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Relative Study between Isoniazid Loaded Chitosan with the Gold Encapsulated with Isoniazid Loaded Chitosan Nanoparticles

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ABSTRACT

In our study, we load first line Anti tubercular drug Isoniazid in Chitosan nanoparticles and Chitosan coated gold nanoparticles by Ionic Gelation Method to enhance controlled, sustained and targeted drug delivery for pulmonary lung tuberculosis treatment. Chitosan in various concentrations dissolved in glacial acetic acid and D.I. water. Isoniazid (INH) taken in similar concentration dispersed in Chitosan solution followed by Tween80 was added, later Sodium tripolyphosphate (TPP) was added at room temperature for Isoniazid loaded Chitosan nanoparticles preparation. Similar procedure was followed for Isoniazid loaded chitosan encapsulated on gold nanoparticles. In which gold nanoparticles and TPP was added simultaneously. In both preparations, Formulations F_6 showed good entrapment efficiency and used for further experimental studies. UV-visible spectrophotometer analysis is to calculate percentage (%) drug entrapment. XRD and FT-IR analysis confirmed the presence of Chitosan coated on the drug and gold nanoparticles. TEM and SEM analysis used to characterize particle size determination. Stability of formulations was studied at different temperature conditions.

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INTRODUCTION

World Health Organization (WHO) declared Tuberculosis (TB) as a global emergency; TB control has become a greatest concern among the national, international and local health authorities. A total of 196 (out of 212) countries reported that these countries collectively account for 99.6% of estimated TB cases in the world 1-2. WHO is struggling with the highly burden Countries in the world, Countries have launched TB control programs but so far improvements in achieving the desired targets of detection is 70% and cure rate is 85% of TB cases. So, WHO's standard therapy is still quiescent 3. Because of low spending and poor political attention TB control programs have been widely suffered in these highly burden countries 4. The major aim of targeted drug delivery system is to target, prolong, localize, reduce in cost of therapy and has a protected drug interaction with the diseased organs. Due to the above said reasons, targeted drug delivery system has achieved a greater potential for the treatment of pulmonary lung Tuberculosis therapy. It is a method of delivering medication to the patient in a manner that increases the concentration of the medication in some parts of the body relative to the others. It is highly required in various disciplines; such as chemists, biologists and nanotechnologists, to join forces to optimize this system.

Isoniazid is a first-line Anti tubercular drug used in the medication to treat the Pulmonary Lung Tuberculosis. It is a pro-drug, activated by catalase-peroxidase (a bacterial enzyme) present in Mycobacterium tuberculosis. Chitosan is a linear polysaccharide which received a considerable attention in potential drug delivery carrier system because of its biocompatibility and biodegradability and shows a relevance hydrophilic carrier system 5. Chitosan nanoparticles were prepared by ionic gelation method. Because of its non-toxicity it can act as carrier delivery systems for proteins and peptides through the pulmonary route 6-8. It is bio adhesive which binds to the negatively charged surfaces like mucosal membranes 9-10. Hence it enhances the transport of polar drugs across epithelial surfaces. Sodium Tripolyphosphate is an inorganic compound of the polyphosphate penta-anion which strongly binds to metal cations and which is widely used as gelating agent in Chitosan nanoparticles preparation. Tween 80 is water soluble, non-toxic and is a non-ionic surfactant which acts as a reducing agent. Colloidal gold nanoparticles have potential attention in areas of bio sensing 11-12, bio imaging 13, catalytic reactions 14 and targeted drug delivery system. Drug loaded chitosan nanoparticles and metal incorporated chitosan nanoparticles prepared by ionic gelation method in which we try to enhance targeted drug delivery system and controlled drug release in our present work.

MATERIALS AND METHODS

MATERIALS

Isoniazid drug was obtained from the manufacturers of Sigma-Aldrich, Bangalore, India. Chitosan, Tween 80 are also purchased from the Sigma-Aldrich, Bangalore. Sodium Tripolyphosphate (TPP) was obtained from Alfa Aesar, Hyderabad. Auric chloride obtained from Loba Chemie, Chennai. Trisodium citrate dihydrate purchased from manufacturers of RFCL Ltd., New Delhi. Disodium hydrogen phosphate obtained from Merck, Mumbai. Potassium dihydrogen phosphate, Sodium chloride purchased from manufacturers of Sisco Research and Laboratories, Mumbai, India. All the other chemicals and reagents used were of analytical grade.

PREPARATION METHODS

PREPARATION OF GOLD NANOPARTICLES BY CITRATE REDUCTION METHOD

Add 20 ml of 1.0 mM $HAuCl_4$ to a 50 ml beaker in a magnetic stirrer placed on the hot plate and boil it for 70ºC. To this solution, add 2 ml of 1% solution of Tri sodium citrate dihydrate, $Na_3C_6H_5O_7\ 2H_2O$. The citrate reduces to gold solution gradually to form the gold (III). The color change is from pale yellow to wine red. Remove from heat when the solution has turned deep red or 10 minutes has elapsed 14.

PREPARATION OF ISONIAZID LOADED CHITOSAN NANOPARTICLES BY IONIC GELATION METHOD

Chitosan nanoparticles were prepared by ionic gelation method. By cross linking of chitosan solution with TPP prepared in the presence of Tween 80 as a suspending agent to prevent particle aggregation, at ambient temperature while stirring 15. Isoniazid loaded chitosan nanoparticles were prepared as described above by dissolving 10 mg of Isoniazid in 10 ml of chitosan solution (1mg/ml) dissolved in 0.1% glacial acetic acid and mixed with D.I. water (concentrations of Isoniazid : chitosan ratio are taken in different ratios as shown in (Table-1). The formulation was then kept overnight in homogenizer to obtain evenly distributed nanoparticles in the suspension. The formulated nanoparticles are further subjected to characterization.

PREPARATION OF GOLD ENCAPSULATED ISONIAZID LOADED CHITOSAN NANOPARTICLES

The Isoniazid loaded Chitosan nanoparticles is prepared and the best ratio was found by entrapment efficiency. From that Formulation F_6 is taken and added 5ml of gold nanoparticles simultaneously with TPP (0.25% w/v). The nanoparticulate suspensions were centrifuged at 15000 rpm for 30 min. The supernatant was analyzed by UV spectrophotometer to calculate the percentage (%) drug entrapment.

Formulation code	Isoniazid (mg)	Chitosan (mg)	TPP (% w/v)	Ratio of Isoniazid and Chitosan
F_1	10	10	0.25	1:1
F_2	10	20	0.25	1:2
F_3	10	30	0.25	1:3
F_4	10	40	0.25	1:4
F_5	10	50	0.25	1:5
F_6	10	60	0.25	1:6
F_7	10	70	0.25	1:7

Table 1 Shows the optimization of formulation with different ratios of Isoniazid and chitosan.

RESULTS

CHARACTERIZATION OF NANOPARTICULATE FORMULATIONS

UV-VISIBLE SPECTROSCOPY ANALYSIS

Gold nanoparticles can be easily quantified with a UV/ visible spectrophotometer (V-570 UV/VIS/NIR, JASCO Corporation, Japan) based on the Beer–Lambert's Law and its extinction coefficient. Total gold concentration was measured according to this method. Initially, gold was extracted from the nanoparticles using n-hexane and ethanol as the organic solvents 16.

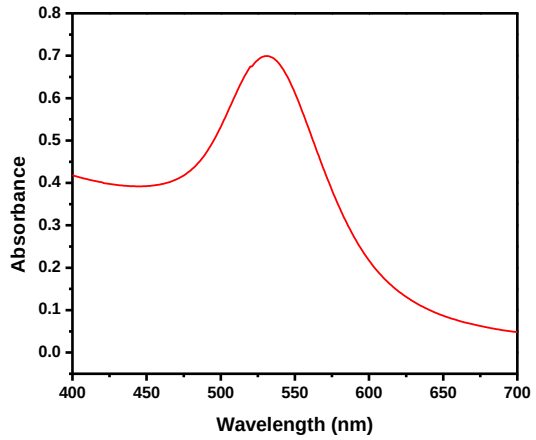


Figure 1 UV spectrum of gold nanoparticles

X-RAY DIFFRACTION (XRD) ANALYSIS

Powder X-ray diffraction patterns (PXRD) were taken with a PANalytical Xpert pro X-ray diffractometer by using a Ni-filtered Cu Kα radiation over the 2 theta range of 10–90°. Samples were finely ground in a glass substrate and the experimental parameters were set as: voltage, 40 kV; current, 20 mA; angular speed, 4°/min. The PXRD patterns Isoniazid loaded Chitosan nanoparticles and gold encapsulated Isoniazid loaded chitosan was recorded.

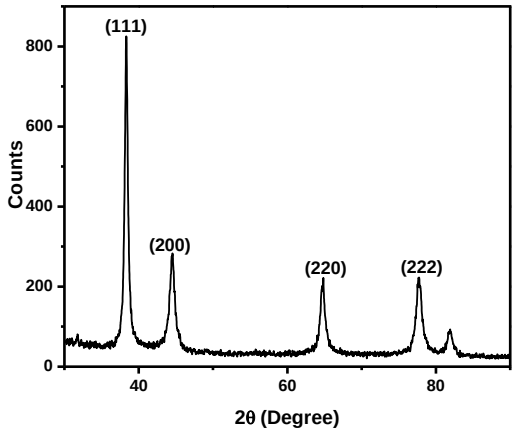


Figure 2 XRD spectrums of Gold nanoparticles.

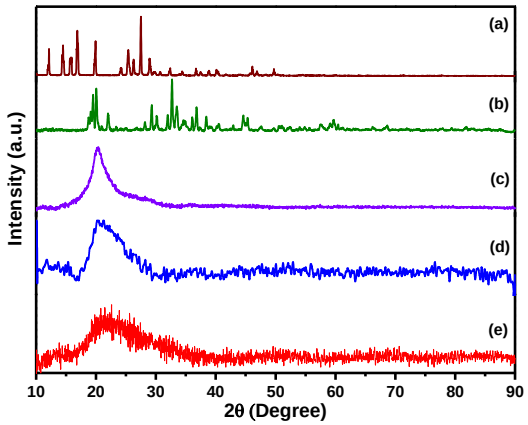


Figure 3 XRD of **(a)** Isoniazid **(b)** TPP **(c)** Chitosan **(d)** Isoniazid loaded Chitosan nanoparticles **(e)** Gold encapsulated Isoniazid loaded Chitosan nanoparticles.

FOURIER TRANSFORMS INFRARED SPECTROSCOPY (FTIR) ANALYSIS

Fourier Transform Infra-red spectroscopy determines drug-polymer interaction and the presence of functional group analysis based on vibrational motion of the molecules. Samples can be milled with Potassium bromide (KBr) to form a very fine powder. This powder is then compressed into a thin pellet which can be analyzed. The scanning range was 400–4000 cm^{-1} and the resolution was $1cm^{-1}$17.

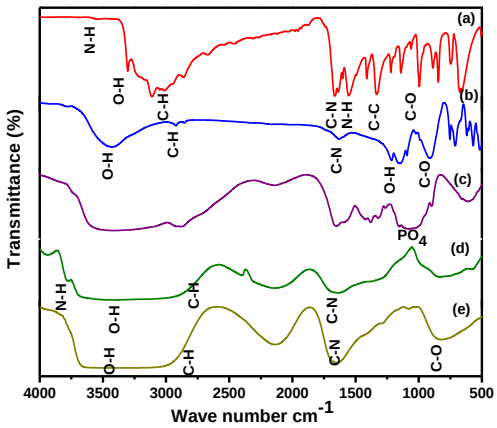


Figure 4 FTIR spectrum of **(a)** Isoniazid **(b)** Chitosan **(c)** TPP **(d)** Isoniazid loaded Chitosan nanoparticles **(e)** Gold encapsulated Isoniazid loaded Chitosan nanoparticles.

TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS

The morphology of the Gold nanoparticles was examined by Transmission Electron Microscopy (FEG Japan). The sample is diluted in the ratio of 1: 5 and placed on a copper grid and observed under the microscope.

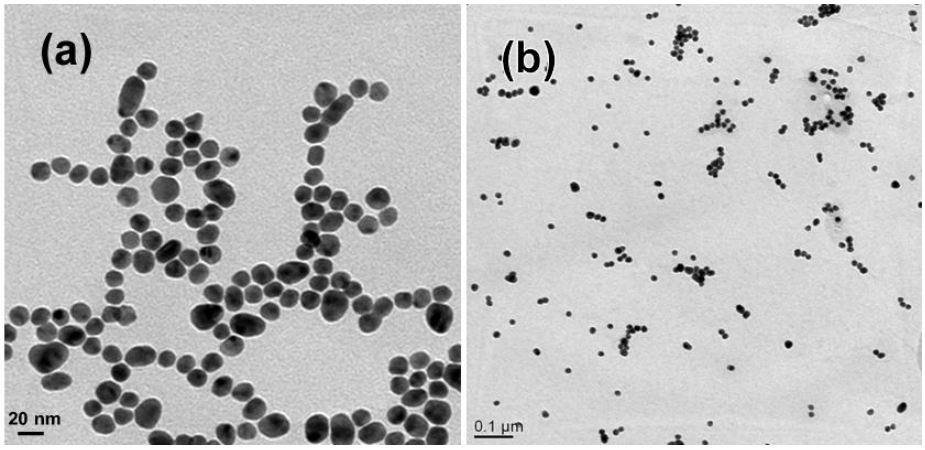


Figure 5 TEM images for gold nanoparticles

SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

The particle size of the formulation of Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles were viewed and photographed using Scanning Electron Microscopy (SEM**)** (QUANTA 3D FE- SEM). Nanoparticles was transferred to a glass slide which is cut in the diameter of 20×20mm, which is then mounted on an aluminum stub using double sided carbon tape. The solution was slowly evaporated at room temperature. The image was captured on SEM mode at desired magnification.

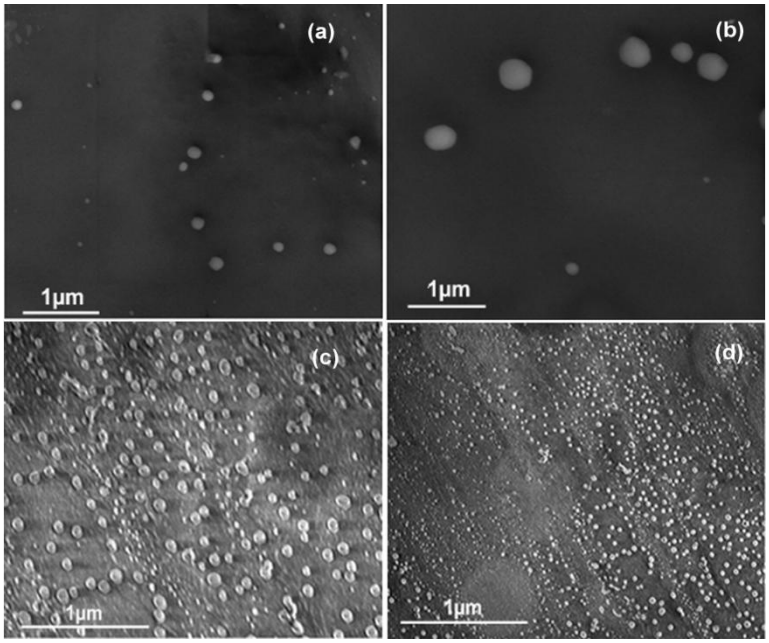


Figure 6 (a) and **(b)** are the SEM images of Isoniazid loaded Chitosan nanoparticles, **(c)** and **(d)** are the SEM images of Gold encapsulated Isoniazid loaded chitosan nanoparticles.

DRUG ENTRAPMENT EFFICIENCY

Drug entrapment efficiency was determined by the amount of freeze-dried formulated nanoparticles was digested with minimum amount of ethanolic solution (water/ethanol in 7:3 ratios) 18. The digested homogenates were centrifuged at 15,000 rpm for 30 min and supernatant was analyzed for drug entrapment. The drug entrapment was measured at 263 nm using JASCO UV/Vis spectrophotometer (Table-2). The percentage drug entrapment was determined using following equation.

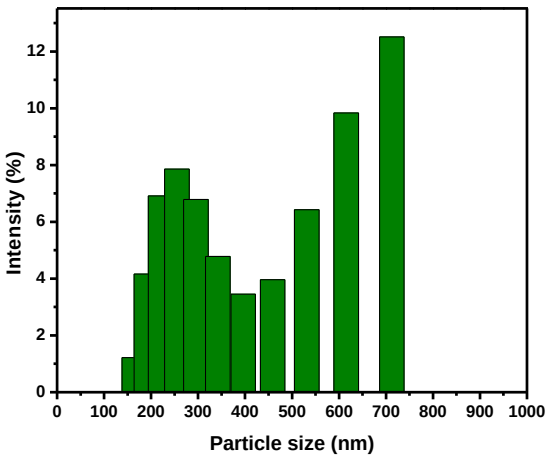
$$\text{Entrapment efficiency} = \frac{\text{Total amount of drug} - \text{Amount of unbound drug} \times 100}{\text{Total amount of drug}}$$

S.No.	Formulations	F_1 (%)	F_2 (%)	F_3 (%)	F_4 (%)	F_5 (%)	F_6 (%)	F_7 (%)
1.	Isoniazid loaded chitosan nanoparticles	83.90	84.22	87.77	89.89	90.99	92.43	83.54
2.	Gold encapsulated Isoniazid loaded chitosan nanoparticles	83.21	84.7	86.75	88.90	90.25	91.97	82.08

Table 2 Drug entrapment efficiency of Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles.

Particle Size Analyzer

The Malvern particle size analysis was done for the optimized formulation of Isoniazid loaded chitosan nanoparticles (F_6) and Gold encapsulated Isoniazid loaded chitosan nanoparticles. The obtained average particle size of the formulated Isoniazid loaded chitosan nanoparticles (F_6) and the Gold encapsulated Isoniazid loaded chitosan nanoparticles as shown in the figure 7 (a) and (b).



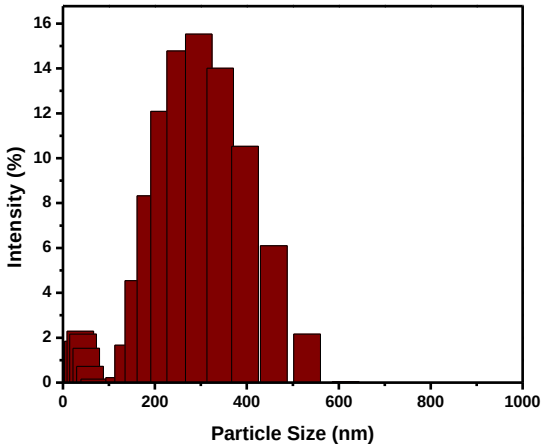


Figure 7 (a) Isoniazid coated chitosan nanoparticles **Figure 7 (b)** gold encapsulated Isoniazid loaded chitosan nanoparticles

IN-VITRO DRUG RELEASE CHARACTERISTICS

In-vitro drug release was studied by most probably using Franz diffusion cell (FDC). The FDC enables *in-vitro* analysis of drug movement across a membrane using a two-compartment model. The donor compartment contains the dose, recipient compartment contains phosphate buffer saline (PBS) pH 7.4 and a non-rate limiting membrane (semi permeable membrane) separates the compartments and supports the dose. Thus, drug-release profiles can be produced for controlled-drug release formulations 19-20. The samples were withdrawn from the sampling port at regular time intervals and the drug release was quantified using UV spectrophotometer.

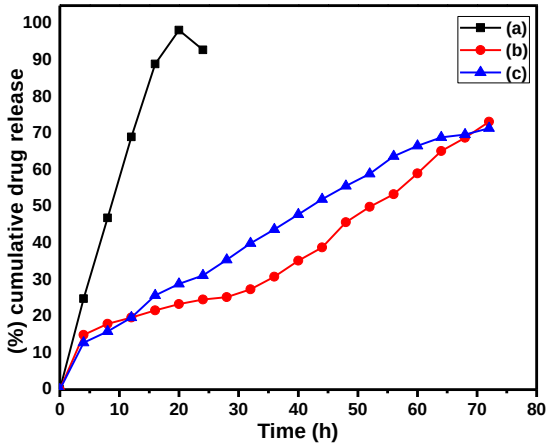


Figure 8 shows comparative studies for **(a)** Isoniazid free drug release **(b)** *In- vitro* release of Isoniazid loaded Chitosan nanoparticles **(c)** Gold encapsulated Isoniazid loaded chitosan nanoparticles.

STABILITY STUDIES

Formulations were stored at different conditions to determine the stability. Six test tubes were filled with 3 ml of the formulation of both Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles and then closed tightly. Two tubes were stored vertically at room temperature (20°C±2); two tubes were stored in a hot air oven at 40°C±2 and two tubes at 4°C ±2. Observations were made for each week and can be done for 8 weeks.

DISCUSSION

The Formulations of Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles were prepared successfully by using ionic gelation method. We have taken different ratio of polymer to optimize the formulation based on the better entrapment efficiency. The absorbance of gold nanoparticles was measured at 528nm as shown in the Figure 1. Fig. 2 shows the XRD of gold nanoparticles which is analyzed with JCPDS No. 4-0783. In the X-ray diffraction of gold nanoparticles shows sharp peaks at a diffraction angle of 2θ are 38.36°, 44.64°, 64.92°, 77.84°, 81.97° are present which suggest that the gold nanoparticle is present as a crystalline material. The XRD of Isoniazid Figure 3(a) shows peak at different 2θ angles are 20.20°, 29.64 °, 32.75 °, 36.77°, 44.66 ° and 59.84°. The XRD of sodium tripolyphosphate Figure 3(b) shows peak at different 2θ angles 20.16 °, 21.80 °, 29.58 °, 33.42 °, 36.84 °, 38.68 °, 44.72 ° and 59.77° .

The XRD of chitosan Figure 3(c) shows a sharp peak at 20.270^{o} which is amorphous in nature. The formulated Isoniazid loaded chitosan nanoparticles F_6 Figure 3(d) and gold encapsulated Isoniazid loaded chitosan nanoparticles Figure 3(e) showed a single characteristic peak of chitosan at 20.27 ^{o} and the other peaks were missing. This indicates the phase conversion of the formulation from crystalline to amorphous state and the polymer has coated uniformly. XRD depends only on crystalline nature of the sample. The dissolved Isoniazid and in molecular state shows crystallinity whereas, the formulation F_6 shows amorphous state, as the individual drug molecules are coated by Chitosan polymer. Overall the XRD graph, says that it is unable to identify the signals, so we obtained noise in the formulation (F_6). As the same peak obtained in the gold encapsulated Isoniazid loaded chitosan nanoparticles formulation also exit in the amphorous form. Thus indicates Chitosan successfully coated over Isoniazid and gold nanoparticles.

From the FTIR spectrum of Isoniazid Figure 4(a) shows characteristics peak at 3538 cm^{-1} for N-H stretching, 3307 cm^{-1} for O-H symmetric Stretching, 2930 $cm^{-1}$for C-H stretching, 1670cm^{-1} for C-N stretching, 1551cm^{-1} for N-H bending, 1340cm^{-1} for C-C bending and 990cm^{-1} for C-O Stretching. The FTIR spectrum of chitosan Figure 4(b) shows peaks at 3443 cm^{-1} for O-H stretching, 2893 cm^{-1} for C-H Stretching, 1629 cm^{-1} for C-N stretching, 1212 cm^{-1} for O-H bending and 908 cm^{-1} for C=O bending vibrations. The FTIR spectrum of sodium tripolyphosphate Figure 4(c) shows peak at 1069cm^{-1} for PO_4 group ions. The Isoniazid loaded chitosan nanoparticles F_6 Figure 4(d) shows the characteristics peak of N-H stretching at 3789 cm^{-1}, 3386cm^{-1} for O-H stretching, 2732cm^{-1} for C-H stretching, 1663cm^{-1} for C-N stretching vibrations and Gold encapsulated Isoniazid loaded chitosan nanoparticles Figure 4(e) shows the characteristics peaks of O-H stretching at 3439 cm^{-1}, 2805cm^{-1} for C-H stretching vibrations, 1657 cm^{-1} for C-N stretching and 831 cm^{-1} for C-O bending vibrations. The spectral analysis indicates that the functional groups present in the formulations almost resemble the characteristics of the pure drug Isoniazid and chitosan. From this graph, it shows that no intermolecular interaction occurred between the polymer and the drug.

The Figure 5 (a) and (b) shows that the TEM Images of the gold nanoparticles were morphologically discrete, evenly distributed without agglomeration which are in size ranges from 10-20nm. From SEM analysis the formulations of F_6 containing Isoniazid loaded Chitosan nanoparticles Figure 6 (a) and (b). Gold encapsulated Isoniazid loaded chitosan nanoparticles (Figure 6(c) and (d)) were morphologically discrete which have particles size ranges from 180-310nm. The drug entrapment efficiency of formulation F_6 of Isoniazid loaded Chitosan nanoparticles shows maximum entrapment of 92.43% and the formulations of Gold encapsulated Isoniazid loaded chitosan nanoparticles show the maximum entrapment of 91.97% when compared to all other formulations as it is shown in the Table (2). The particle size of the formulation was determined and From the Figure 7(a) and 7(b) show the particle size analysis of Isoniazid loaded chitosan nanoparticles F_6 and Gold encapsulated Isoniazid loaded chitosan nanoparticles. The maximum number of particles size and size distribution were in the range of 200-700 nm.

The Figure 8 shows the *in-vitro* release profile of nanoparticulate F_6 formulations of Isoniazid loaded Chitosan nanoparticles shows 73.23% and Gold encapsulated Isoniazid loaded chitosan nanoparticles releases 71.49% by evaluating the sample for every four hours using UV/Vis spectrophotometer. The formulations F_6 shows controlled drug release for 3days when compared with the free Isoniazid drug release studies, as it shows 98.31% for 5 hours. From stability analysis, Isoniazid loaded Chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles found to be more stable at refrigeration condition (4°C ±2) and stable at room temperature (20°C±2) but the drug degradation takes place at increased temperature (40°C±2).

CONCLUSION

The formulation of Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles was prepared successfully by using Ionic Gelation method. The Isoniazid drug, chitosan, TPP was found to be biocompatible, biodegradable and is used for the treatment of tuberculosis as a nanoparticulate system. The experimental data shows that the particle size, entrapment efficiency and *in-vitro* release characteristics of the formulated nanoparticles as it shows better performance, reproducibility, controlled and sustained in action. These formulations F_6 is an alternative of target drug delivery system which enhance drug transport and within lungs for the Anti tuberculosis treatment. These prepared formulations; Isoniazid loaded chitosan nanoparticles and Gold encapsulated Isoniazid loaded chitosan nanoparticles mainly used to increase half life, bioavailability and achieved the maximum therapeutic efficacy, reduce side effects and adverse effects. The nanoparticulate formulations shows good controlled release of drug than free Isoniazid; it decreases the frequency of dose and also reduces the cost of the therapy. The colloidal gold nanoparticles accumulated to the affected tissue shows considerable advantage in bio imaging analysis for our future *in-vivo* studies.

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