Journal of Pharmaceutical and Scientific Innovation
www.jpsionline.com
Research Article

DEVELOPMENT AND CHARACTERIZATION OF CHITOSAN NANOPARTICLES LOADED WITH ISONIAZID

Junise V*[1], R Saraswathi[2]
[1]Research scholar, Karpagam University, Coimbatore, Tamil Nadu, India
[2]Professor in Pharmaceutics, Jazan University, Jazan, KSA
*Corresponding Author Email: junooz10@gmail.com

DOI: 10.7897/2277-4572.04342

Received on: 15/04/15 Revised on: 29/05/15 Accepted on: 09/06/15

ABSTRACT

Only a small fraction of the total dose reaches the lungs after oral administration of Anti-tubercular drug. Pulmonary route can avoid the daily dosing, as it deliver drug directly to the diseased organ and also reduce systemic toxicity of the drugs. Drug-loaded nanoparticles have the potential to be used for pulmonary delivery of therapeutics for treating lung diseases**.** 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. Nanoparticles were formulated by ionotropic gelation method using different concentration of chitosan [2:3, 3:3, 4:3, 5:3 %w/v (chitosan-drug ratio)]. Drug was dispersed in Chitosan in acetic acid solution, into which Sodium Tripolyphosphate solution is added drop wise with continuous stirring and followed by sonication for 5 min. The resulting Chitosan nanoparticle suspension was centrifuged at 16,000 rpm for 30 min and Nanoparticles were collected after freeze drying. Formulation no 2 (F2) shows maximum drug loading and In vitro drug release. The positive zeta value was obtained due to positive charge of polymer used in preparation of dispersion.SEM study revealed that the cross linked chitosan nanoparticles have smooth surface. Pharmacokineticevaluationshowsalltheformulationshowsfirstorder ratereleaseprofile and release mechanism from nanoparticles is diffusion controlled. Stability studies conducted as per ICH guidelines 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. 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: Nanoparticles, Chitosan, Isoniazid

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) are very effective, but is still associated with a number of significant drawbacks. More than 80% of TB cases are of pulmonary TB and high drug doses are required to be administered since only a small fraction of the total dose reaches the lungs after oral administration. Anti-Tubercular drug delivery systems which can be administered via the Pulmonary route 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 atthe site of infection[1]. 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[2].

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 Nano delivery 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[3].Nanoparticles range in size from10 to1000 nm whereas micro-particles lie In the size range of 1to1000 µm. The difference between micro particles and nanoparticles lies not in the size, but also in the ability of nanoparticles to achieve a high drug loading, minimized consumption of polymers, and elicit a better therapeutic response[4,5].Furthermore, inhalable nanoparticles stand better chances of mucosal adherence, particle(s) delivery and hence net drug delivery to the lungs[6].A possible obstacle to use Nanocarriers for Pulmonary delivery is that the mass median aerodynamic diameter ,an essentialparameterfor the particle deposition in the lungs, is often too small[2].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 aerolization[2]. A drug may also be delivered to the lungs directly, i.e. without prior aerosolization, using a device called an insufflators. Compared with a nebulizer, a DPI is more efficient in terms of drug delivery and less time consuming [7].

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. NH is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH (PKa = 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[8] 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 de

acetylation of naturally occurring chitin. It is also hypo allergenic 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[9,10,11].

Spontaneous emulsification method is a low energy emulsification method of adding a mixture of surfactant, oil and water miscible solvent into aqueous phase. It diverts physicochemical properties of components in Nano-emulsion formulation and expends low-energy in Nano emulsion formulation. It has sub-micron sized particles and narrow size distribution.

MATERIALS AND METHODS

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, Banglore)
Chitosan (CIF, Cochin)
Span 80 (Lobachemie)
Linseed oil (National chemicals, Vadodara)
Glutaraldehyde (Nice chemicals)
Toluene (Nice chemicals)

Method

The Chitosan nanoparticles containing the drug Isoniazid were prepared by the method of spontaneous emulsification. 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. Then the solution 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 drop wise 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 Nanoparticle 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[12].

Evaluation of nanoparticles

Determination of drug-loading capacity: 50 mg of drug-loaded Chitosan Nanoparticles were digested with 20ml of a mixture of 0.1 N HCl and Ethanol (1:1 vol/vol) for 24 hours. The particles were then 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. [11]

$$\text{Drug loading capacity} = \text{mass of drug in nano particle} / \text{mass of drug used in formulation} \times 100$$

Swelling index: The 300 mg of prepared product 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:[12]

$$SI = Ws - Wd / Wd \times 100$$

Assessment of the Mucoadhesive Strength: The nanoparticles were immersed in a 50 mL glass beaker at37^{0}C containing a Phosphate buffer solution (pH 7.4) for 5 min in such a way that the solution just covered the Nanoparticles. After 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 ^{0}C till constant weight. The percent of adhered nanoparticles (AN) was estimated using the following equation:[14]

$$\% AN = Wo - Wt / Wt \times 100$$

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

Differential light scattering and zeta potential: Particle size and Zeta potential of Nanoparticles were measured by using Zeta sizer .Nano (Malvern instrument UK) at a fixed angle of 90^0 using a Helium- Neon laser at 633nm.The parameters of particle size analyzer were set as Temperature at 25^{0}C, Viscosity at 0.933centipoiseand index of refraction at 1.333.Each sample was diluted in distilled water and appropriate concentration particle was achieved to avoid multi scattering events. The obtained homogenous suspension was examined to determine the volume in diameter, size distribution and poly disparity. Each sample is repeated for 3 times and the values were expressed in mean value. Similarly Zeta potential was measured by same equipment. [12]

Scanning electron microscopy: Samples were prepared by finely spreading the dried sample of F6, over slabs and by drying them under the vacuum.The samples were then coated in a cathodic evaporator with a fine gold layer using anion sputter. Coating was provided at 20m A for4min and observed at 520.0k Vin SEM using a JSM-5581 scanning electron microscope (JEOL, Tokyo, Japan)
.
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 10^0 C/min over a temperature range of 30-24^{0}C under nitrogen purging (80 ml/min) in pin-holed aluminum pans.

***In vitro* 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 7.4 phosphate buffer. 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 37°C. 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[12].

Kinetic analysis: To analyze the in-vitro release data various kinetic models were used to describe the release kinetics. The zero order rates describe the systems where the drug release rate is independent of its concentration. The first order describes the release from system where release rate is concentration dependent. Higuchi described the release of drugs from insoluble matrix as a square root of time dependent process based on fickian diffusion. The Hixson-Crowell cube root law describes the release from

systems where there is a change in surface area and diameter of particles or tablets.

Stability studies: The samples were taken in Borosilicate glass vials and sealed, and the vials were stored in room temperature (25°C±2°C/60%RH± 5%RH) and 40°C±2°C/70%RH±5%RH) over a period of 3 months. Samples were evaluated 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[12-14].

RESULTS AND DISCUSSION

The Percentage Drug Loading for all the formulation was calculated and the result analysis shows that the Formulation 6(F6) having maximum drug loading of 71.78% and F1 shows minimum drug loading of 63.33%.

Swelling index indicates the hydrophillic property of polymer.Swelling index found to be in the range of 47.44 - 49.65%w/w. The concentration of chitosan is not varying in the formulation, hence value of swelling index doesn't vary considerably.

Mucoadhesive strength for the all formulations was calculated and the percentage adherence was found to be differing in each formulation. F5 (98.2%) having the minimum value and F6 (99.5%) having the maximum value. Polymer swelling permits a mechanical entanglement by exposing the bio adhesive sites for hydrogen bonding and electrostatic interaction between the polymer and the mucous network.

All the formulations show positive Zeta potential value and were found to be in the range of 20.56 - 22.34. (F6) along with F(2) shows maximum Zeta potential. The positive zeta value was obtained due to positive charge of polymer used in the preparation

The DLS plot of Formulations shows that Average particle diameter is in the range of 661.8(F1) - 823.5(F6) nm. The size range of Nanoparticle for good pulmonary deposition is in the range of 500 to 5000 nm. Hence, all the formulations are satisfactory for pulmonary deposition.

The DSC Thermo gram of Isoniazid shows the onset at 170^{O}C and the midpoint was found to be 171^{0} C and the peak was found to be in 172^{0} C. The DSC Thermo gram of Chitosan shows onset at 87.90^{0}C and peak at 90.28^{0}C and ends at 96.65^{0}C. DSC Thermo gram of Formulation 6 (F6) shows first peak at 90.28^{0}C corresponding to Chitosan and second one at 171.08^{0} C corresponding to Isoniazid. It shows that there are no such characteristic change in the purity of both drug & polymer. The surface morphology of nanoparticle (F6) is shown in Fig. no: 3, the study revealed that the SEM micrographs of cross linked Chitosan nanoparticles have smooth surface. The SEM micrograph also indicates the aggregation of chitosan nanoparticles due to existing weak inter particle bonding.

In vitro drug releases for the formulations were in the range of 76.77- 91.39% with F6 having the maximum drug release and F1 having the minimum drug release.

Pharmacokinetic evaluation shows that all the formulations follow firstorder 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

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.

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%							

Table 2: Evaluation parameters of Isonaizid Nanoparticles

Formulation code	Drug loading (% w/w)	Swelling index (% w/w)	Mucoadhesive strength (%)	Zeta potential (MV)	Average diameter (nm)	In vitro release (24 hrs) %
F1	63.33	48.78	99.1	21.67	678.0	82.05
F2	65.52	47.44	98.8	22.34	683.5	79.28
F3	65.55	48.99	98.6	20.56	661.8	89.46
F4	67.18	49.20	98.4	21.97	724.3	83.83
F5	68.24	48.78	98.2	21.89	661.8	83.13
F6	71.78	49.40	99.5	22.21	823.5	91.39
F7	69.19	49.65	98.3	20.78	747.8	88.76

Table 3: *In vitro* drug release kinetic data

Sl. No	Formulation	Zero order		First order		Korsmeyer- Peppas	Higuchi	
		n value	R^2	n value	R^2	n value	n value	R^2
1	F1	2.372	0.972	-0.026	0.996	0.038	14.43	0.984
2	F2	2.226	0.970	-0.024	0.987	0.565	13.32	0.965
3	F3	2.535	0.914	-0.039	0.944	0.612	15.53	0.940
4	F4	2.325	0.859	-0.030	0.873	0.608	14.55	0.922
5	F5	2.224	0.987	-0.022	0.958	0.570	13.31	0.950
6	F6	2.797	0.954	-0.042	0.976	0.594	16.85	0.948
7	F7	2.097	0.942	-0.026	0.981	0.571	12.72	0.950

Table 4: Drug loading data in stability studies

	Zero Month	First Month	Second Month	Third Month
Drug Loading (25°C ± 2°C / 60% RH ± 5 % RH	57.24	56.98	56.34	55.87
Drug Loading 40°C ± 2°C / 70 % RH ± 5% RH	57.24	55.89	55.02	54.67

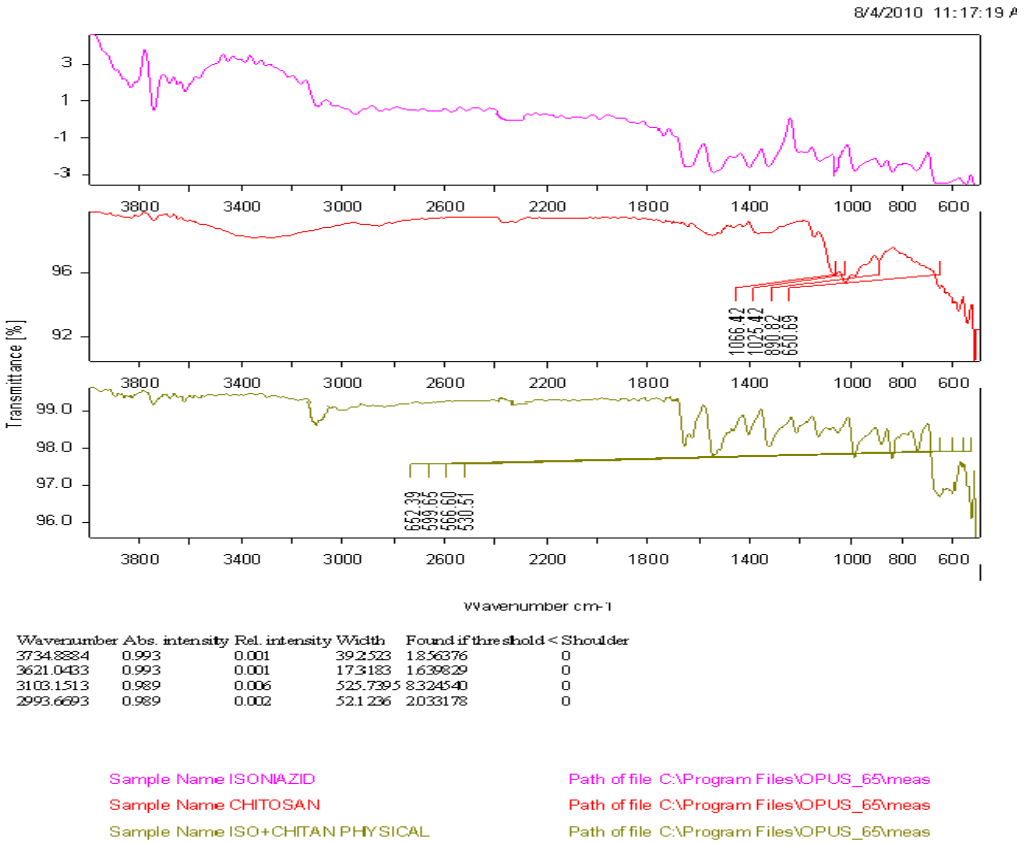

Figure 1: Drug polymer compatibility study using IR spectroscopy

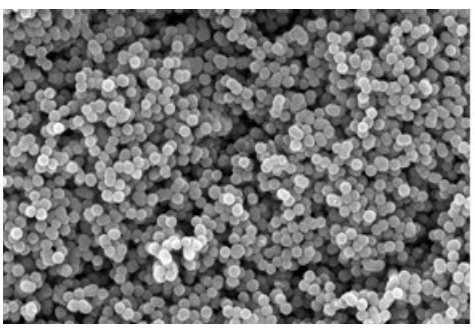

Figure 2: SEM image of Formulation F6

Results

		Diam. (nm)	% Volume	Width (nm)
Z-Average (d.nm): 823.9	Peak 1:	881.4	100.0	227.4
PdI: 0.193	Peak 2:	0.000	0.0	0.000
Intercept: 0.959	Peak 3:	0.000	0.0	0.000
Result quality : Good				

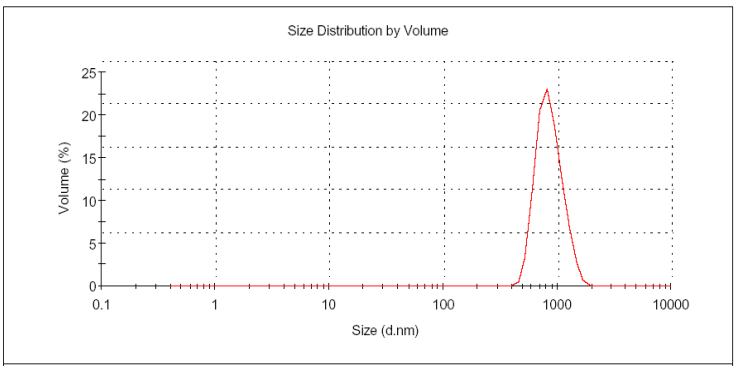

Figure 3: DLS plot of Formulation F6

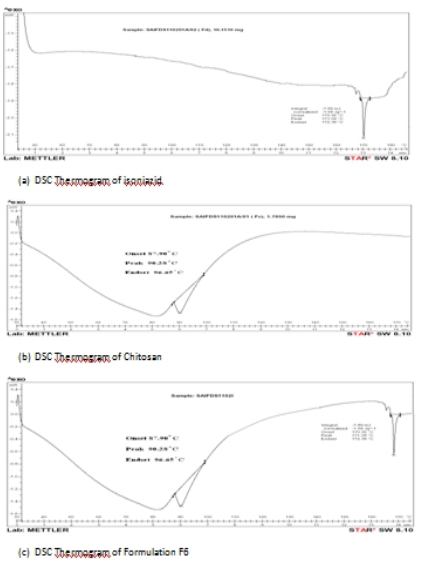

Figure 4: DSC Thermo gram of Isoniazid, Chitosan and Formulation F6

CONCLUSION

The advent of nanotechnology has reignited interest in the lungs as a major route of drug delivery for both systemic and local treatments. The large surface area of the lungs and the minimal barriers impeding access to the lung periphery make this organ a suitable portal for a variety of therapeutic interventions. Nanoparticle formulations have many advantages over traditional dosage forms, such as enhanced dissolution properties and the potential for intracellular drug delivery.The goal of the present investigation was to formulate and evaluate chitosan loaded nanoparticles with isoniazid for TB. Nanoparticles of isoniazid were prepared using chitosan by spontaneous emulsification method. The concentration of the polymer Chitosan was selected based on the results on preliminary screening. 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).. All formulations consist of fine and free flowing powders. The nanoparticles prepared were evaluated for drug polymer inter action, morphology, loading efficiency, *in vitro* release. The particle shape and morphology of the prepared isoniazid nanoparticles were determined by SEM & DLS analysis .It shows that the size of the particles were reduced and formulation no 6 having optimum Nanonised particle. An interaction study like UV and IR spectra (Figure 1) shows that there were no interaction between drug and carrier used. DSC studies confirmed that there is no interaction with drug and polymer. The Amount of isoniazid entrapment in the nanoparticles was calculated by the difference between the total amount of drug added to the nanoparticle and the amount of non-entrapped drug remaining in the aqueous supernatant. It shows that formulation 6 having maximum drug entrapment efficiency. The swelling index decreases as the ratio of chitosan decreases and all the formulation shows good mucoadhesive property. Among the seven formulations Formulation 6 showed maximum drug release profile in dissolution studies. Pharmacokinetic evaluation of all the formulations shows first order rate release profile. Stability studies show that the prepared nano particle is stable for 3 months. From all

these results, it shows that formulation no 6 is the best formulation and which is recommended for future studies.

REFERENCES

1. Shirzad Azarmi, Wilson H. Roa, RaimarLöbenberg , Targeted delivery of nanoparticles for the treatment of lung diseases.Adva Drug Del Rev 2008;60:863-875.
2. Catarina Pinto Reis, Ronald J. Neufeld, Anto´ nio J. Ribeiro, Francisco Veiga, Nano encapsulation. Methods for preparation of drug-loaded polymeric nanoparticles. Nanomed: Nanotech Biol. Med2006;2:8–21.
3. Barnabas Wilson, Malay K. Samanta, Kumaraswamy Santhi, K.P. Sampath Kumar, Muthu Ramasamy, Bhojraj Suresh-Chitosan nanoparticles as a new delivery system for the anti Alzheimer's drug tacrine. Nanomed: Nanotech Biol. Med 2010; 6(1):144-152.
4. Akbar Bayat, Bagher Larijani. Preparation and characterization of insulin nanoparticles using chitosan and its quaternized derivatives Nano: Nanotechnol, BiolMed2008;4:115-120.
5. Pandey R, Khuller GK, Polymer based drug delivery systems for mycobacterial infections, Current Drug Del 2004; 1:195-201.
6. RaoG C, Kumar MS, MathivananN. Et al., Nanosuspensionsas the most promising approach in nanoparticulate drug delivery systems, Pharmazie2004;59:5-9.
7. Wareetiyaboonchai, Chitosan nanoparticles: A promising system for drug delivery, NaresuanUni J 2003; 11(3): 51-66.
8. Ahsan F, Rivas I.P, Khan M.A et al., Targeting tomacrophages: role of physicochemicalproperties of particulate carriers liposomes andmicrospheres on thephagocytosisby macrophages, JContRel 2002;79:29– 40.
9. P.Sabitha, J. VijayaRatna and K. Ravindra Reddy, Design and evaluation of controlled release chitosan-calcium alginate microcapsules of anti tubercular drugs for oral use,.Inter JChemTech Res 2010;2(1),88-98.
10. AlessandroNasti, NohaM.Zaki, Chitosan/TPP and Chitosan/TPP-hyaluronic Acid Nanoparticles, J Pharm Res2009;26(8):1918-1930.
11. Somwanshi SB, DolasRT, SiddheshwarSS, MerekarAN, Nanoscale drug delivery technologies: introduction and recent developments. J Pharm Sci Innov 2013;2(4):18-23.
12. AnaGrenha,CarmenRemunanLopez,EdisonL.S.Carvalho,Begon˜aSeijo,Microspherescontaininglipid/chitosannanoparticlescomplexesforpulmonary delivery of therapeutic proteins, European J PharmBiophar2008;69:83-93.
13. Sofia Papadimitriou , Dimitrios Bikiaris, Konstantinos Avgoustakis, Evangelos Karavas, Manolis Georgarakis Chitosan nanoparticles loaded with dorzolamide and pramipexole, Carbohydrate Polymers 2008;73: 44-54.
14. Gurpreetarora, Karan Malik, Inderbir Singh, Sandeep Arora, Formulation and Evaluation of Controlled Release Mucoadhesive Matrix Tablets: Assessment of Myrrh Oleo Gum Resin as a Natural Pharmaceutical Excipient, Inter JPharmSci Drug Res 2011; 3(2):84-88.

Source of support: Nil, Conflict of interest: None Declared

QUICK RESPONSE CODE	ISSN (Online) : 2277 –4572
	Website http://www.jpsionline.com

How to cite this article:
Junise V, R Saraswathi. Development and characterization of chitosan nanoparticles loaded with isoniazid. J Pharm Sci Innov. 2015;4(3):190-195 http://dx.doi.org/10.7897/2277-4572.04342