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Alginate microspheres of isoniazid for oral sustained drug delivery

R. Rastogi [a], Y. Sultana [b,*], M. Aqil [b], A. Ali [b], S. Kumar [c], K. Chuttani [c], A.K. Mishra [c]

[a] *Centre for Biomedical Engineering, Indian Institute of Technology-Delhi, Hauz Khas, New Delhi 110016, India*
[b] *Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, Hamdard Nagar, New Delhi 110062, India*
[c] *Department of Radiopharmaceuticals, Institute of Nuclear Medicine and Allied Sciences (INMAS), Brig. S.K. Majumdar Road, Delhi 110054, India*

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Abstract

In the present study, spherical microspheres able to prolong the release of INH were produced by a modified emulsification method, using sodium alginate as the hydrophilic carrier. The shape and surface characteristics were determined by scanning electron microscopy using gold sputter technique. Particle sizes of both placebo and drug-loaded formulations were measured by SEM and the particle size distribution was determined by an optical microscope. The physical state of the drug in the formulation was determined by differential scanning calorimetry (DSC). The release profiles of INH from microspheres were examined in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 7.4). Gamma-scintigraphic studies were carried out to determine the location of microspheres on oral administration and the extent of transit through the gastrointestinal tract (GIT). The microspheres had a smoother surface and were found to be discreet and spherical in shape. The particles were heterogeneous with the maximum particles of an average size of 3.719 μm. Results indicated that the mean particle size of the microspheres increased with an increase in the concentration of polymer and the cross-linker as well as the cross-linking time. The entrapment efficiency was found to be in the range of 40–91%. Concentration of the cross-linker up to 7.5% caused increase in the entrapment efficiency and the extent of drug release. Optimized isoniazid-alginate microspheres were found to possess good bioadhesion (72.25 ± 1.015%). The bioadhesive property of the particles resulted in prolonged retention in the small intestine. Microspheres could be observed in the intestinal lumen at 4 h and were detectable in the intestine 24 h post-oral administration, although the percent radioactivity had significantly decreased ($t_{1/2}$ of ^{99m}Tc = 4–5 h). Increased drug loading (91%) was observed for the optimized formulation suggesting the efficiency of the method. Nearly 26% of INH was released in SGF pH 1.2 in 6 h and 71.25% in SIF pH 7.4 in 30 h. No significant drug–polymer interactions were observed in FT-IR studies. Dissolution and γ-scintigraphy studies have shown promising results proving the utility of the formulation for enteric drug delivery.

Keywords: Isoniazid; Microspheres; Modified emulsification method; Gamma scintigraphy; Sodium alginate

1. Introduction

Isoniazid (INH) is a widely used antimycobacterial agent for first line therapy of tuberculosis. The drug is characterized by a short half-life ranging from 1 h to 4 h, depending on the rate of metabolism. INH has a pronounced absorption from all the three sections of the small intestine (Mariappan and Singh, 2003) and from IM injection sites. INH is inactivated in the liver, mainly by acetylation and dehydrazination; the rate of acetylation is genetically determined and subject to individual variation. Long-term continuous therapy with INH leads to hepatotoxicity and peripheral neuritis. It is thus, important to have a drug formulation with controlled release of INH, especially in the small intestine.

Sodium alginate, is a sodium salt of alginic acid, a natural polysaccharide found in all species of brown algae and certain species of bacteria. It is a linear polymer of β (1 → 4) mannuronic acid (M) and α (1 → 4) guluronic acid (G) residues in varying proportions and arrangements. The homopolymer regions composed of M blocks and G blocks are interspersed with MG heteropolymeric regions. Sodium alginate is soluble in water and can be cross-linked with divalent or polyvalent cations to form an insoluble meshwork. Ca^{2+} and Zn^{2+} have been reported for cross-linking of acid groups of alginate (Chan et al., 2002). However, Ca^{2+} are preferred as they selectively bind to the guluronic acid units to form an 'egg-box' model (Grant et al., 1973). Alginate has been extensively utilized for

* Corresponding author. Tel.: +91 9811660105.
E-mail address: yas2312003@yahoo.com (Y. Sultana).

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oral immunization of humans and ruminals by targeting to the Peyer's patches in small intestine.

Reports mention the preparation of alginate microspheres by ionotropic gelation and cross-linking with calcium salts and polyions such as polylysine, chitosan, etc. (Hari et al., 1996a,b; Gonzalez Ferreiro et al., 2002; Gonzalez-Rodrıguez et al., 2002; Lucinda-Silva and Evangelista, 2003). Studies have shown the preparation of alginate microspheres for INH by syringe extrusion method. However, due to the high aqueous solubility of INH, low entrapment efficiency was observed (Ain et al., 2003). In the present study, an improved and simplified emulsification method for the preparation of alginate microspheres has been described.

2. Materials and methods

Isoniazid was a kind gift sample from August Pharmaceuticals, New Delhi, India. Sodium alginate (molecular weight 3.2×10^5, determined by viscosity), analytical grade was purchased from Central Drug House, Mumbai, India. All other reagents were of analytical grade and purchased from the local market. Technetium-99m pertechnate (^{99}Mo/^{99m}Tc generator) and CITC-DTPA was obtained from INMAS, New Delhi, India. Instant Thin Layer Chromatography-Silica Gel (ITLC-SG) strips were purchased from Gellman, Germany.

2.1. Preparation of microspheres

The emulsification method was utilized for the preparation of microspheres followed by cross-linking with calcium chloride (Poncelet et al., 1992, 1999; Wan et al., 1994; Fundueanu et al., 1998; Heng et al., 2003). Core material, INH (100 mg) was dispersed in 5% aqueous solution of sodium alginate (10 ml). The aqueous phase was emulsified in light liquid paraffin in the ratio 1:10 containing 2% (v/v) Span 80 using a mechanical stirrer (Remi Motors, India) at 1500–2000 rpm for 60 min to it 5 ml of 0.2 M calcium chloride dissolved in a mixture of methanol and isopropyl alcohol (2:3) was added slowly to the emulsion and stirred to assure efficient cross-linking. Microspheres were collected by filtration in vacuum, washed with isopropyl alcohol thrice and finally air-dried at room temperature. Various variables like polymer concentration, drug-polymer ratio, concentration of cross-linking agent and time required for cross-linking were considered in the optimization of the formulation.

2.2. Characterization of the microspheres

2.2.1. Determination of entrapment efficiency

The drug content of the microspheres was determined spectrophotometrically (λ_{max} = 263 nm; Shimadzu Model 1601, Japan). The alginate microspheres (10 mg) loaded with INH were dissolved in 10 ml of isotonic phosphate buffer pH 6.8 under sonication for 20 min. The solutions were filtered through 0.22 μm Millipore filters and the amount of INH was measured. Preliminary UV studies showed that the presence of dissolved polymers did not interfere with the absorbance of the drug at 263 nm.

$$\%\ \text{Drug entrapment} = \frac{\text{Mass of drug present in microparticles}}{\text{Mass of drug used in the formulation}} \times 100$$

2.2.2. Surface morphology and particle size analysis

The shape and surface characteristics were determined by scanning electron microscopy (SEM) (Leo 435 P) using gold sputter technique. The particles were vacuum dried, coated with gold palladium and observed microscopically. Particle sizes of both placebo and drug loaded formulations were measured by an optical microscope fitted with an ocular and stage micrometer and particle size distribution was calculated. In all measurements at least 100 particles in 5 different fields were examined.

2.2.3. Differential scanning calorimetry (DSC) analysis

The physical state of the drug in the samples was determined by DSC (Perkin Elmer equipped with software Pyris 6.0). Samples containing 3 mg of the drug/placebo/formulation were placed in aluminium pans and heated from 30 °C to 225 °C at a heating rate of 10 °C/min under inert atmosphere flushed with nitrogen at the rate of 20 ml/min.

2.2.4. Fourier transform-infra red (FT-IR) spectroscopic analysis

One to 2 mg of INH, placebo microspheres, INH-alginate microsphere samples were weighed and mixed perfectly with potassium bromide (0.3–0.4 g) to a uniform mixture. A small quantity of the powder was compressed into a thin semi-transparent pellet by applying pressure. The IR spectrum of the pellet from 4000 cm^{-1} to 670 cm^{-1} was recorded (Hitachi, Japan) taking air as the reference and compared to study any interference.

2.2.5. Measurement of bioadhesion

In vitro bioadhesion (in triplicate) was determined for alginate microspheres by following a previously reported method (Ranga Rao and Buri, 1989). Microspheres (50 mg) were placed on albino rat small intestine (2 cm) and kept for 20 min in a humidity temperature control cabinet (Metrex International, India) at 75% relative humidity and temperature of 25 ± 2 °C to allow hydration of the microspheres. This is followed by thorough washing of the mucosal lumen with isotonic phosphate buffer pH 6.8. The washings were then dried at 70 °C in a hot air oven. Percent bioadhesion was determined by the following formula:

$$\%\ \text{bioadhesion} = \frac{\text{Weight of adhered microspheres}}{\text{Weight of applied microspheres}} \times 100$$

2.2.6. In vitro drug release

The release profiles of INH from microspheres were examined in simulated gastric fluid (SGF pH 1.2) and simulated intestinal fluid (SIF pH 7.4). The drug-loaded microspheres (equivalent to 10 mg of INH) filled in empty capsule shells were put into the basket (50 rpm) and placed in 500 ml of the dissolution medium, thermostated at 37 °C. At scheduled time intervals

agitation was stopped, the samples (2 ml) were withdrawn and replaced with fresh medium. The samples were diluted, filtered and the drug content determined spectrophotometrically at 263 nm.

2.2.7. In vivo study

Gamma-scintigraphic studies were carried out to determine the location of microspheres on oral administration and the extent of transit through the gastrointestinal tract (GIT). Microspheres were labelled using 99m-Technetium (^{99m}Tc) and administered to adult Wister rats.

CITC-DTPA was used to provide the necessary ^{99m}Tc species for radiolabelling the microspheres. One millilitre of distilled water was added to approximately, 2 mg of microspheres in a sterile glass vial. In another similar vial, 2–3 ml of ^{99m}Tc was taken and 50 μg of stannous chloride dihydrate (1 mg/ml in 10% acetic acid) was added. The pH was adjusted to 7.5 with 0.5 M sodium bicarbonate. The reduction of ^{99m}Tc was assured by ITLC-SG. When the reduction was found to be 99%, the suspended microspheres were added to it and the mixture was left for 2 min to complete the reaction.

The radiolabelling efficiency was evaluated with ITLC-SG strips as stationary phase and acetone 100% as the mobile phase.

$$\%\ \text{Radiolabelling} = \frac{\text{Radioactivity (counts) retained in the lower half of the strip}}{\text{Initial radioactivity associated (total count present) with the strip}} \times 100$$

Radiochemical impurity that is likely to exist in the form of unconjugated technetium was determined by ascending ITLC-SG (Saha, 1992).

$$\%\ \text{R/H technetium} = \frac{\text{Counts present in the lower part of strip}}{\text{Total count present in the strip}} \times 100$$

In order to determine the extent of localization of alginate microspheres in the GIT, imaging studies were performed using high specific activity of ^{99m}Tc-labelled microspheres. Nine adult Wistar Rats, either sex (average weight 300–350 g; 15–20 weeks old) were used, each group of treated and untreated animals comprising of at least three rats. The microspheres were administered by gavage, a dose of 2 mg/ml, after overnight fasting for 8–10 h. Animals were given free access to water, but food was restored 1–2 h after dosing. The animals were anaesthetized with diazepam and serial scintigraphic examination was done at 4 and 24 h to assess the mobilization of the microspheres in the GIT, using a large field view gamma camera (Siemens) equipped with a high-resolution, parallel-hole collimator and interfaced to a dedicated computer. Images were recorded for a preset time of 5 min/view with a 15% window centered to include the 140 keV photopeak of ^{99m}Tc.

2.3. Statistical analysis

Statistical analysis of the results was carried out using Student's *t*-test. The *in vitro* release profile was compared with zero order, first order and Higuchi's matrix models.

3. Results and discussion

3.1. Size analysis of microspheres

The high shearing rate required for emulsification caused the breakdown of the viscous aqueous alginate solution to fine globules resulting in small microspheres. The microspheres had a smoother surface and were found to be discreet and spherical in shape (Fig. 1A). No change in the morphology was observed in drug-loaded microspheres (Fig. 1B). As reported in literature, use of isopropyl alcohol resulted in dehydration of the particles with decrease in their particle size (Zheng et al., 2004). The particles were heterogeneous with the maximum particles of an average size of 3.719 μm (Table 1) (Fig. 2). Results indicated that the mean particle size of the microspheres increased with increase in the polymer concentration due to subsequent increase in the viscosity with the formation of larger aqueous droplets in emulsion. Increase in particle size was also observed with increase in the concentration of the cross-linker and the cross-linking time. However, concentration of the cross-linker above 10% caused formation of irregular lumps due to extensive cross-linking of the guluronic acid unit of sodium alginate.

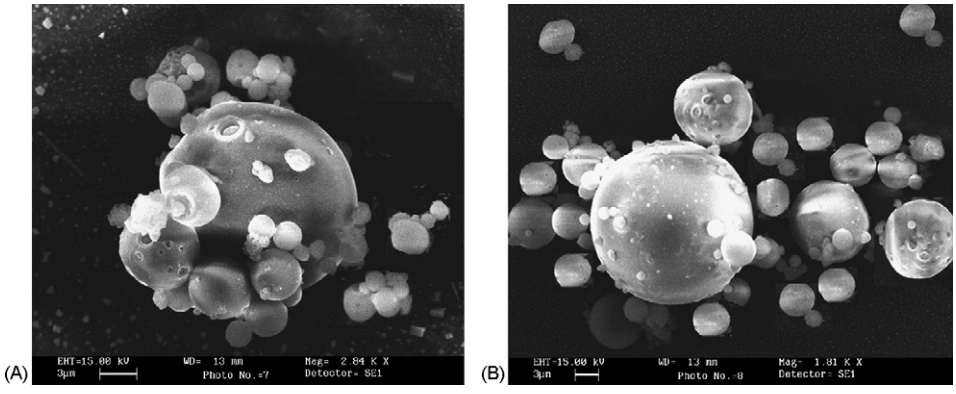

Fig. 1. SEM photograph of placebo (A) and drug loaded (B) microspheres.

Table 1
Particle size distribution of optimized alginate formulation

Size range	Mid size	d (μm)	No. of particles	nd	% Particles in range	Dav = Σnd/Σn
0–1	0.5	1.5625	195	304.68	56.19597	
1–2	1.5	4.6875	88	412.50	25.36023	
2–3	2.5	7.8125	32	250.00	9.221902	3.71958
3–4	3.5	10.9375	14	153.12	4.034582	
4–5	4.5	14.0625	10	140.62	2.881844	
			Σn = 339	Σnd = 1260.92		

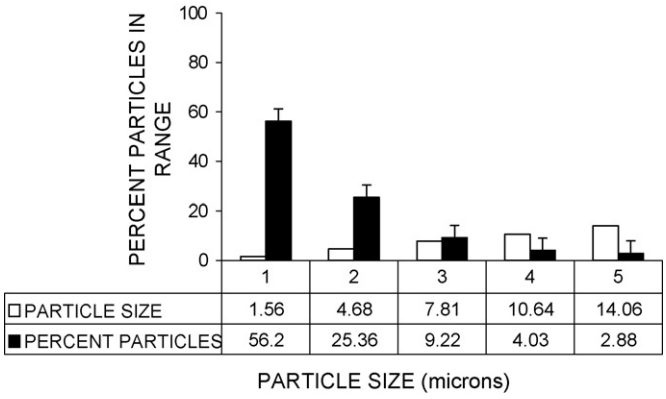

Fig. 2. Particle size frequency distribution of optimized alginate formulation.

Table 2
Preparation and characteristics of alginate microspheres with INH

Formulation variables	Formulation				
	A1	A2	A3	B1	B2
Drug:polymer ratio	1:5	1:5	1:5	1:10	1:2
Cross-linker concentration (%)	7.5	10	20	7.5	7.5
Entrapment efficiency (%)	79.95	50.51	44.94	76.25	42.05

3.2. Determination of entrapment efficiency

The entrapment efficiency was found to be in the range of 40–91%, as shown in Tables 2 and 3. This value of entrapment efficiency is significantly higher as compared to the reported value of the ionotropic gelation method (Ain et al., 2003). The difference is due to the high aqueous solubility of INH resulting in high concentrations of the drug present in the preparation medium in the gelation method. Loss of the drug in the emulsification method can be accounted in the hardening, washing and filtering processes only.

Table 3
Effect of cross-linking time on the entrapment efficiency

Formulation and process variables	Formulation		
	C1	C2	C3
Drug:polymer ratio	1:5	1:5	1:5
Cross-linker concentration (%)	7.5	7.5	7.5
Cross-linking time (min)	10	20	30
Entrapment efficiency (%)	40.30	91.00	79.95

The method adopted for the preparation of microspheres could also be responsible for the observed higher incorporation efficiency. The added calcium chloride solution was merged with the internal aqueous phase of alginate-containing drug by use of methanol resulting in the formation of gel instantaneously with entrapment of the drug in the planar two-dimensional lattice of the cross-linked alginate to produce the 'egg-box' structure. The presence of isopropyl alcohol in the calcium chloride solution could result in dispersion of calcium chloride in the external phase also. This can account for the quick hardening of the gel preventing the escape of INH back into the aqueous phase of the emulsion (Lemoine et al., 1998).

3.3. In vitro drug release

Fig. 3 showed the release behavior of INH from alginate microspheres in SGF, pH 1.2 and SIF, pH 7.4. Approximately, 25.47% of the drug was released in the SGF, pH 1.2 over a period of 5 h and 71.25% in SIF, pH 7.4 in 30 h. It is generally seen that when microspheres of hydrophilic polymers are immersed in water, they swell and form a gel diffusion layer that hinders the outward transport of the drug, hence, producing a controlled release effect. However, at acidic pH the alginate microspheres shrink due to tightening of the gel meshwork. The polymer is eroded at alkaline pH and the contents are released in a sustained manner by both diffusion and slow erosion of polymer matrix (Kim and Lee, 1992). The best-fit model for drug release from microspheres was the Higuchi's matrix model (Table 4).

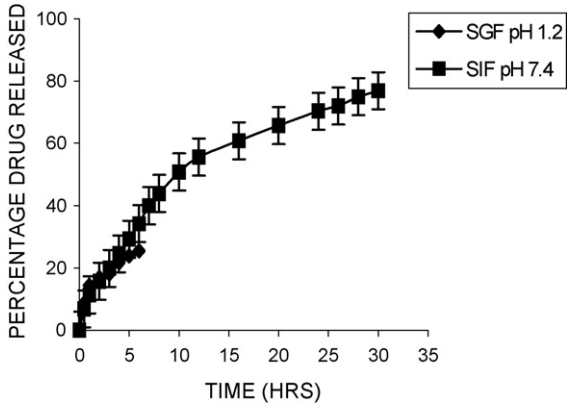

Fig. 3. Drug release profile of optimized formulation in SGF pH 1.2 and SIF pH 7.4.

Table 4
In vitro release kinetic models

Models	Dissolution medium	Release constants	Regression coefficients
Zero order	SGF pH 1.2	0.0377 mg min^{-1}	0.8293
	SIF pH 7.4	0.7809 mg h^{-1}	0.7293
First order	SGF pH 1.2	−0.0013 min^{-1}	0.9348
	SIF pH 7.4	−0.0467 h^{-1}	0.9588
Higuchi's matrix model	SGF pH 1.2	0.4371 mg $min^{-1/2}$	0.9829
	SIF pH 7.4	3.6053 mg $h^{-1/2}$	0.9726

3.3.1. Effect of cross-linker concentration

Concentration of the cross-linker up to 7.5% caused increase in the entrapment efficiency and the extent of drug release (Table 2 and Fig. 4A). However, further increase in calcium chloride concentration resulted in increase in the particle size and decrease in the entrapment efficiency. This could be due to the instant gelling of sodium alginate on addition of calcium chloride and squeezing out of the aqueous phase from the gel lattice. INH being highly soluble in water comes out of the gel along with the squeezed aqueous phase.

3.3.2. Effect of cross-linking time

Variations in the cross-linking time were also studied for selecting the best optimized formulation. Cross-linking time of 20 min was found to be sufficient for good entrapment efficiency (Table 3); less cross-linking time resulted in incomplete gelling of sodium alginate. Increasing the cross-linking time greater than 20 min, however, caused no significant change ($p > 0.05$) in the amount of drug release (Fig. 4B).

3.3.3. Effect of drug–polymer ratio

The effect of drug–polymer ratio on INH release from different batches of microspheres is shown in Fig. 4B. Although the release profile was found to be controlled in all the cases reported, drug-polymer ratio of 1:5 resulted in the highest concentration of INH in the selected media. A decrease in the rate and extent of release was observed with relative increase in the polymer concentration in microspheres. This can be attributed to the increase in the density of the polymer matrix with increased polymer concentration (1:10). However, drug polymer ratios below 1:5 showed low entrapment efficiency (Table 2) due to insufficient concentration of the polymer.

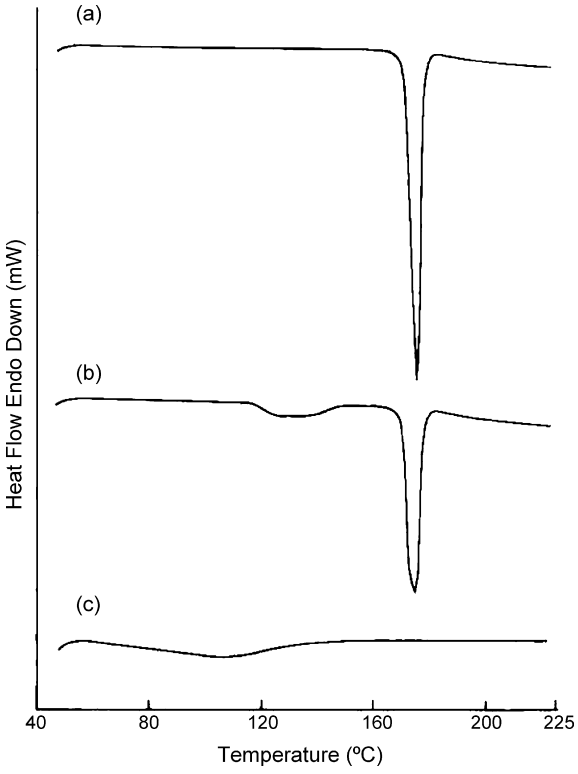

Fig. 5. DSC curves of INH (a), INH loaded microspheres (b) and blank microspheres (c).

3.4. Drug–polymer interaction studies

DSC and FT-IR were performed on the raw materials and on the microspheres to detect interactions between the drug and the excipients. The DSC endotherm of INH showed a sharp melting endotherm at 175 °C. The intensity of the peak was slightly diminished in the formulation, which can be due to the dilution factor (Fig. 5). The FT-IR spectrum of INH showed a strong C=O stretch band (Amide I) around 1650 cm^{-1} and an Amide II due to N–H bend at 1620 cm^{-1}. These peaks were, however, completely masked in the FT-IR spectrum of the drug-loaded microspheres (Fig. 6).

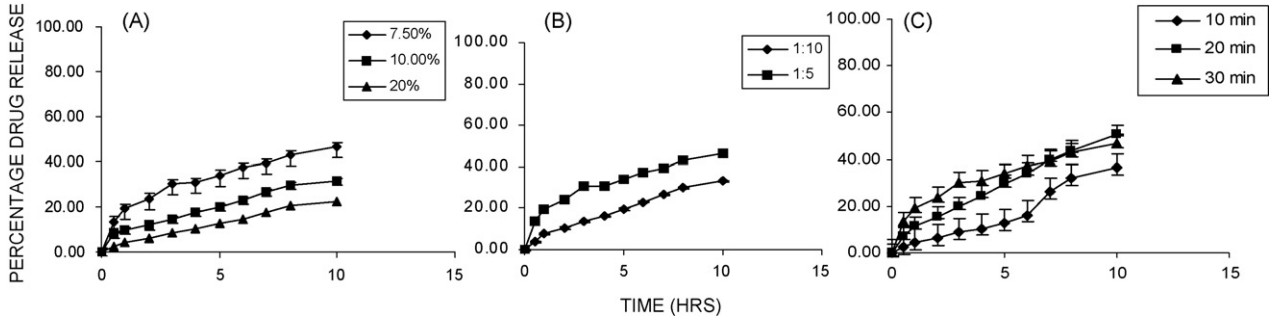

Fig. 4. The release profiles of INH from alginate microspheres in SIF pH 7.4 under variable cross-linker concentration (A), drug:polymer ratios (B) and cross-linking times (C).

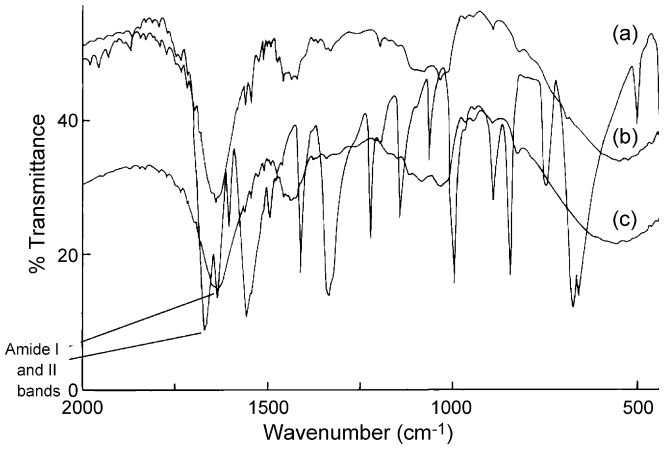

Fig. 6. FT-IR graphs of INH (a), drug-loaded micropheres (b) and placebo alginate microspheres (c).

3.5. Bioadhesion study

Optimized isoniazid-alginate microspheres were found to possess good bioadhesion (72.25 ± 1.015%). The bioadhesive property of these particles resulted in prolonged retention in the small intestine which was also supported by the γ-scitigraphy studies.

3.6. Radiolabelling of microspheres

3.6.1. Quality control

Amount of reduced/hydrolyzed (R/H) ^{99m}Tc was determined using pyridine:acetic acid:water (PAW) in the volume ratio of 3:5:1.5 as mobile phase and ITLC-SG strip as the stationary phase. The mobile phase may normally be found involving ethanol and ammonia as the first two components of the mobile phase, however, in the same volume ratio (Throll et al., 1976).

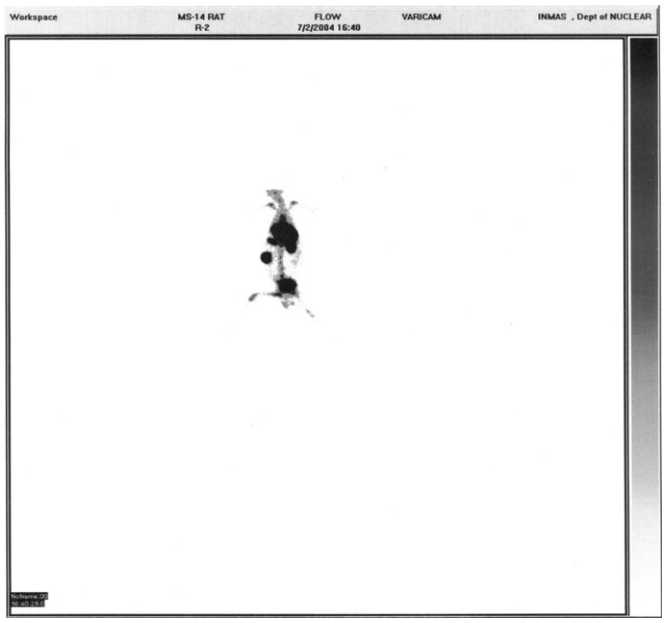

Fig. 7. Representative gamma camera image of an adult Wistar rat after injection of ^{99m}Tc after 2 h. High accumulation of radioactivity could be observed in the lungs, liver and spleen.

Their replacement, respectively with pyridine and acetic acid while maintaining the volume ratio and volume fraction contribution was invariably noticed to produce better separation and therefore, reproducible results, and hence employed as the constituent of the mobile phase for this part of the experiment. The reduced/hydrolyzed technetium remained at the point of application whereas free pertechnetate and labelled complex moved with the solvent front.

3.6.2. γ-scintigraphy studies

Fig. 7 depicted the passage of ^{99m}Tc administered by the i.v.-route. Retention of radioactivity was observed in the thoracic

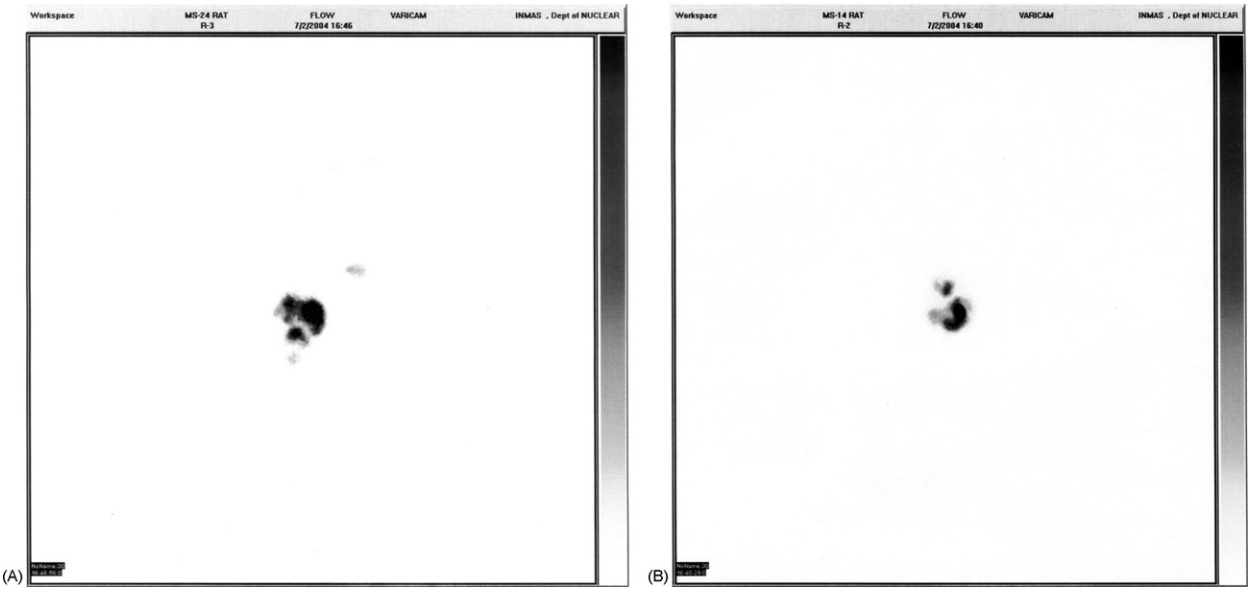

Fig. 8. (A and B) Gamma camera image of an adult Wistar rat 4 h post-oral administration of ^{99m}Tc-labelled alginate microspheres. Localization of radioactivity was observable in the small intestine.

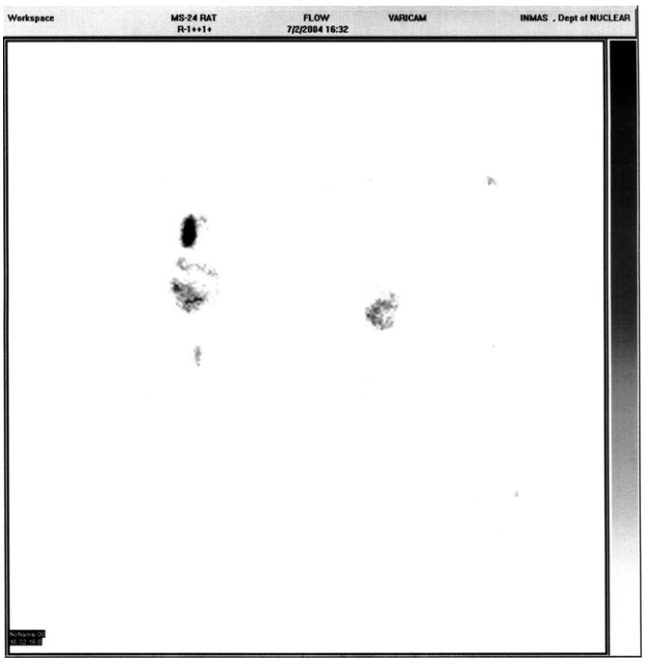

Fig. 9. Gamma camera image of an adult Wistar rat after 24 h post-oral administration of ^{99m}Tc-labelled alginate microspheres showing presence of particles in the small intestine.

and upper abdominal region and in the tail region. High levels of ^{99m}Tc were detected in the lung, liver and spleen immediately after injection. This image was taken as the standard for marking the position of radiolabelled microspheres in the GIT after oral administration.

The presence of microspheres could be marked in the intestinal lumen 4 h post-oral administration (Fig. 8A and B). Fig. 7 showed the contamination of the windpipe in one of the animals during oral administration. Microspheres could also be detected in the intestine after 24 h (Fig. 9) although the percent radioactivity had significantly decreased ($t_{1/2}$ of ^{99m}Tc = 4–5 h). Presence of microspheres in the GIT could not be assessed after 24 h of administration due to negligible radioactivity.

4. Conclusion

In the study, alginate microspheres of isoniazid were prepared by a single without emulsion-crosslinking method for prolonged enteric release. Various variables such as the drug-polymer ratio, cross-linker concentration and the cross-linking time were considered. High entrapment efficiency (91%) was obtained by this method as compared to the previously reported methods. The drug release from the microspheres was affected by the pH of the dissolution medium. FT-IR and DSC studies did not reveal any significant drug interactions. Due to prolonged drug release alkaline pH as suggested by the dissolution and γ-scintigraphy studies, alginate microspheres can be used for enteric delivery of drugs. Moreover, this method could be useful for encapsulation of hydrophilic bioactives without much loss of the active principle.

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