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Formulation and characterization of isoniazid loaded chitosan microspheres

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Abstract
Microencapsulation for oral use has been employed to sustain the drug release and to reduce or eliminate gastrointestinal tract irritation. The objective of the present study was to prepare microspheres with acceptable and suitable specifications by solvent evaporation using Isoniazid (INH) as model drug, chitosan as encapsulating polymer. Isoniazid loaded chitosan microsphere are orally administered to treat tuberculosis (TB) caused by Mycobacterium tuberculosis. Chitosan is used as polymer to improve the release of drug in a controlled manner. In the present study microspheres were prepared by solvent evaporation method and were evaluated for physicochemical parameters such as drug excipient interaction by FTIR, particle size distribution, encapsulation efficiency, *in-vitro* drug release studies. Optimisation was based on controlled release time (72hours) with acceptable cumulative percentage drug release. The FTIR study revealed that there is no drug – drug and drug excipients interaction. From the study it is revealed that a promising controlled release microsphere drug delivery of isoniazid can be developed.

Keywords: microencapsulation, isoniazid, chitosan, controlled release

Introduction
Tuberculosis (TB) is a contagious disease spread by inhalation of the airborne droplets containing tubercle bacilli, mycobacterium tuberculosis. Isoniazid is used alone or with other drugs to treat tuberculosis (TB) and to prevent it in people who have had contact with tuberculosis bacteria. It eliminates only active (growing) bacteria. Since the bacteria may exist in a resting (non-growing) state for long periods, therapy with isoniazid (and other ant tuberculosis drugs) must be continued for a long time (usually 6-12 months).
Isoniazid was selected as a model drug for dry powder formulation in treatment of tuberculosis. INH 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 site specific release formulation especially in case of tuberculosis to deliver it in lungs. Chitosan is a bio adhesive, biocompatible and biodegradable polymer. The release modifying and mucoadhesive property of chitosan appears to be a good choice of preparing sustained release formulations for lung delivery via inhalation.
Microencapsulation for oral use has been employed to sustain the drug release, and to reduce or eliminate gastrointestinal tract irritation. Due to its small particle size, they are widely distributed throughout the gastro intestinal tract which improves the drug absorption and reduces side effects due to localized build-up of irritating drugs against the gastrointestinal mucosa.
Synthetic polymeric microspheres are widely used in clinical application, also used as bulking agent, fillers, embolic particles, drug delivery vehicles etc and proved to be safe and biocompatible. But the main disadvantage of this kind of microspheres is that they tend to migrate away from injection site and lead to potential risk, embolism and further organ damage.
Isoniazid (INH) is a bactericidal agent active against the organism of the genus Mycobacterium specifically M. tuberculosis, M. Bovis, M. Kansasii. The drug is a bactericidal to rapidly dividing Mycobacteria, but is bacteriostatic if the Mycobacterium is slow growing. Isoniazid is highly specific being active against only a subset of the Mycobacteria and largely ineffective against other micro organisms.
It is a prodrug activated by catalase-peroxidase hemoprotein KatG. INH inhibits InhA, NADH- specific enoyl-acyl carrier protein (ACP) reductase involved in fatty acid synthesis.
The prepared microspheres may provide constant and prolonged therapeutic effect, reduces the dosing frequency and thereby improve the patient compliance. They could be injected into the body due to the spherical shape and smaller size. Better drug utilization will improve the bioavailability and reduce the incidence or intensity of adverse effect. The microsphere morphology allows a controllable variability in degradation and drug release.

Materials and Methods
Isoniazid was a gift sample obtained from Themis lab, Mumbai; chitosan from central institute of fisheries technology, Cochin; and all other chemicals and reagents were of laboratory grade.

Standardisation of drug [4]
10mg of isoniazid was dissolved in 100ml of 0.1 N HCL,

and further dilutions were made by using 0.1N HCL to obtain concentrations ranging from 2 to 10 µg/ml. The absorbance of solution was measured at 266.5nm using UV – Visible Spectrophotometer.
10mg of isoniazid was dissolved in 100ml of 6.8 phosphate buffer, further dilutions were made by using 6.8 phosphate buffer to obtain concentrations ranging from 5 to 25µg/ml. The absorbance of solution was measured at 265.5nm using UV- Visible Spectrophotometer.

Preparation of microspheres by solvent evaporation method
The polymer is dissolved in a suitable water immiscible solvent and the medicament is dispersed or dissolved in this polymeric solution. Bioactive compound is added in to the solution of the matrix material by either co dissolution in a common solvent, dispersion of finely pulverized solid material or emulsification of an aqueous solution of the bioactive compound immiscible with the matrix material solution. Dispersion of solid or dissolved bioactive material into the matrix solution can be done with the help of impeller or static mixing, high speed – stator mixing or micro fluidization.

Droplet formation
This is the step that determines the size of resulting microspheres. The size of microsphere affects the rate of drug release and drug encapsulation efficiency. The following procedures are used in droplet formation.

Stirring
The extraction phase is filled into a vessel & agitated by an impeller. The drug/matrix dispersion was then added, drop wise or all at once, under agitation at a speed sufficient to reach the desired droplet size.

Static mixing
Static mixer consists of baffles or other flow obstacles installed in a tube. The baffle arrangement repeatedly splits and recombines the stream of fluid passing through the tube.

Extrusion
It involves feeding of drug/matrix dispersion through single or multichannel pathways directly into the continuous extraction phase. When drug/ matrix dispersion leaves the pathways, discrete droplets are formed within the slow flowing continuous phase. In extrusion, flow is laminar, the droplets are formed at the site of introduction of drug/matrix dispersion into continuous phase, due to which there is no effect on size of droplets formed thereafter. Where as in static mixing, turbulent flow occur which constantly act on the disperse phase and thus there is a continuous change in the size of droplets.

Dripping
Microspheres were prepared by dripping 10% and 15% (w/w) solution of poly (ethylene-co-acetate) in DCM, containing dispersed protein particles from a needle into an electric field. The droplet formed was dethatched from the needle by electrostatic forces.

Solvent Removal
Solvent removal can be achieved either by evaporation or extraction. In both processes the drug/matrix dispersion should be slightly soluble in the continuous phase so that partitioning into continuous phase can occur that leads to precipitation of the matrix material. The two ways of solvent removal are as follows:

Solvent Evaporation
In this method, the capacity of the continuous phase is insufficient to dissolve the entire volume of disperse phase solvent. Thus, solvent evaporates from the surface of the dispersion to obtain hardened microsphere.

Solvent Extraction
This is a two step process. Firstly, the drug/matrix dispersion is mixed with a small quantity of continuous phase to yield desired size of droplets. Then secondly further more continuous phase and/or additional extraction agents are added at an amount sufficient to absorb the entire solvent leaching from droplets of drug/matrix. This results into formation of solid microspheres.

Microsphere drying
Solidified microspheres were separated from the continuous phase either by filtration or centrifugation. Then the particles were rinsed with suitable liquids to remove adhering substance such as dispersion stabilizers or non encapsulated drug. Finally these microspheres were dried at elevated temperature or under reduced pressure to yield free flowing powder.

Table 1: Drug polymer ratio

Formulation code	Drug (INH)	Polymer (chitosan)	Drug polymer ratio
F	150mg	-	-
F1	150mg	150mg	1:0.25
F2	150mg	225mg	1:0.5
F3	150mg	300mg	1:1
F4	150mg	375mg	1:1.5
F5	150mg	450mg	1:2

Results
Compatibility Studies
The infrared (IR) spectra recorded using an FTIR spectrophotometer (Shimadzu IR affinity-1) in the wavelength region between 500 and 4000cm^{-1} shows no interaction between the drug excipients.

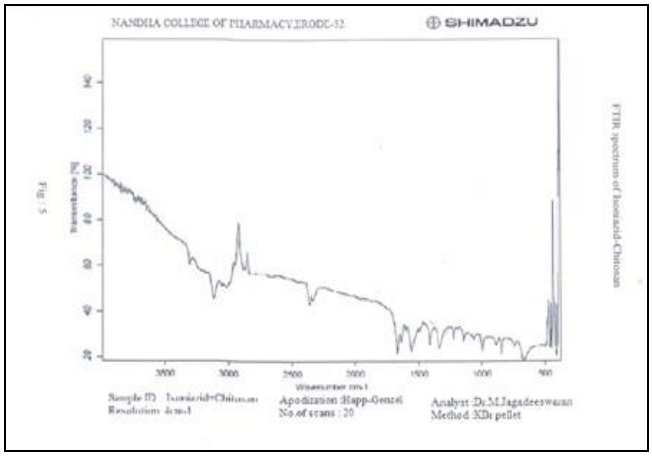

Fig 1: IR Spectra of Drug and Excipient

The study results show that all the Pre-formulation and Post-

formulation parameters were within the permissible limits as shown in table 2 - 3.

Table 2: Pre-formulation parameters

Formulation code	Average particle size (µm)	Angle of repose ± SD (˚)	Bulk density ± SD (gm/cm$^{3)}$
F1	68.58	27˚ 24' ±1.18	0.57±0.018
F2	75.17	26 ˚56±1.09	0.58±0.040
F3	83.41	26˚ 23' ±0.58	0.55±0.017
F4	81.50	31˚ 15' ±0.70	0.57±0.019
F5	66.41	26 ˚56' ±1.00	0.56±0.031

Table 3: Encapsulation Efficiency

Formulation	Entrapment efficiency (%)	Drug content ± SD (%)
F1	53.75	70.62±1.23
F2	57.20	72.00±1.10
F3	69.05	77.92±1.07
F4	68.00	74.98±1.32
F5	66.25	71.24±1.03

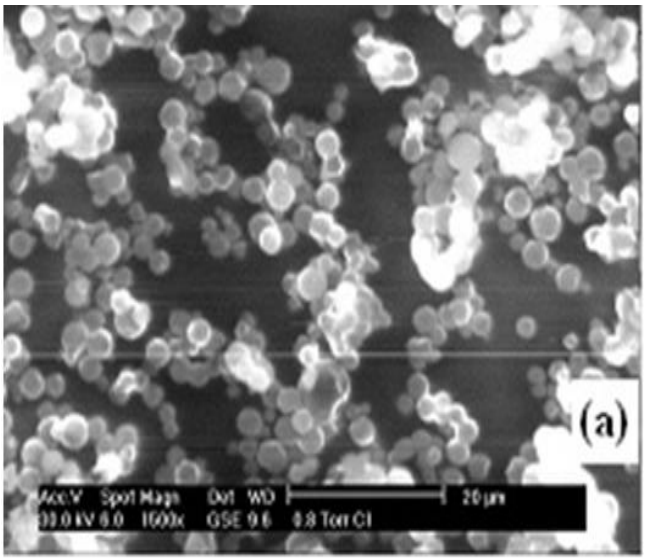

Fig 2: SEM image of Isoniazid loaded chitosan microspheres for formulation F1

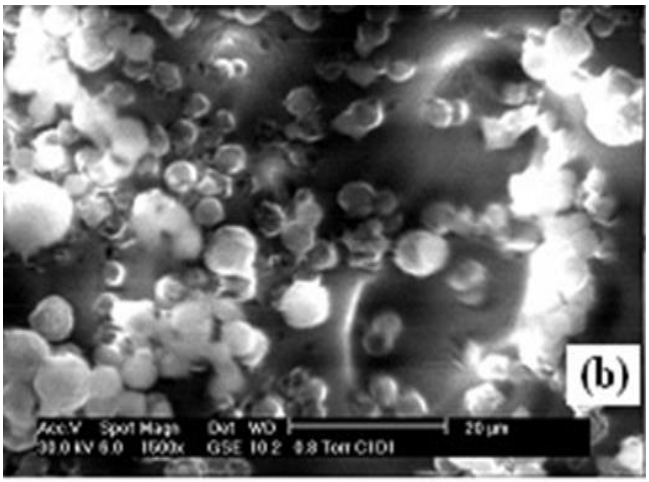

Fig 3: SEM image of Isoniazid loaded chitosan microspheres for formulation F2

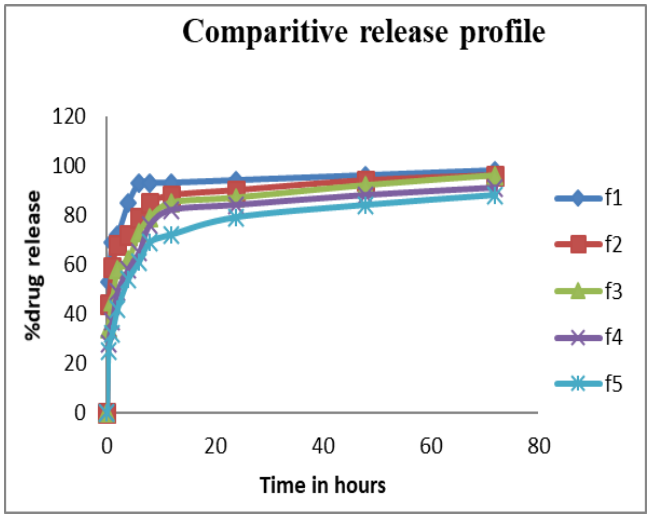

Fig 4: In-vitro drug release profile of different formulations

Discussion

The degradation of isoniazid in the stomach that results in poor bioavailability from currently available dosage forms remains a concern for effective control of tuberculosis.

In past several years many approaches were attempted by researchers. Chitosan was chosen as polymer for preparation of microspheres as the polymer is natural, biodegradable, free from toxicity and ideal for chronic infections like tuberculosis. Pre-formulation studies were performed and the results showed isoniazid and chitosan were satisfactorily compatible without any change in the chemical nature of the drug and also different polymer ratio was used to study the drug release with respect to time.

The morphology microspheres done by SEM analysis showed that the microspheres are spherical with smooth surfaces and with no aggregation. The particle size distribution of all the formulations were determined and mean average particle size of isoniazid loaded chitosan microsphere was in the range of 66.41 to 83.41µm. The angle repose of different formulations was within the range of 26˚ to 31˚. The bulk density of microsphere was in the range of 0.55 to 0.58gm/cm^3. Standard calibration curve for the drug obtained by measuring absorbance at 262nm and by plotting the graph of absorbance versus concentration in pH 0.1N, pH 6.8 was between 2 to10 µg/ml concentrations.

Drug entrapment efficiency was in the range of 53% to 67%. The drug entrapment efficiency of micro particles increases with the increase in concentration of chitosan. *In-vitro* isoniazid release was found to be successful from the formulations. It shows that decrease in release was observed with increase in percentage of polymer with increase in concentration with time. The study also indicated that the time required for drug release increases with and decreases in the polymer concentration.

Conclusion

It has been observed that microspheres are better choice of drug delivery system than other types of drug delivery system because it is having the advantage of target specificity and better patient compliance. From the study it

is revealed that a promising controlled release microsphere drug delivery of isoniazid can be developed. Further study is recommended to perform in vivo investigation using animal model to establish the efficacy of these formulations.

References

1. Pejman Sadegh Pourshahab, Kambiz Gilani, Esmaeil Moazeni, Hamideh Eslahi, Mohammad Reza Fazeli, Hossein Jamalifar. Preparation and characterization on spray dried inhalable powders containing chitosan nanoparticles for pulmonary delivery of isoniazid. Journal of microencapsulation. 2011; 28(7):605-613.
2. Prasanth VV, Akash Chakraborthy Moy, Sam T Mathew, Rinku Mathapan. Microspheres- An Overview. International Journal of Research in Pharmaceutical and Biomedical sciences. 2011; 2(2).
3. Kataria Sahil, Middha Akanksha, Sandhu Premjeet, A jay Bilandi, Bhawana Kapoor. Microspheres – A review. International journal of research in pharmacy and chemistry IJRPC. 2011; 1(4).
4. Amol V Pande, Pravin D Vaidya, Aseem Arora, Madhura V Dhoka. In vitro and in vivo evaluation of ethyl cellulose based floating microspheres of cefpodoxime proxetil. Int J Pharm Biomed Res. 2010; 1(4):122-128.
5. Gangadharappa HV, Srirupa Biswas, Anil Getyala, Vishal Gupta N, Pramod Kumar TM. Development, In vitro and In vivo Evaluation of Novel Floating Hollow Microspheres of Rosiglitazone Maleate. Der Pharmacia Lettre, 2011; 3(4):299-316.
6. Kataria Sahil, Middha Akanksha, Sandhu Premjeet, Ajay Bilandi, Bhawana Kapoo. Microsphere: a review. International journal of research in pharmacy and chemistry. IJRPC. 2011, 1(4).
7. Jaya Raja Kumar, Selvadurai Muralidharan, Subramani Parasurama. In Vitro and In Vivo Evaluation of Microspheres Loaded Topical Gel Delivery System of Ketoconazole in Male Rats against Candida Glabrata, Sci. & Res. 2014; 6(11):376-381.
8. Pavani Sriram, Deepak Kamlekar, Sujitha Hazari. Preparation and in vitro evaluation of chitosan microspheres of eplerenone. International Journal of Pharmacy and Pharmaceutical Science. 2013; 5(Suppl 3).
9. Nagda C, Chotai N, Patel S, Nagda D, Patel U, Soni T. Chitosan microspheres of aceclofenac: in vitro and in vivo evaluation. Pharm Dev Technol. 2010; 15(5):442-51.
10. Ranjan OP, Shavi GV, Nayak UY, Arumugam K, Averineni RK, Meka SR, Sureshwar P. Controlled release chitosan microspheres of mirtazapine: in vitro and in vivo evaluation. Arch Pharm Res, 2011; 34(11):1919-29.
11. Himansu Bhusan Samal. formulation, charcterization and in-vitro evalution of floating microspheres of nateglinide. International Journal of Pharma and Bio Sciences. 2011; 2(1).
12. Venkata Ramana Reddy K, Pratap Kumar Patra, Divakar K, Venkateswara B. Formulation and in vitro studies of Carvedilol microspheres with its characterization. International Journal of Pharmacy and Pharmaceutical Sciences. 2014; 6(4).
13. Arifa Begum SK, Basava Raju D, Rama Rao N. Simultaneous estimation of rifampicin and isoniazid in combined dosage formby a simple UV spectrophotometric method. Der Pharmacia Lettre, 2013; 5(3):419-426.
14. Saviae R, Eisenberg LLA, Maysinger D. Micellar nanocontainers distribute to defined cytoplasmic organelles, Science. 2003; 300:615-618.
15. Pisal S, Shelke V, Mahadik K, Kadam S. Effect of organogel components on in vitro nasal delivery of propranolol hydrochloride. AAPS Pharm Sci Tech. 2004; 5:63.
16. Rossi S, Bonferoni MC, Ferrari F, Caramella C. Drug release and washability of mucoadhesive gels based on sodium carboxymethylcellulose and polyacrylic acid. Pharmaceutical development and technology. 1999; 4(1):55-63.
17. Shiraishi S, Imai T, Otagiri M. Controlled release of indomethacin by chitosan polyelectrolyte complex: optimization and in vivo/in vitro evaluation. J Control Release. 1993; 25:217-225.
18. Warren SJ, Kellaway IW. The synthesis and in vitro characterization of the mucoadhesion and swelling of poly (acrylic acid) hydrogels. Pharm Dev Technol. 1998; 3(2):199-208.
19. Lim ST, Martin GP, Berry DJ, Brown MB. J. Control Rel. 66, 281-292. U.S. Koff (1963). Patent, March 2, 1963, 3, 080, 292, 2000.
20. Leitner VM, Guggi D, Bernkop-Schnürch A. 5th Central Eur. Symp. Pharm. Technology, Ljubljana, Slovenia, 2003.
21. Huang YC, Yen MK, Chiang CH. Formulation factors in preparing BTM-chitosan microspheres by spray drying method. Int J Pharm. 2000; 242:239-42.
22. Rao YM, Devi KM, Rameshachary B. Stability study of Rifampicin mucoadhesive nasal drops. Indian J Pharm Sci. 1999; 61:366-70.