Formulation and Evaluation of Chitosan Microspheres Containing Isoniazid

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The present investigation was designed to prepare chitosan microspheres and evaluate the *in vitro* release pattern of the drug isoniazid. The microspheres were formulated by glutaraldehyde cross-linking method using various concentrations of chitosan. The prepared microspheres were evaluated for drug content, particle size distribution, stability studies and compared with marketed tablets. The percentage of entrapment obtained was 60 %. *In vitro* release studies were carried out in 0.1 N HCl and 88.1, 83.8, 79.3 and 73.0 % of drug was released from 0.5, 1.0, 1.5 and 2.0 % of chitosan microspheres respectively after 8 h. Stability studies were carried out at different temperatures and found that all the formulations were more stable at 4° and room temperature.

Microspheres can be defined as solid, approximately spherical particles ranging from 1 to 1000 μm. They are made of polymeric, waxy or other protective materials, i.e. biodegradable synthetic polymers and modified natural products such as starch, gums, proteins, fats and waxes. The natural polymers include albumin and gelatin; the synthetic polymers include polylactic acid and polyglycolic acid. Targeting is the controlled distribution of drug carriers in the body at specific sites. It not only reduces the dose of the drug, reaching to the effective biological sites rapidly but also results in reduced toxicity. Various attempts have been made in the field of targeting but in past few years, pharmacists have focused their research in colloidal drug delivery system/colloidal carriers like liposomes, microspheres and nanoparticles as targeting carriers which has given selective targeting. Chitosan is used as hypocholesterolemic[1] and hypolipidemic agent, liposome stabilizer[2] and for making contact lenses[3]. Chitosan microspheres are used in delivering anticancer[4] and antibacterial agents and for developing orthopaedic materials[5]. Chitosan beads and granules for oral sustained delivery of nifedipine have been developed[6]. Chitosan matrix[7] is also used for the oral sustained delivery of ampicillin. In cancer chemotherapy, chitosan gel microspheres were used for the delivery of anticancer agents to the tumour target cells in sufficient amount for a desired period of time without any severe side effects[8]. Microsphere drug administration offers a number of advantages in therapeutics, where the controlled release of drug delivery as well as the predictable and reproducible drug release kinetics is important features of them. The aim of this work is to investigate the possibility of obtaining a prolonged, relatively constant effective level of isoniazid from the microsphere formulations using chitosan as carrier. Although the drug is highly effective, it suffer from the major drawbacks such as delayed onset of action, bitter in taste, incidence of side effects because of frequent doses, low biological half life and less protein binding.

0.5, 1.0, 1.5 and 2.0 % solutions of chitosan (Central Institute of Fisheries Technology, Cochin) were prepared in aqueous acetic acid (10 %). Isoniazid (Novartis, Mumbai) 200 mg was dispersed in this solution and mixed well. This solution was added to liquid paraffin to form water-in-oil (w/o) emulsion. The dispersion was stirred at 700 rpm for 30 min after the addition of glutaraldehyde (5 % v/v) solution. The product was filtered and washed with chloroform several times and finally with water and dried at 50°. All batches were prepared at least three times. The prepared microspheres were first evaluated for drug content to estimate the amount encapsulated.

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The dialysis tube diffusion technique[9] was used to determine the rate of release of isoniazid. 250 ml of 0.1 N HCl was used as the dissolution medium. Microspheres, equivalent to 100 mg of isoniazid were suspended in 5 ml of the dissolution medium and then the dispersion was placed in a dialysis bag. The bag was placed in a basket rotated at 100 rpm (rewarmed to 37±2°). The samples were withdrawn at predetermined time intervals and assayed spectrophotometrically at 263 nm[10] using the corresponding medium as blank and the release study was also compared with pure drug and marketed tablets of isoniazid.

For determining the size distribution of microspheres, they were separated by sieving using a set of standard sieves. The resulting fractions remaining on the sieves were weighed. The data obtained was subjected to graphical treatments in order to find the mode of size distribution. The mean microsphere diameter was calculated after sieving[11]. The scanning electron microscopic (SEM) photographs of the microsphere and its surface were obtained by using a Scanning Electron Microscope (Jeol, JSM-840A, Japan) with 20 kV accelerating voltage, which are used to evaluate the shape and surface characteristics of the microspheres. The content uniformity test was done to evaluate whether the prepared microspheres having uniformity in the drug content by assaying three different samples for each batch. In order to check the integrity of the drug in the formulatuions, IR spectra of the drug and microspheres were obtained and compared using FTIR spectrophotometer (FTIR-8201 PC, Shimadzu Corporation, Japan) KBr pellet method. All the formulations were studied for stability profile for 6 months at different environmental conditions such as 4°, room temperature, 45° and 30°/85 % RH[9,12].

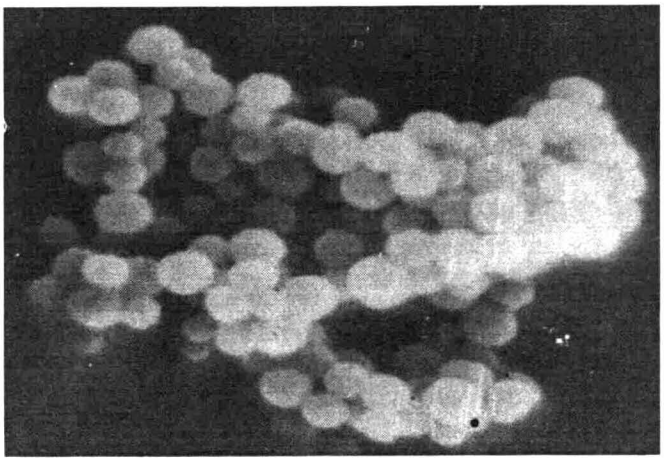

Fig. 1: SEM photomicrograph of the microspheres prepared with 0.5 % chitosan.

The percentage of entrapment was found to be around 60 % for all the formulations. The drug content uniformity was found to be 92±8 % for all the batches. SEM photographs indicated that the microspheres were spherical and discrete. The SEM photomicrograph of the prepared microspheres is shown in fig. 1. Micromeritic analysis revealed that the mode of size distribution was log normal in all batches with the average particle diameter of 423.9 μm. The IR spectra of isoniazid and its microspheres were found to be identical and indicating no chemical interaction between the drug and the excipients. In the *in vitro* dissolution release studies, the pure drug releases 98.6 % of the drug within 10 min and the marketed isoniazid tablet releases 97.5 % of the drug within 20 min. The microsphere formulation of isoniazid using chitosan retards the release of isoniazid from the microsphere and produces the sustained action. The microspheres prepared with 0.5 % of chitosan releases 88.1 % of isoniazid at 8 h and 1.0, 1.5 and 2.0 % chitosan microspheres releases the drug isoniazid 83.8, 79.3 and 73.0 % at 8th h, respectively. The fig. 2 shows the in vitro release profile of isoniazid from microspheres formulated with various concentrations of chitosan and 'T' values are shown in Table 1. The increase in concentration of chitosan causes the decreasing rate of drug release. There was no significant change in the release pattern when it was stored at 4° and room temperature. The release rate was affected very little when it was stored at 45° and 30°/85 % RH (Table 2).

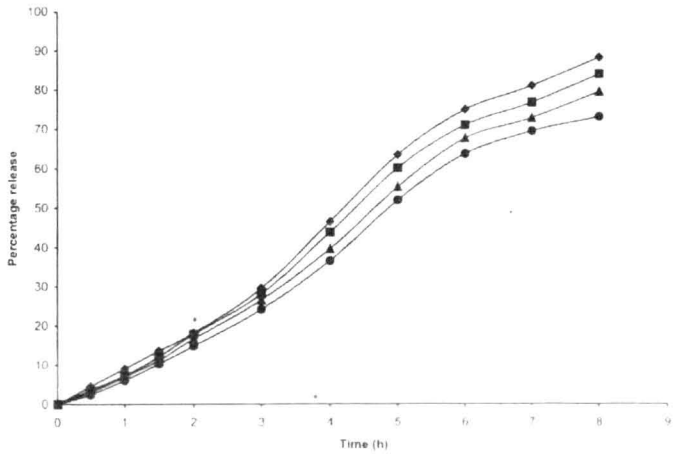

Fig. 2: *In vitro* release profile of isoniazid from chitosan microspheres.

In vitro release of isoniazid from microspheres containing 0.5 (-◆-), 1.0 (-■-), 1.5 (-▲-) and 2.0 % (-●-) chitosan in a medium containing 0.1 N HCl over a period of 8 h.

TABLE 1: 'T' VALUES OF VARIOUS FORMULATIONS.

'T' Values	Formulations					
	Pure drug	Marketed formulation (Tablet)	F_1	F_2	F_3	F_4
T_{90}	6.5	16	510	570	630	720
T_{75}	4	12.5	360	390	441	510
T_{50}	3	8	249	261	279	294
T_{25}	1.5	4	159	165	171	186
T_{10}	0.75	1.75	66	78	84	90

F_1, F_2, F_3 and F_4 are microspheres formulated with 0.5, 1.0, 1.5 and 2.0 % of chitosan. T_{90}, T_{75}, T_{50}, T_{25} and T_{10} are the time taken to release 90, 75, 50, 25 and 10 % of the drug from the formulations.

TABLE 2: THE STABILITY OF MICROSPHERE FORMULATIONS AT VARIOUS TEMPERATURES.

Formulation	Amount of drug present in the formulation after 6 months*			
	4°	Room temperature	45°	30°/85 % RH
F_1	96.79	91.26	78.21	82.16
F_2	95.12	92.51	80.39	79.59
F_3	96.38	89.05	78.73	77.14
F_4	94.61	90.23	78.66	78.48

* Average of three values. F_1, F_2, F_3 and F_4 are microspheres formulated with 0.5, 1.0, 1.5 and 2.0 % of chitosan.

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