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Preparation and In-Vitro /In-Vivo Evaluation of Chitosan Based Microspheres as Respirable Slow Release Isoniazid Formulation

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ABSTRACT

The present work was aimed to prepare respirable slow release formulation for isoniazid by spray drying method using hydrophilic carrier, chitosan. The chitosan microspheres were crosslinked with glutaraldehyde to modify release characteristics. Microspheres were prepared and evaluated for % yield, drug loading, moisture content, morphological characteristics, particle size, tapped densities, in vitro drug release, in vitro aerosolization and pharmacokinetic parameters. The scanning electron microscopy revealed that microspheres produced were spherical shaped with slight rough surface. The drug loading efficiency of microspheres showed drug loading efficiency was in the range of 84.44 % to 98.24 %. The in-vitro drug deposition revealed that mass median aerodynamic diameter of crosslinked chitosan microspheres (2.82 µm) was better than the uncrosslinked chitosan microspheres (3.85µm). The complete drug release was seen with uncrosslinked microspheres in two hour while crosslinked chitosan microspheres showed sustained drug release for more than 12 hrs. All the formulation batches showed Carr's index in the range of 28.7 to 34.3 %. The fine particle fraction for crosslinked chitosan microspheres was found to be 69.1%. Pharmacokinetic differ among the free, crosslinked and uncrosslinked formulations. The crosslinked chitosan microspheres showed sustained drug release lasted for more than 3 days with half life of 31.67 hrs. In vitro and in vivo evaluation studies suggested that chitosan microspheres prepared with spray drying method showed promising aerosol properties having potential to use as sustained drug release formulation for isoniazid as inhalable microparticles.

Keywords: Microspheres, Isoniazid, hydrophilic carrier, dry powder inhalation, Sustained drug release.

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INTRODUCTION

Isoniazid is an antibiotic. Isoniazid (INH) is used to treat tuberculosis and other mycobacterial infections. Isoniazid is the most effective antituberculosis drug currently available. The drug is characterized by short half life ranging from 1hr to 5 hr depending on the rate of metabolism. Isoniazid is metabolized in the liver by acetylation and dehydrazination[1]. Recent studies indicated that the INH related liver toxicity is caused by the major metabolite of INH, acetyl hydrazine, which is formed by metabolic hydrolysis of Ac-INH [2]. The rate of acetylation is genetically determined and subject to individual variation. INH is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH[1]. Hence a drug formulation with controlled release of INH is required especially in the small intestine. Therefore it can be considered as a choice of drug to be delivered at site specific to lung. The hepatic metabolism can be overcome if the drug is delivered directly to the lung which offers a largest surface area for absorption of drug. Inhalation route is an effective method to deliver therapeutic agents to respiratory tract. Local and systemic delivery of drug through the lung is highly desirable, especially in case of patient with pulmonary disease. The delivery of drugs in small dose to lung also improves the therapeutic index of drug compared to larger dosed given by other route of administration. Dry powder inhalers (DPI) seems to be most popular amongst the current drug delivery device to lung compared with nebulizers and pressurized metered dose inhalers. The other important aspect of dry powder inhalers is its design which offers physicochemical and microbiological stability of drug. The dry powder inhalers offer comparatively high reach of drug concentration to the site of action. This benefits associated with dry powders makes DPI more successful in treatment of local and systemic diseases[3,4]. DPI formulations generally consist of at least one other component as carrier to facilitate aerosolization of the therapeutic agent. Carrier particles are used to improve the flowability and thus dosing accuracy with minimum variability of dose. Inhalable particles in general need to fulfill the requirement of optimum particle size with diameter <10 preferably within 1-5 μm for deep lung deposition. Lactose monohydrate, mannitol, trehalose and amino acids are generally used as flowability enhancers and bulking agents [5-7]

One of the strategies to deliver drug with potential implication of inhalation delivery is to deliver drug encapsulated and to be delivered in the form of microparticles or nanoparticles. The incorporation of drug to polymeric matrix can lead to improve the therapeutic index of drug compared to free drug administered. There are many advantages of preparing sustained release

dosage formulations for pulmonary delivery including reduced frequency, improved patient compliance and reduction in side effects. Besides the advantage of providing sustained release, microparticulate preparation can target alveolar macrophages that harbor the Tubercular bacteria [8,9]. Chitosan being a natural polymer with drug release modifying properties and mucoadhesive properties appears to be a useful excipient for inhalation of therapeutic agent. Chitosan is able to form microparticulate carriers with good attractive bioadhesive properties related to attractive electrostatic forces between the negatively charged glycoprotein of mucin and the positively charged amino groups of the polymer[10]. Further more chitosan is less toxic than other polymers and natural antibacterial properties make it an interesting candidate for investigation for respiratory delivery of drug [11-14]. Other polymers such as hyaluronan, poly (lactic acid), poly (D, L lactic, co-glycolic acid), hydroxyl propyl cellulose, carbomers, sodium carboxymethyl cellulose, sodium alginate and gelatin have been studied by many researchers for inhalation therapy [15-20].

The Aim of the present research work to develop INH loaded crosslinked chitosan microspheres by spray drying method combining the use of crosslinking agent to achieve sustained drug delivery and targeting INH to alveolar macrophages. The prepared microspheres were characterized for their physicochemical properties like size, drug loading, morphology, densities. The suitability of microspheres as sustained release formulation system for Isoniazid was assayed by in vitro deposition study and in vivo pharmacokinetic studies.

MATERIAL AND METHODS

Materials

Isoniazid was obtained as a gift sample from Lupin laboratories, Aurangabad (Inida), chitosan was generously provided by Central institute of fisheries technology, Cochin, India. Glutaraldehyde 25% aqueous solution was purchased from S D fine Chemicals, Baroda. L Leucine was purchased from Renkem, India. α, mono lactose was generously provided by Meggle, Wasserburg Gmbh and Co., Germany. All Other chemicals and solvents used were of analytical grade.

Preparation of spray dried microspheres

Isoniazid, leucine and lactose were dissolved in 300ml of 1% w/v Chitosan solution. Chitosan solution was prepared by dissolving chitosan flakes in 1% v/v acetic acid solution by stirring at 50°C overnight. The drug polymer solution was stirred mechanically at 500 rpm for 5 minutes. Glutaraldehyde (GH) (0.125, 0.25, 0.5 and 1 ml) was added drop wise into chitosan-drug

solution and stirred for 30 minutes at 1000 rpm. The prepared drug-chitosan-GH-drug solution was spray dried to obtain the crosslinked chitosan microspheres loaded with drug. Spray drying was performed using lab Ultima spray drier (LU 222-advance spray drier) with a standard 0.7 mm nozzle. The spray drying conditions such as inlet temperature, temperature, pump prate, pressure and aspirator setting were set as 140°C, 5 ml/min, 3 kg/cm^2 and 45 m^3/hr, respectively. These spray drying conditions resulted in outlet temperature of 75-80ºC. The chitosan microspheres were produced by spray drying 300ml of aqueous chitosan solution containing 0.5 %w/v Isoniazid, 1 % w/v chitosan, 0.4 % leucine and 0.1% w/v lactose.

Determination of % yield

The % yields of the microspheres obtained by spray drying were calculated as the percentage ratio of weight of recovered spray dried powder to the total amount of dry solids in the initial feed solution.

% yield = *[Mass obtained / Theoretical mass]* x 100

Determination of drug loading

To calculate drug loading, 25 mg of microspheres were accurately weighed and dissolved in 50 ml of 0.1 N HCl in volumetric flask. The resulting solution was kept on shaking for 1 hour and then passed through 0.22 µm membrane filter (Whatman filters, UK, No 41). The drug content was determined by measuring the absorbance at 265 nm by UV spectrophotometer after suitable dilution

Drug loading: (amount of drug calculated/ amount of microsphere) x100

Determination of moisture content

The residual water content in the microspheres was determined by keeping microspheres in the desiccators containing charged silica beads. 100 mg of sample was placed in a desiccator. The dry weight was determined at specific intervals until constant weight obtained. The difference between Initial and final weight was calculated and moisture content was calculated by equation below,

Moisture content (MC) = 100 x $[(W_0 - W_t)/ W_t]$

Where W_0 is initial weight and W_t is final weight

Differential Scanning calorimetric (DSC) analysis

Differential scanning calorimetry **(DSC)** experiments were carried out in order to characterize the physical state of the drugs. DSC scans of the drug loaded microspheres were performed using DSC- PYRIS-1 (Perkin-Elmer Thermal Analysis). The Test samples were placed in aluminium pans and thematically sealed. The experiment was carried out at heating rate of 10ºC per minute

using nitrogen as the purge gas. The analysis was performed with a heating range of 50 - 300°C.

Morphological characterization

Microspheres prepared were examined for their surface properties and shape by means of scanning electron microscope (ESEM TMP with EDAX, Philips, and Holland). Spray dried powders were mounted onto separate, adhesive coated 12.5 mm diameter aluminium stubs. Excess powder was removed by tapping the stubs sharply and then gently blowing a jet of particle free compressed gas across each. The specimens were examined using EDAX SEM. The SEM was operated at high vacuum with accelerating voltage of 5-15 KV and specimen working distance of 12mm.

Determination of particle size by laser diffraction method

The particle size of the spray dried powder was also measured by laser diffraction (HELOS particle size analyzer VIBRO/RODOS dry dispersion system: Sympatec gmbh system partikel technik, clausthal zelerfeld, Germany), equipped with a computer-controlled image analysis System. Approximately 100mg of powder was used to achieve the required obscuration of 5%.

Determination of powder densities and aerodynamic diameter

The poured density of the spray dried powder was determined by pouring known mass of powder (approximately 0.5g) under gravity into a calibrated measuring cylinder and recording the volume occupied by the powder. The tapped densities of the spray dried powders were determined by volume measurement of tapped mass until no further change a in the powder volume was observed. Measurement was performed in triplicate (n = 3). The tapped density was calculated by the following equation

Carr's Index = [1- (bulk density /Tapped density)] x100

Theoretical estimation of the particle primarily aerodynamic diameter $MMAD_t$ was derived from the sizing ($\mathbf{d_{0.5}}$) and tapped density data ($\boldsymbol{\rho}$) as follows[21]

$MMAD_t = \mathbf{d_{0.5}}\,(\boldsymbol{\rho} / \boldsymbol{\rho_1})^{0.5}$ ***where*** $\boldsymbol{\rho_1}$ is $1 gm/cm^{-3}$

Characterization of Aerosol performance of microspheres

An eight stage Andersen cascade impactor was used to determine the fine particle fraction of the INH formulation. Eight metal plates were installed on the impactor before use. A formulation containing 10 mg equivalent of isoniazid was filled in HPMC capsule and loaded directly into the chamber with the help of the respirable device, cyclohaler and dispersed at an aspiration rate of 60L/min for an inhalation time of 10 sec[22]. Powder deposited on each plate and throat piece was extracted with 0.1 N HCl and analyzed by UV spectroscopy at the wavelength of 265nm after suitable dilution. The Emitted dose (ED), Fine Particle Fraction (FPF), fine particle dose

(FPD) and Mass Median Aerodynamic Diameter ($MMAD_{ae}$) were determined. The emitted dose was calculated as the percentage of total loaded powder mass exiting the capsule. The % FPF was determined as the total percentage deposition at stages 2–7 of the cascade impactor. FPD was calculated from the ratio of total mass of powder having particle size below 5µm, found on the stages 2-7 of the apparatus to the number of doses. $MMAD_{ae}$ of the powders was also derived, defined as the particle size at the 50% mark of a plot of cumulative fraction vs. effective cut-off diameter.

In vitro drug release studies

Dissolution testing was performed as described in our earlier studies[23]. Briefly, Dissolution test was performed on a USP Type II dissolution test apparatus at a stirring speed of 150 rpm. A dialysis membrane was cut into equal pieces of about 5 cm x 3 cm and pre-treated. Microspheres (50 mg) were accurately weighed out on the pre-treated dialysis membrane and sealed with clips. The pouch thus formed was attached to the paddles of the apparatus using cotton threads over the clips. 900 ml of phosphate-buffer pH of 7.4 was used as a dissolution medium to ensure sink conditions. Samples were withdrawn for analysis at specified time points and assessed for Isoniazid content by UV spectroscopy (Shimadzu UV-1700, Japan) at 265 nm. Each dissolution experiment was performed in triplicate.

Pharmacokinetic studies

Experimental design

Wistar albino rats (250-300g) were used for animal experiments. Rats were housed in a room maintained on a 12 hr light/dark cycle at 23 ± 2 ºC with free access to food and water. The animal experimental procedures were approved by Institutional Animal Ethics Committee (IAEC). The animals are divided into four (4) groups, each group containing 6 animals (n=6). Animal of group 1 was kept at controlled. Animals of the group 2 received drug via oral route in solution form. The other two groups, group 3 and group 4 received drug formulations via pulmonary route through insufflation. The rats were fasted for 1 day before the experiments and kept on water intake only. The rats were anesthetized by intraperitoneal injection of 50 mg/kg ketamine: 5 mg/kg xylazine.

The powder formulation (5 mg/kg) was insufflated using 3 ml of air by insufflator syringe. The powder was introduced to the rat lung which was maintained at an angle of 80°. Drug solution in PBS (1 mg/0.5 ml) was orally administered by oral feeding needle. After the administration of formulations the rats were positioned to an angle of 10ºC. Volume of dose and air in syringe were kept constant in each case. Serial blood samples were collected periodically by puncturing

of retro orbital plexus using heparinzed capillaries and collected in heparinzed centrifugation tubes with aseptic precautions under mild ether anesthesia. Blood samples of 1ml volume were collected at prespecified time intervals (at 0.5, 1, 2, 4, 6, 8, 12, 24 and 72 hrs) and cold centrifuged for 30 minutes at 5000 rpm below 4 º C. The drug concentration in plasma was determined by method as described by Rohit Bhandari et al[24] with slight modification. To 200µL aliquots of plasma a 300 µL of methanol was added as deprotinizeing agent and the dispersion was then vortexed for 2 minute. The sample was then centrifuged at 10000 rpm for 15 minutes at 4 ºC. The supernatant was then collected and vacuum dried at 40º C. Residue after complete drying was reconstituted with 50µl of methanol: water (75:25). The samples were then filtered (0.20 µm nylon filters). The drug concentration in reconstituted samples were determined by HPLC method, setup consist of flow rate 1ml/min with C18 column (RP-C_{18} 2.27µm size, 250 mm 4.6 mm) and the eluent was monitored by UV detector at λ_{max} 254 nm. The mobile phase was consist of Methanol (75 %) and 0.02 M disodium hydrogen orthophosphate (25%), adjusted to pH 4.5. The calibration curve of isoniazid in plasma was linear in the range of 100ng to 10µg/ml (r^2 = 0.9995). The assay was validated to ensure both accuracy and precision. The intraday precision (coefficient of variation) and accuracy of the quality control (QC) samples were 4.65 to 9.52 % and 98.3 to 102.4% respectively.

RESULT AND DISCUSSION

The chitosan microspheres were prepared with Spray drying method. Spray drying is one step method which is having the advantage of producing dry powders with desired characteristics from drug excipient solution or suspensions. Recently, a number of articles have been published describing the preparation of microspheres from natural or semi synthetic polymers by spray drying. The capacity of producing micron sized, spherical and ability to manipulation of physical properties makes it suitable to produce particles for pulmonary inhalation. Many researchers are involved in exploration of production of respirable powder from the natural origin polymers by spray drying method. Chitosan is a natural bio polyamino- saccharide, obtained by the alkaline deacetylation of chitin. Number of applications of chitosan as polymer is explored due to its relative biodegradability, low toxicity and biocompatibility[25]. Production of microparticles is desirable as altering a number of process parameters can be conveniently control particle size[26]. A repsirable fraction (<6.4µm) of 11-18 % was reported for albumin microspheres containing tetradine, anti silicotic agent, which although low, therapeutically provided greater lung concentration that those achievable with oral dosing[27].

Preparation and characterization of spray dried chitosan microspheres

The physical properties of spray dried chitosan microspheres loaded with drug is presented in table 1 and 2. The % yield of the chitosan microspheres produced by spray drying method was ranged between 29.8 to 36.2 % of the total anticipated solids in manufacturing process. There is little improvement in % yield was seen with addition of crosslinking agent. Although, under the present spray drying conditions the % yield of chitosan microspheres were no much high. It was noticed that the chitosan formulation had lesser moisture content that was in line with the outcomes that indicated moisture content of the spray dried powders to be up to 7%. The moisture uptake was remained below 4 % for all formulation batches. The particle size analysis showed microspheres produced had particle size below 6 µm. It was noted that inclusion of crosslinking agent resulted in decrease in particle size. As the amount of crosslinking agent increased in the formulation the particle size was found to be decreased. The surface morphology of spray dried chitosan and chitosan-GH-microspheres are shown in figures 1.

Table 1: % yield, moisture content and drug loading of chitosan microspheres

Formulation code	Glutaraldehyde (ml/300ml)	(%)Yield	Drug loading (%)	Moisture content (%w/w)
IM1	----	29.8 ± 2.8	24.56 ± 1.05	3.92 ± 0.03
IM2	0.125	32.4 ± 3.1	23.05 ± 1.36	3.17 ± 0.04
IM3	0.25	33.7 ± 2.9	22.66 ± 0.77	3.40 ± 0.05
IM4	0.5	36.2 ± 3.1	21.11 ± 1.67	3.49 ± 0.09
IM5	1	34.8 ± 2.5	21.53 ± 2.00	3.55 ± 0.04

Table 2: Powder properties of cross linked chitosan microspheres

Formulation code	Bulk density (g/cm^3)	Tapped density(g/cm^3)	Carr's Index (%)	$MMAD_t$	Particle size (µm)
IM1	0.152 ± 0.002	0.222 ± 0.014	31.8	2.55	5.8 ± 0.3
IM2	0.156 ± 0.005	0.238 ± 0.017	34.3	2.49	5.1 ± 0.3
IM3	0.138 ± 0.011	0.208 ± 0.009	33.3	2.19	4.8 ± 0.4
IM4	0.147 ± 0.006	0.217 ± 0.004	32.3	2.00	4.3 ± 0.2
IM5	0.151 ± 0.002	0.212 ± 0.006	28.8	1.75	3.8 ± 02

The spray drying process has produced the microspheres with spherical and slight depressed surfaces either crosslinked or uncrosslinked. The particle size of chitosan microspheres loaded with isoniazid was less than 10µm and having good particle distribution as seen in SEM image(Figure 1). The SEM image also depicts the aggregation of chitosan microspheres. There was no sign crystallization of drug on the surface of microspheres that indicated towards the effective encapsulation of drug into the polymer matrix. The aggregation of particles may be linked to the responsive surface properties like cohesive and adhesive forces exist between the small particles. In presence of sufficient amount of GH, fusion of smaller microspheres has given

rise to chitosan microspheres of a larger size. The laser diffraction studies revealed that the particle size of spray dried powders were under 10µm. The $D_{0.5}$ values for particle size were in the range of 3.8 to 5.8 µm. The amount of glutaraldehyde used to crosslink chitosan microspheres was found to influence the particle size.

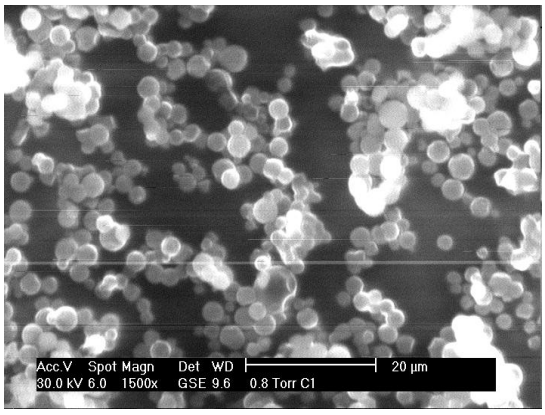

Figure 1: Scanning electron micrograph of spray dried crosslinked chitosan microspheres

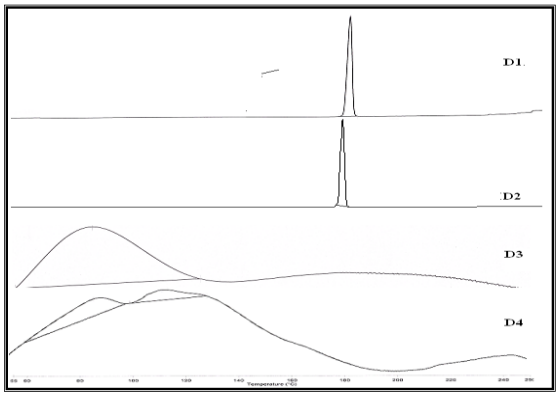

Figure 2: Comparative thermal behavior of raw INH (D1), Physical mixture of INH with chitosan (D2), chitosan blank microspheres (D3), and Chitosan microspheres containing INH (D4).

The tapped density is important physical property of dry powders. The tapped density provides significant information about the flow properties of the particle from the inhaler device, the porosity of particles, particle size distribution and interparticulate cohesive and adhesive forces[28]. The bulk density and tapped density of each batch is shown in table 2. The tapped densities were in the range of 0.212 to 0.238 g/cm^3. Carr's index is a indirect but good indicator of powder flowability. The powders meant for dry powder inhalation must possess a good flowability for accurate and efficient drug deposition to lung. The Carr's index of various chitosan microsphere formulations was found to be in the range of 28.7 to 34.3. The values of Carr's index are higher which was due to the very fine size of microspheres that offers compaction on flow.

In vitro drug release studies

The in vitro drug release studies were carried out in USP dissolution type 2 apparatus maintaining the sink conditions. Many parameters determine the drug release from the chitosan microspheres, these includes concentration and molecular weight of chitosan, type and extent of crosslinking and drug to chitosan concentration. The desired drug release pattern can be obtained by using different crosslinking agents at different crosslinking extent. The release of Isoniazid from crosslinked and non crosslinked chitosan microspheres is shown in figure 3.

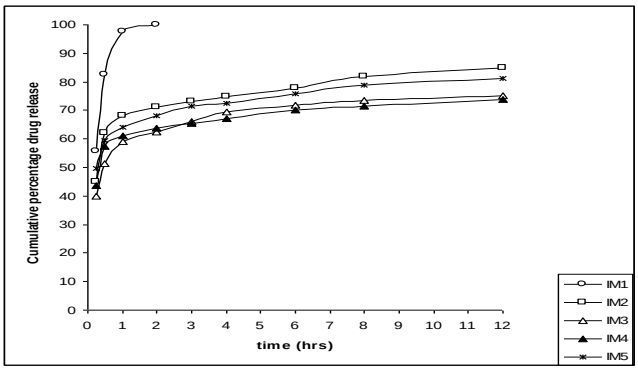

Figure 3: Comparative in vitro dissolution studies of crosslinked chitosan microspheres containing Isoniazid in phosphate buffer 7.4

Table 4: The kinetic constants (k), diffusional exponent (n), Correlative coefficient (r) and time taken for 50% drug release

Formulation	R^2 value	n value	K value	$t_{50\%}$ (min)	$t_{70\%}$ (min)
IM1	0.8786	0.3221	1.8770	25	47
IM2	0.8773	0.1279	1.8620	30	90
IM3	0.9423	0.1460	1.8628	45	330
IM4	0.8710	0.1148	1.8865	33	378
IM5	0.9700	0.1200	1.8712	23	152

R^2: correlation coefficient, $t_{50\%}$ and $t_{70\%}$ time at 50% and 70% drug release, respectively.

A significant bust effect was observed for all the formulation batches particularly at higher GH concentration (1ml). The data regarding $t_{50\%}$ and $t_{70\%}$ are shown in table 4. The drug release from uncrosslinked chitosan microspheres containing isoniazid was complete in nearly two hours while from crosslinked chitosan microspheres the drug release was found to give sustained release for more than 12 hrs. The faster dissolution in uncrosslinked chitosan microspheres was due to faster solubility of small chitosan microspheres, where microspheres were unable to retain shape integrity; rapidly swelled and dissolved. The sustained release form crosslinked microspheres attributed towards the slow chain relaxation which again dependent of the crosslinking extent. To explore the drug release mechanism the Ritger Peppas equation was

introduced. The logarithm of the accumulated release was plotted as a function of logarithm of time (figure 4).

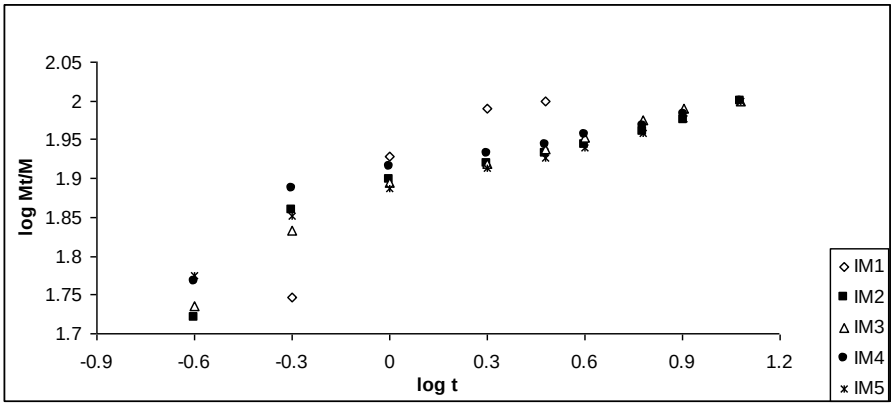

Figure 4: Plot of drug release data lg (Mt/M) versus lg t for crosslinked drug loaded chitosan microsphere formulations

The linearity was observed with crosslinked chitosan microspheres after initial burst release. The mechanism parameters, n values, correlation coefficient (R^2 values) and rate of drug release (k values) obtained from the intercept are presented in table 4. It was seen that the n values were in the range of 0.3221 to 0.12 which indicated the fickian diffusion controlled drug release. As reported by Ritger-Peppas, the n value is an empirical value which characterizes the drug release mechanism. On the basis of diffusion exponent, n value below 0.45 indicates the drug release mechanism approaches that of Fickian diffusion controlled release. An n value of 0.45 to 0.89 represents a drug release mechanism for non Fickian diffusion or chain relaxation controlled release. While n value above 0.89 indicates that the drug release approaches zero order release[29].

Aerosol performance of chitosan microspheres

The Aerodynamic properties of prepared chitosan microspheres were carried out on two selected batches IM1 and IM5. The comparative evaluation was done on IM1 (non crosslinked) and IM5 (crosslinked) formulation. Each batch containing 10 mg equivalent to isoniazid was subjected for in vitro lung deposition studies. the mass deposition on each stage of Andersen cascade impactor after inspiration were further used to calculate various aerosol properties like emitted dose (ED), fine particle fraction (FPF), fine particle dose (FPD), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD). The values for different aerosol parameters measured are shown in table 3. The crosslinked chitosan microparticles produced slightly better aerosol properties compared to uncrosslinked as per data shown in table. The improve in aerosol properties of batch IM5 may be due to the particle size and size distribution as the particle size of crosslinked microspheres was less compared to uncrosslinked microspheres. The % mass

deposition of spray dried microspheres of batch IM5 in different stages of ACI is shown in figure 5. The MMAD values obtained from experimental were larger than the theoretical aerodynamic diameter, 2.55 to 3.85 and 1.75 to 2.82 for IM1 and IM5 respectively. MMAD value of less than 5µm is prerequisite for the powder formulation to deposit the active constituent to lower region of the lung. The GSD values were found to be near 2 which indicate the homogenous distribution of particles. The difference in $MMAD_t$ and MMAD values explains the individual behavior of particle on aerosolization.

Table 3: Comparative aerosol properties of spray dried non crosslinked and crosslinked chitosan microspheres loaded with Isoniazid

formulation	Recovery (%)	Emitted dose (ED)(%)	FPD (mg)	FPF (%)	MMAD	GSD
IM1	95.70 ± 3.21	84.6 ± 2.1	5.13 ± 0.011	54.8 ± 0.22	3.85 ± 0.65	2.44
IM5	95.52 ± 2.07	83.8 ± 2.3	6.68 ± 0.191	69.1 ± 2.88	2.82 ± 0.21	1.82

*MMAD: mass median aerodynamic diameter, FPD: fine particle dose, FPF: fine particle fraction, GSD: Geometric standard deviation

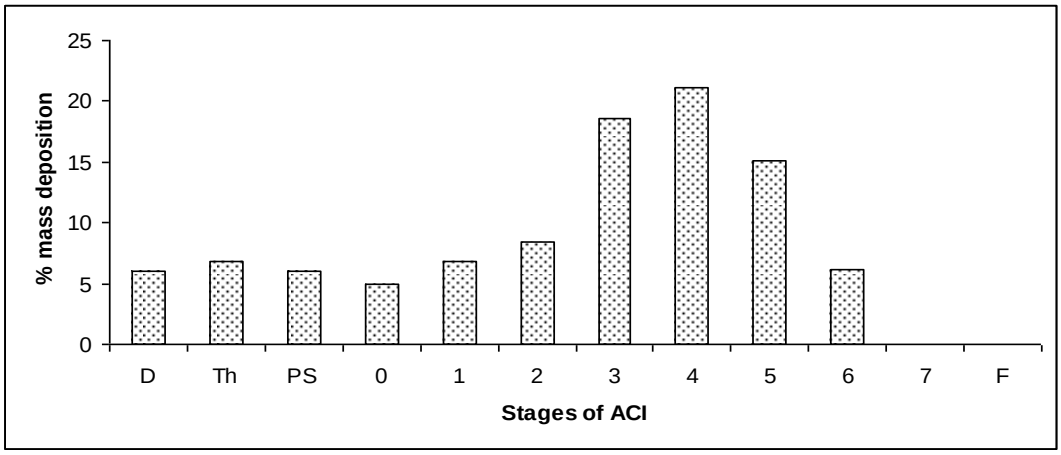

Figure 5: % mass deposition of crosslinked chitosan microsphere on stages of impactor for formulation IM5.

Pharmacokinetic analysis

Tubercular mycobacterium is known to infect alveolar macrophages and affect pathogenesis of TB. There has been renewed interest in developing formulations of anti-TB drugs that can directly target the alveolar macrophages. The pharmacokinetic studies were included to access the potential difference in a pulmonary absorption by evaluating area under curve under the concentration time curve and Cmax estimations. Figure 6 shows the average plasma concentration to the function of time after oral and pulmonary instillation of free and the selected sustained release isoniazid formulation (IM1, IM5). In agreement with the slower absorption,

values of C_{max} after all formulation under study were lower than the administration of free Isoniazid. The C_{max} values were further decreased with crosslinked microsphere formulation (IM5). The AUC_{0-t} of the chitosan microspheres was comparable with the unencapsulated free drug given by oral route. The AUC_{0-t} (134.26 µg h/mL) of formulation IM5 was significantly higher than that of the uncrosslinked chitosan microspheres (11.08 µg h/mL). The crosslinked chitosan microsphere formulation was able to sustain the drug concentration in plasma with elimination rate constant (Ke) of 0.021 hr^{-1} which was comparatively lesser than Ke of uncrosslinked chitosan microspheres, 0.053 hr^{-1}. The prolonged plasma retention of drug encapsulated in chitosan matrix compared with free drug might be attributed toward their longer half life coupled with sustained release potential.

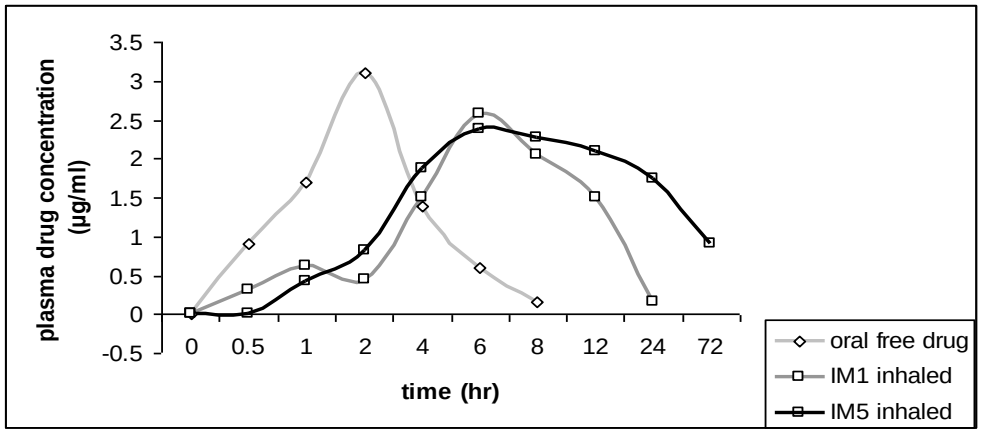

Figure 6: Plasma concentration versus time profile of Isoniazid formulations

Table 5: Pharmacokinetic parameters after oral free drug and pulmonary administration of Isoniazid formulations.

Pharmacokinetic Parameter	Free drug (Oral)	IM1	IM5
C_{max} (µg/mL)	3.1	2.57	2.26
T_{max} (hr)	2.0	8.0	6.0
$AUC_{0\rightarrow t}$ (µg·h/mL)	11.08	79.68	134.26
$AUC_{0\rightarrow\infty}$ (µg·h/mL)	11.225	80.06	145.68
K_e (h^{-1})	0.4117	0.053	0.021
$T_{1/2}$ (hr)	1.68	13.08	31.67
AUMC (µg·h^2/mL)	19.47	106.12	197.12
MRT (h)	1.76	1.33	1.47

*$MMAD_t$: theoretical mass median aerodynamic diameter**,** $AUC_{0\rightarrow\infty}$,: area under curve for time 0 to ∞, K_e, :elimination rate constant, $T_{1/2}$,: half life, AUMC: area under mean curve, MRT: mean residence time, C_{max}: maximum concentration, T_{max},: time to achieve maximum concentration, $AUC_{0\rightarrow t}$,: Area under curve from time 0 to t, AUMC: Area under first movement curve

CONCLUSION

Our study on preparation of isoniazid loaded microspheres, demonstrated that the desired particles encapsulating drug for inhalation can be prepared using chitosan as a carrier by spray drying method**.** The microspheres prepared were also effectively crosslinked with different Glutaraldehyde concentration which resulted in prolongation of drug release from the chitosan matrix. The drug release was extended from 2 hrs to more than 12 hrs. The drug release mechanism when studies using Rotger and Peppas equation showed fickian diffusion drug release kinetics. The prepared microspheres showed comparable aerosol properties where MMAD of 2.82 and GSD of 1.82 were achieved which indicated toward good lung deposition with homogenous particle distribution of microspheres. The in vivo animal studies showed promising prolongation in drug release, although the toxicity associated with glutaraldehyde is needed to be studied

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