required to be administered because only a small fraction of the total dose reaches the lungs after oral administration. ATD delivery systems which can be administered via the pulmonary route and can avoid the daily dosing, because they would help in: (i) direct drug delivery to the diseased organ; (ii) targeting to alveolar macrophages which are used by the mycobacteria as a safe site for their prolonged survival; (iii) reduced systemic toxicity of the drugs; and (iv) improved patient compliance (v) Higher drug concentration at the main site of infection. Moreover, in contrast to the oral route of administration, inhaled drugs are not subjected to first-pass metabolism. But the retention property of liquid or suspension, two commonly used formulations in Inhalational method, is not satisfying because they cannot persistently stay in the lung. Owing to this limitation, Controlled delivery systems have to be studied for Inhalation administration (Kawashime et al 1998).

Drug delivery research is clearly moving from the micro to the nano size scale. Nanotechnology is therefore emerging as a field in medicine that is expected to elicit significant therapeutic benefits. The development of effective nanodelivery systems capable of carrying a drug specifically and safely to a desired site of action is one of the most challenging tasks of pharmaceutical formulation investigators (Sahso et al 2007). Nanoparticles range in size from 10 to 1000 nm whereas micro- particles lie in the size range of 1 and 1000 μm . The difference between microparticles and nanoparticles lies not merely in the size, but also in the ability of nanoparticles to achieve a high drug loading, minimize the consumption of polymers, cross permeability barriers and elicit a better therapeutic response (Pandey and Rao et al 2004). Furthermore, inhalable nanoparticles stand better chances of mucosal adherence, particle(s) delivery and hence net drug delivery to the lungs. A possible obstacle to use nanocarriers for pulmonary delivery is that their mass median aerodynamic diameter, an essential parameter for the particle deposition in the lungs, is often too small.

A convenient way of delivering drugs to the lungs is the aerosolization of the drugs as fine powders with the aid of Dry Powder Inhalers (DPIs). Alternatively, the drug may be first solubilized/suspended in an aqueous medium and subsequently aerosolized (liquid aerosolization or nebulization) through a nebulizer. A nebulizer requires a dispersing force (either a jet of gas or ultra- sonic waves) for aerosolization. A drug may also be delivered to the lungs directly, i.e. without prior aerosolization, using a device called an insufflator. Compared

with a nebulizer, a DPI is more efficient in terms of drug delivery and less time consuming.

Isoniazid is the first line medication in prevention and treatment of tuberculosis. It inhibits the synthesis of mycolic acid required for the mycobacterium cell wall. INH is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH ($pK_a = 2$). Therefore, it can be considered as a good candidate for the development of a site-specific release formulation especially in case of Tuberculosis to deliver it in lung. (Ahsam et al. 2002, Sabitha et al 2010). Chitosan is a biodegradable, biocompatible, cationic hydrophilic polymer with low toxicity, mucoadhesive properties, biodegradability and ability to enhance the penetration of large molecules across mucosal surfaces obtained through deacetylation of naturally occurring chitin. It is also hypoallergenic and has natural antibacterial properties. The release modifying and mucoadhesive property of chitosan appears to be a good choice for preparing sustained release formulation for lung delivery via inhalation (Alessandro et al 2009, Anne et al 2010).

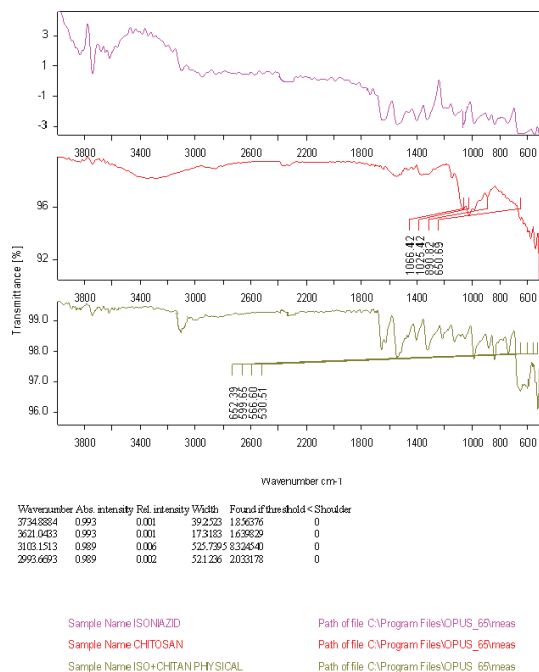


Figure 1: FT-IR spectrum of Isoniazid, Chitosan and Physical mixture.

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2. MATERIALS AND METHODS/ EXPERIMENTAL SECTION

Instruments: Instruments used were UV-visible spectrophotometer, FT-IR Spectrophotometer, sonicator, SEM and cellulose dialysis bag.

Materials: All chemicals used were of either analytical or pharmaceutical grade, such as

Isoniazid (Micro labs, Bangalore)

Chitosan (CIF, Cochin)

Span 80 (Loba chemie)

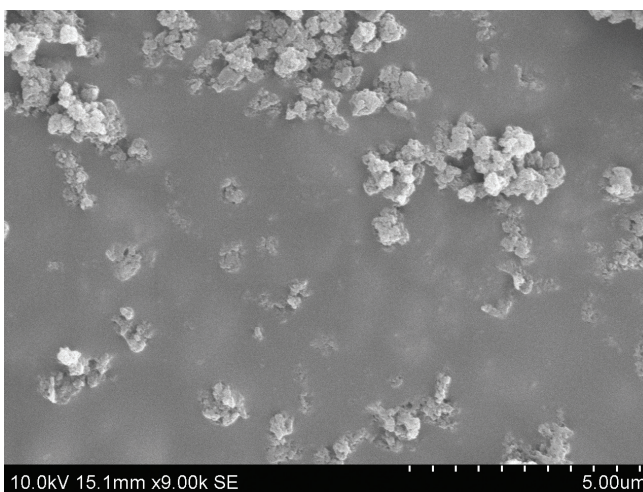


Figure 2: SEM photograph of formulation 6.

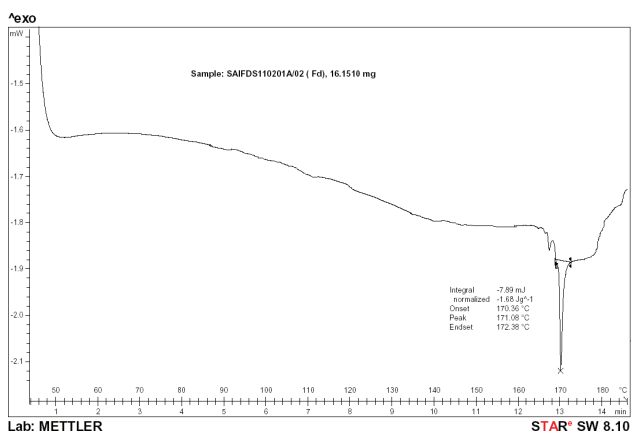


Figure 2: DSC thermogram of Isoniazid.

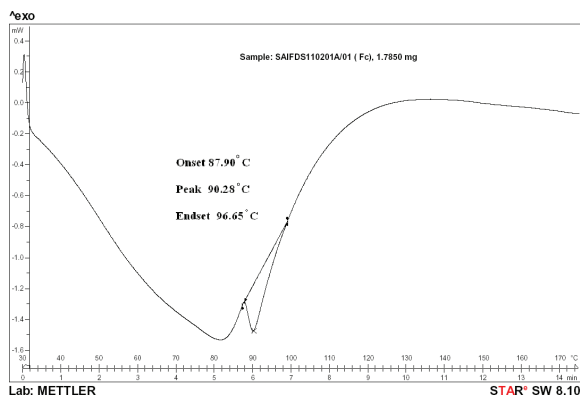


Figure 3: DSC thermogram of Chitosan.

Linseed oil (National chemicals, vadodara)

Glutaraldehyde (Nice chemicals)

Toluene (Nice chemicals)

3. METHOD

The chitosan nanoparticles containing the drug isoniazid were prepared by the method of spontaneous emulsification which is affordable to do in the laboratory setup. Required quantities of chitosan and NaCl (2%) were dispersed in required quantities of 3% (v/v) glacial acetic acid and stirred for 2 hours continuously to obtain chitosan gel, which was kept overnight to obtain clear chitosan gel. The drug was dissolved separately in 5 ml of chitosan gel (drug-to-polymer ratios of 1:0.5, 1:1, 1:1.5, 1:2, 1:2.5, 1:3, 1:4 and 1:5) under magnetic stirring. Chitosan gel containing the drug was added dropwise into 10 ml of linseed oil containing 2% vol/vol of Span 80 under magnetic stirring. To this 5 mL of acetone were added drop by drop (2 ml/ min). The system was maintained under stirring for 1 hour while covering it with aluminum foil. Then 5 ml glutaraldehyde - saturated toluene was slowly added to the system and continuously stirred for 2 hours. The nanoparticles suspension obtained was centrifuged at 5000 rpm and washed with toluene and dried. Drug-free chitosan nanoparticles were also prepared in the same manner by omitting the drug. The entire process was carried out in dark room to avoid exposure to sunlight (Wilson et al 2010).

4. EVALUATION OF NANOPARTICLES

4.1 Determination of drug-loading capacity

Drug loading was determined by procedure suggested by Barnabas Wilson et. al. 50 mg of drug-loaded chitosan nanoparticles; these were digested with

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20 mL of a mixture of 0.1 N HCl and ethanol (1:1 v/v) for 24 hours. Then the particles were separated by centrifugation at 10,000 rpm, and the drug content in the supernatant was analyzed by ultraviolet (UV) spectrophotometry at 262 nm against dummy nanoparticles, which had also been prepared as reagent blanks and treated similarly to the drug-loaded nanoparticles.

$$\text{Drug Loading Capacity} = \frac{\text{Mass of drug in Nanoparticle}}{\text{Mass of Drug used in formulation}} \times 100$$

4.2 Swelling index

The prepared product 300 mg was weighed initially (W_d) and immersed in 250 mL deionized water at ambient temperature for 24 hours. The swollen weight (W_s) was obtained by gently removing the surface water with blotting paper. Swelling index (SI) was then calculated using the following formula:

$$\text{SI} = \frac{W_s - W_d}{W_d} \times 100$$

4.3 Assessment of the mucoadhesive force

The nanoparticles were immersed in a 50 mL glass beaker at 37 °C containing a phosphate buffer solution (pH 7.4) for 5 min in such a way that the solution just covered the nanoparticles. After nanoparticles wetting, a round fresh pig intestinal mucosa (PIM) with a diameter similar to that of glass beaker was placed on nanoparticles surface so as to cover all the nanoparticles and remained for 5 min in contact with the nanoparticles. The intestinal mucosa with the attached nanoparticles was removed and the remaining nanoparticles on the glass beaker were dried at 60°C till constant weight. The percent of adhered nanoparticles (AN) was estimated using the following equation (Papadimitriou et al 2008):

$$\%AN = \frac{W_0 - W_t}{W_t} \times 100$$

Where W_0 is the initial weight of nanoparticles and W_t the remained unattached weight of nanoparticles

4.4 Differential light scattering and zeta potential

Particle size and zeta potential of nanoparticles were measured by using Zetasizer nano (Malvern instrument UK) at a fixed angle of 90° using a Helium- Neon laser at 633 nm. The parameters of particle size analyzer

were set as Temperature at 25⁰ C, Viscosity at 0.933 centipoise and index of refraction at 1.333. Each sample was diluted in distilled water and appropriate concentration particle was achieved to avoid multy scattering events. The obtained homogenous suspension was examined to determine the volume in diameter, size distribution and poly dispersity. Each sample is repeated for 3 times and the values were expressed in mean value. Similarly zeta potential was measured by same equipment.

4.5 Scanning electron microscopy

Samples were prepared by finely spreading the dried sample of F6, over slabs and by drying them under vacuum as a routine procedure. The samples were then coated in a cathodic evaporator with a fine gold layer using an ion sputter. Coating was provided at 20mA for 4 min. and observed at 520.0 kV in SEM using a JSM-5581 scanning electron microscope (JEOL, Tokyo, Japan).

4.6 Differential scanning calorimetry

DSC analyses were performed using 821e model instrument from Mettler Toledo (Schwerzenbach, Switzerland). The same was operated using STAR software version 5.21 under Solaris operating system. The samples were exposed to a heating rate of 10⁰C/min over a temperature range of 30-24⁰C under nitrogen purging (80 ml/min) in pin-holed aluminium pans.

4.7 *Invitro* release of isoniazid from nanoparticles

The release of the drug Isoniazid from chitosan nanoparticles of different drug-to-polymer ratios was studied by dialysis method in pH of the body fluid i.e 7.4 phosphate buffers. 50mg nanoparticles were placed in a cellulose dialysis bag which was then sealed at both ends. The dialysis bag was dipped into the receptor compartment containing the dissolution medium, which was stirred continuously at 100 rpm and maintained at 37⁰C. The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were withdrawn at regular time intervals, and the same volume was replaced with fresh dissolution medium. The samples were measured by UV spectrophotometry at 262 nm for isoniazid against dummy nanoparticles, which had also been prepared as reagent blanks and treated similarly to the drug loaded nanoparticles.

4.8 Short Term Stability studies

The samples were taken in borosilicate glass vials and sealed, and the vials were stored in room temperature (25⁰C±2⁰C/60%RH± 5%RH) and 40⁰C±2⁰C/70%RH±5%RH) over a period of 3 months. Samples were evaluated

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at 0, 1, 2, and 3 months for their drug content as well as any changes in their physical appearance. Chemical stability during the storage was checked Fourier transform-infrared (FT-IR) studies after 3 months of storage.

5. RESULTS AND DISCUSSION

The percentage Drug Loading for the all formulation was calculated and the result was found to be that all the formulations are varying in the values. Formulation 1(F1) shows maximum drug loading of 78.64% and F3 shows minimum drug loading of 53.33%. Swelling index found to be varying from 33.03 to 75.57 percentage. Formulation seven (F7) shows minimum value of 33.03 % .As the concentration of chitosan increases the values of swelling index also increased up to 75.57 in formulation one(F1). Mucoadhesive force for the all 7 formulation was calculated and the percentage adherence was found to be differing in each formulation. The minimum value and maximum vales are 98.2% and 99.5% respectively. The zeta potential value was found to be in the range of +18.45 to 38.9. The positive zeta value was obtained due to positive charge of polymer used in preparation of dispersion. The DLS plot of Formulations shows that Average particle diameter are in the range of 661.8-823.8nm. The size range of nano particle for good pulmonary deposition is in the range of 500 to 5000 nm. Hence, all the formulations are satisfactory for pulmonary deposition. The surface morphology of nanoparticles is shown in Figure no: 2. The study revealed that the SEM micrographs of cross linked chitosan nanoparticles have smooth surface. The

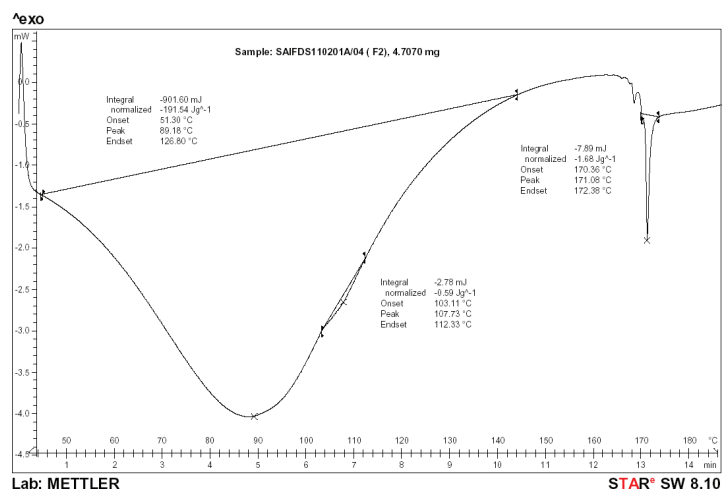


Figure 4: DSC thermogram of Chitosan and Isoniazid.

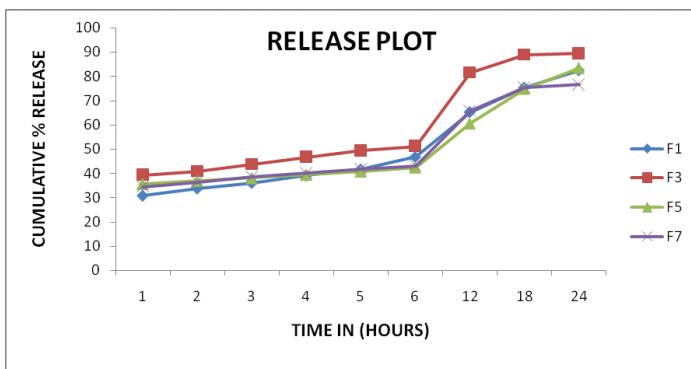


Figure 5: Release profile from F1, F2, F5, F7.

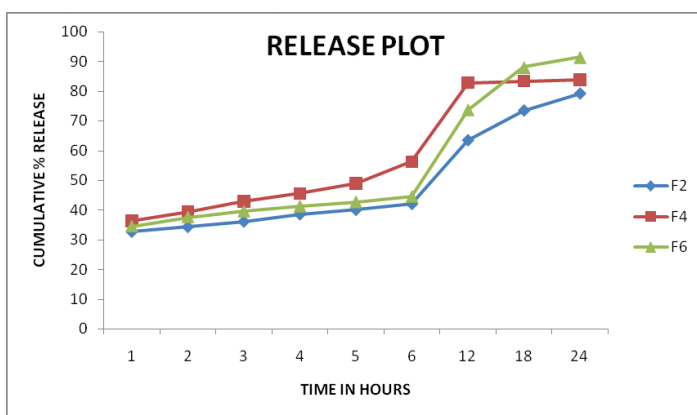


Figure 6: Release profile from F1, F2, F6

Table 1: Formulation table for Isoniazid nano particles.

Ingredients [#]	Formulation Code						
	F1	F2	F3	F4	F5	F6	F7
	1:0.5	1:1	1:1.5	1:2	1:3	1:4	1:5
Chitosan (mg)	400	400	400	400	400	400	400
Isoniazid (mg)	200	400	600	800	1200	1600	2000

[#]Every formulation code has: Linseed oil-20ml, Span 80-2%, Sodium Chloride-200mg, Glutaraldehyde-10ml, Acetone-10ml, Acetic acid-3%

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Table 2: Evaluation parameters of Isonaizid nanoparticles.

Formulation code	Drug loading (%w/w)	Swelling index (%)	Mucoadhesive force (%)	Zeta potential (%)
F1	78.64 ± 1.572	75.57 ± 1.32	99.1 ± 1.992	23.60±0.012
F2	55.55 ± 1.111	73.04 ± 1.28	98.8 ± 1.972	18.45±0.008
F3	53.33 ± 1.066	69 ± 1.25	98.6 ± 1.982	21.67±0.013
F4	57.18 ± 1.143	66.02 ± 1.15	98.4 ± 1.921	26.98±0.056
F5	59.19 ± 1.183	59.34 ± 1.03	99.5 ± 1.978	31.00±0.016
F6	57.24 ± 1.144	49.40 ± 0.98	98.2 ± 1.996	38.9±0.065
F7	55.52 ± 1.110	33.03 ± 0.57	98.3 ± 1.964	32.78±0.076

Table 3: Evaluation parameters of Isonaizid nanoparticles.

Formulation code	Average diameter (nm)	PDI	In vitro release (24 hrs)
F1	67.80	0.362	82.05
F2	68.35	0.260	79.28
F3	66.18	0.384	89.46
F4	72.43	0.379	83.83
F5	66.18	0.384	83.13
F6	82.35	0.193	91.39
F7	74.78	0.272	76.77

SEM micrograph also indicates the aggregation of chitosan microspheres due to existing weak interparticle bonding. The thermogram of the formulations showed the shifting of endotherm. This indicates the possible change in the release kinetics and bioavailability of the drug. In vitro drug releases for the formulations were in the range of 76.77-91.39% with formulation F6 having the maximum and formulation F7 having the minimum. Pharmacokinetic evaluation shows all the formulation shows first order rate release profile which suggests that the drug release from nanoparticle depend on drug loading. As the best correlation coefficient was observed in Higuchi's plot, the major release mechanism from nanoparticles is diffusion controlled and follows non- fickian transport as observed by Korsmeyer-Peppas plot. Stability studies were conducted as per ICH guidelines. The results indicated

that the developed chitosan nanoparticles are physically and chemically stable and retain their pharmaceutical properties at various environmental conditions over a period of 3 months.

6. CONCLUSION

Studies were undertaken on the development and characterization of chitosan nanoparticle loaded with isoniazid with a view to develop control release formulation. In the preparation of nanoparticle, carrier chitosan was used with different concentration of isoniazid and seven formulation are made (1:0.5, 1:1, 1:1.5, 1:2, 1:3, 1:4, 1:5). Nanoparticles were prepared for all these concentration by Spontaneous emulsification method. All formulations consist of fine and free flowing powders. An interaction study like UV and IR spectra shows that there were no interaction between drug and carrier used. SEM and DLS studies showed that the size of the particles were reduced and formulation no 6 having optimum nanonised particles. Zeta potential report also shows good positive potentials for formulation no 6 and all formulation shows good encapsulation efficiency. DSC studies confirmed the no interaction with drug and polymer. As increasing the concentration of chitosan the swelling index also increased and all the formulation shows good muco adhesive property. Results of dissolution studies shows that formulation 6 gives good release profile compared to other formulations. Pharmacokinetic evaluation shows all the formulation shows first order rate release profile. Stability studies including chemical stability studies shows that the prepared nano particle is stable for 3 months.

From all these results it concludes that formulation no: 6 is the best formulation and which is recommended for future studies like nano dry powder preparation and fill in to capsule (rotacaps) and its evaluation.

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