PREPARATION AND EVALUATION OF CHITOSAN MICROCAPSULES CONTAINING RIFAMPICIN

Reddy P Sabitha[1], Reddy K Ravindra[1] and Vijaya Ratna J *

*Professor, University College of Pharmaceutical sciences, Andhra University, Visakhapatnam - 530 003, India.
[1]Department of Pharmaceutics, P. Rami Reddy Memorial College of Pharmacy, Kadapa - 516 003, Andhra Pradesh, India.

ABSTRACT

The present investigation was designed to prepare chitosan microcapsules and evaluate the *in-vitro* release pattern of the drug rifampicin. The microcapsules were formulated by glutaraldehyde cross-linking method using various core:coat ratios. The prepared microcapsules were evaluated for SEM analysis, sieve analysis, drug content, encapsulation efficiency, swelling studies, *in-vitro* and mucoadhesion test, and were compared with pure drug. The microcapsules obtained were spherical, discrete, free flowing and exhibited a slow and sustained release over a period of 12 hrs. The release depended on core:coat ratio and size of microcapsules. *In-vitro* release studies were carried out in pH 1.2 and pH 7.4 and 60.20%, 43.25% and 39.63% of drug was released from microcapsules having 1:1, 1:2, and 1:3 core:coat ratios respectively after 12 hrs. Drug release was found to be diffusion controlled and followed first order kinetics.

INTRODUCTION

The controlled delivery of antimycobacterial agents may be accomplished by employing various polymeric drug carriers[1]. Although experience with synthetic polymers is extensive and encouraging, the recent trend has been to shift towards natural polymers. The major advantage of natural polymers (chitosan, sodium alginate etc.,) includes their low cost and compatibility with the encapsulation of a wide range of drugs, with minimal use of organic solvents. Furthermore, bio-adhesion, stability, safety and their approval for human use by US FDA are additional advantages[2]. Subsequent work showed that chitosan is not only capable of improving the drug encapsulation efficiency and bioavailability but is also helpful in reducing the dose and dosing frequency. Chitosan is used as hypocholesterolemic[3], hypolipidemic agent, liposome stabilizer[4] and for making contact lenses[5]. Chitosan microspheres are used in delivering anticancer[6] and antibacterial agents and for developing orthopedic materials[7]. Chitosan beads and granules for oral sustained delivery of nifedipine have been developed[8] .Chitosan matrix[9] 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 tumor target cells in sufficient amount for a desired period of time without any severe side effects[10].

Recently numerous natural polymers have been investigated, which are currently used in the design of controlled release systems. Among the natural polymeric materials used for the development of controlled drug delivery systems, chitosan is commanding good interest because chitosan microspheres can be prepared by various methods such as cross-linking with anions[11], precipitation[12], complex-coacervation[13], modified emulsification and ionotropic gelation[14], precipitation- chemical cross-linking[15], glutaraldehyde cross-linking[16]and thermal cross-linking[17]. The cross-linking may be achieved in acidic, neutral, or basic environments depending on the method applied. Usha Yogendra Nayak et al prepared zidovudine-chitosan microspheres by a suspension cross-linking method[18]. SK Jain et al formulated mucoadhesive chitosan microspheres for non-invasive and improved nasal delivery of insulin by emulsification method[19]. Mousumi Kar and Choudhury PK developed chitosan microspheres for a water-soluble antidiabetic drug by denaturation process[20]. Harris et al prepared chitosan microspheres cross-linked with genipin by a spray-drying method[21]. Samad et al designed rifampicin containing microspheres by using a biodegradable and biocompatible polymer, gelatin B, using a thermal gelation method for the treatment of tuberculosis[22]. *Qurrat-ul-Ain et al developed* alginate microparticles as oral sustained delivery carriers for antitubercular drugs in order to improve patient compliance[23]. Ito F and Makino K prepared mono dispersed rifampicin (RFP)-loaded poly(lactide-co-glycolide) (PLGA) microspheres by a solvent evaporation method[24]. Intira Coowanitwong et al developed rifampicin microspheres by spray drying using either poly lactic acid (PLA) or poly(lactic-*co*-glycolic acid) (PLGA) polymers in different drug to polymer ratios (90:10 to 5:95, w/w)[25]. Though substantial work was reported on microcapsules using

*Correspondence : vijaya_ratna@yahoo.com; sabithareddy135@yahoo.co.in

various polymers, not much significant work was done on rifampicin microcapsules using chitosan alone as the polymer. The earlier reports suggest that rifampicin microcapsules were prepared by using more than one polymer which required more number of cross linking agents and stabilizers. Ming-Yue Mo[26] et al formulated rifampicin / chitosan microcapsules by solvent evaporation and emulsifying cross-linking method which shows encapsulation efficiency of 42.67%. In the present study the objective was to prepare rifampicin microcapsules using chitosan alone by emulsification cross linking method and to achieve more encapsulation efficiency by using less cross linking agent.

Rifampicin is a macrocyclic antibiotic obtained from *Streptomyces mediterranei*. Rifampicin is a semi synthetic derivative of rifamycin B. It has broader antimicrobial activity and has profound applications in the treatment of a number of different bacterial infections[27] (mainly in the treatment of tuberculosis). It is well absorbed from the gut and the plasma half-life of rifampicin was found to be 3.5 hrs. In the treatment of tuberculosis a dose of 300-600mg is given orally daily[28].

Microparticle-based drug delivery systems have considerable potential for treatment of tuberculosis. The important technological advantages of microparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration including oral application and inhalation. Microparticles are designed to allow controlled (sustained) drug release from the matrix. These properties of microparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of non adherence to prescribed therapy, which is one of the major obstacles in the control of TB epidemics.

MATERIALS AND METHODS
Materials
Rifampicin was a gift sample from Alkem laboratories limited, Mumbai, chitosan from Alkem laboratories limited, Mumbai, glacial acetic acid (Karnataka fine chem., Bangalore), ascorbic acid (Universal Laboratories, Mumbai), glutaraldehyde (SD fine chem. Limited, Mumbai). All other chemicals and solvents used were of analytical grade. Paddle stirrer (Remi motors), dissolution apparatus (TDP6P Campbell Electronics, Mumbai) and UV-visible spectrophotometer (Systronics, SL 150) were the equipments used in this study.

Preparation of Microcapsules
Microcapsules containing rifampicin were prepared using chitosan as a coat material employing emulsification cross-linking method. Three batches with core: coat ratios 1:1, 1:2 and 1:3 were prepared. Initially chitosan (500mg) was soaked in 5% glacial acetic acid

(17ml) for 12 hours. The active ingredient rifampicin (250mg) was dissolved in 2ml methanol and dispersed uniformly in polymer solution. Then aqueous polymer dispersion was added drop wise with the help of 23 gauge needle into the beaker containing liquid paraffin (250ml) with continuous stirring using overhead stirrer. To this 1ml of 25% v/v glutaraldehyde solution was added to the liquid paraffin as a cross-linking agent. Stirring was continued for 20 minutes. Finally solid polymer microcapsules were separated by decantation, washed with petroleum ether (3x30ml); air dried for 12 hours and was stored in a desiccator.

Characterization of Microcapsules
The microcapsules were found to be discrete and free flowing. The prepared microcapsules were evaluated for surface morphology by SEM analysis, size distribution, drug content, encapsulation efficiency, swelling studies, *in-vitro* bioadhesion and *in-vitro* drug release studies.

SEM analysis
The particle size, shape and surface morphology of microcapsules were examined by scanning electron microscopy. Microcapsules were fixed on aluminum studs and coated with gold using a sputter coater SC502 under vacuum (0.1 mm of Hg). The microcapsules were then analyzed by SEM (model LEICA S-430, London, U.K)[29].

Size analysis
Different sizes in the batch were separated by sieving using a range of standard sieves (USP sieves) 12/16, 16/20, 20/30, 30/40, 40/60 and the amounts retained on different sieves were weighed. Studies were carried out in triplicate. The average sizes of microcapsules were calculated by using the equation.

$$D_{ave} = \frac{X_i f_i}{f_i}$$

Where, X_i = the mean size of the range.
f_i = the percent material retained on the smaller sieve in the size range.

Drug content and encapsulation efficiency
Microcapsules (50mg) were powdered and transferred into a 50ml volumetric flask and the volume was made up to the mark with methanol, kept aside for 12 hours with occasional shaking and filtered. Then the drug content was analyzed spectrophotometrically at 475 nm. Three determinations were carried out for each batch.

The encapsulation efficiency was calculated using the formula.

$$\% \text{ Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

Fourier Transform- Infra Red Spectroscopy

FT-IR spectra were recorded on the Shimadzu Spectrophotometer. Rifampicin and their microcapsules were prepared in KBr discs prepared at a hydrostatic pressure of 5 tones /cm^2 for 2 mins. The scanning range was 450-4000cm^{-1} and the resolution was 1 cm^{-1}.

Swelling studies

A known weight (50mg) of microcapsules were placed in a glass vial containing 10ml hydrochloric acid buffer pH 1.2 and phosphate buffer pH 7.4 at 37 +0.5^{o}C with occasional shaking. The microcapsules were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally the weight of swollen microcapsules was recorded after a time period of 4 hours and the swelling ratio (SR) was then calculated using the formula[30],

$$\text{Swelling Ratio (SR)} = \frac{W_e - W_o}{W_0}$$

Where, Wo = Initial weight of dry microcapsules.
We = weight of the swollen microcapsules at equilibrium swelling in the medium.

***In-vitro* mucoadhesion test[31]**

An Albino rat (215-230 gms) was fasted for 12 hours. Then the animal was sacrificed, the small intestine was excised and washed with phosphate buffer of pH 7.4 to clear the contents. Around 4-5 cm length of everted jejunum was filled with phosphate buffer pH 7.4 and both the ends were tied with thread. Now 20ml capacity of test tube was taken and filled with 5ml of phosphate buffer pH 7.4. The everted jejunum and 100 microcapsules were agitated end to end for 30 minutes at room temperature, then the supernatant liquid and microcapsules bound to sac were separated. The number of particles adhering to intestine and remaining in the test tube was counted. The study was repeated with hydrochloric acid buffer of pH 1.2.

***In-vitro* dissolution studies**

The release of rifampicin from microcapsules was investigated in hydrochloric acid pH 1.2 for 2 hours and then in phosphate buffer of pH 7.4 for 10 hours in dissolution medium (900ml) containing 200 g/ml ascorbic acid as an antioxidant[32], by using rotating basket method specified in USP XXIV. A sample of 100mg of microcapsules was taken in the basket. A speed of 75 rpm and a temperature of 37 ±0.5^{o}C were maintained throughout the experiment. At fixed time intervals, aliquots (5ml) of sample was withdrawn and replaced with fresh dissolution media. The concentration of the drug released at different time intervals was then determined by measuring the absorbance using UV-visible spectrophotometer at 475 nm against blank. The studies were carried out in triplicate. The percent of drug released at various time intervals was calculated and plotted against time. The release of pure drug was also determined in the same way.

Stability studies

Stability studies were performed over a period of 3 months on the promising formulation (1:3 ratio). The microcapsules were packed in screw capped amber colored glass container and kept in a stability chamber at 40^{0}C and 75% RH. These samples were again evaluated for drug content and drug release once in a month.

RESULTS AND DISSCUSION

Rifampicin microcapsules were prepared by emulsification cross-linking method with varying proportions of chitosan and rifampicin. Chitosan is a cationic polymer which can be easily cross-linked with aldehyde group such as glutaraldehyde. Aldehyde group interacts with amine group of chitosan resulting in complex formation (imide group) which is responsible for the controlled release of the drug. The prepared microcapsules were found to be discrete and free flowing.

SEM analysis

SEM was used to investigate the morphology of microcapsules. The microphotographs are shown in Fig.1 and Fig.2 and they indicate that the microcapsules were almost spherical, discrete and covered continuously and completely with chitosan coat material.
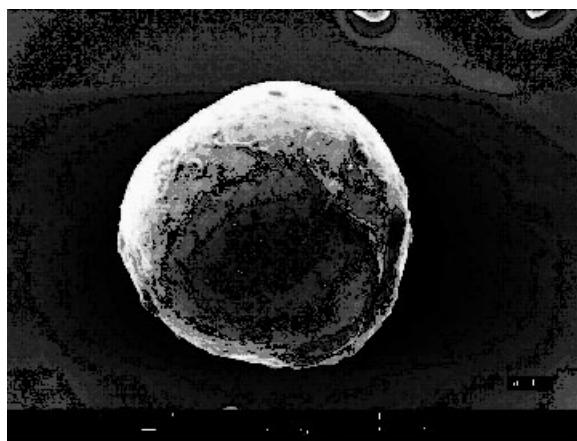

Fig.1: *SEM photograph of rifampicin-chitosan microcapsule of core : coat ratio (1:3), before dissolution studies*

Size analysis

Particle size analysis was carried out using standard sieve set. The size analysis of different microcapsules showed that about 28.514-34.637% were in the size mesh of (**USP**) –20+30. The microcapsules were uniform in size with a mean size of 795.76 ± 1.154 m. Particle size is inversely proportional to surface area and this affects the drug release from microcapsules.

Fig. 2: SEM photograph of rifampicin-chitosan microcapsule of core:coat ratio (1:3), after 12 hours of dissolution studies.

With increase in coat: core ratio, the size of microcapsules prepared was increased. This increase in size with increase in polymer concentration may be due to the greater viscosity of the coating solution with increasing concentrations.

Drug content and encapsulation efficiency
Drug content of microcapsules determines the amount of drug entrapped in the microcapsules. The drug content estimated in each 50mg of various microcapsules was found to be in the range of 4.95-5.94mg in methanol. The encapsulation efficiency represents the percentage of encapsulated drug with respect to the total drug introduced into polymer solution. The encapsulation efficiency ranged from 41-98.62% and the encapsulation efficiency increased with increase in coat proportion.

Fourier Transform- Infra Red Spectroscopy studies.
The compatibility between drug and polymer was confirmed by using IR spectroscopy. Fig. 3 shows that IR spectra of chitosan with its peak at 3433.6 cm^{-1}, 2920.03 cm^{-1}, 1654.81 cm^{-1}, 1423.37 cm^{-1}, 1323.08 cm^{-1}, 1261.36 cm^{-1}, 1026.0 cm^{-1} for coupled NH and OH stretching, C-H stretching, O=C-NH stretching, CH, NH, C-O, C-N, OH, C-O, C-N out of plane respectively. Fig. 4 shows IR spectra of drug Rifampicin with its peak at 3421.48 cm^{-1}, 2970 cm^{-1}, 2881 cm^{-1}, 2850 cm^{-1}, 1712 cm^{-1}, 1654 cm^{-1}, 1562 cm^{-1}, 1377 cm^{-1}, 1458 cm^{-1}, 1253 cm^{-1}, 1689 cm^{-1}, 775 cm^{-1} for O-H stretching, C-H stretching(aromatic), C-H stretching (aliphatic), O=C-N stretching, C=N stretching, C=C stretching of ethylene / aromatic, C-O stretching, C-H deformation, O-H deformation, O=C-NH weak stretching, benzene out of plain deformation respectively. Fig. 5 shows IR spectra of microcapsule (1:3 ratio) with its peak in the same region as that of individual components suggesting no interaction or degradation.
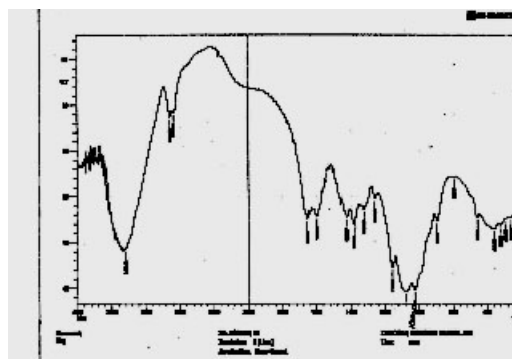
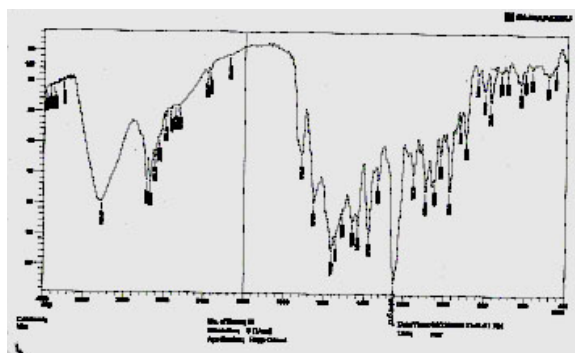

Fig. 3 : IR spectra of Chitosan
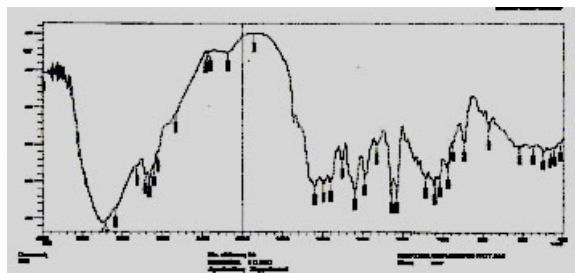

Fig. 4 : IR spectra of Rifampicin

Fig. 5: IR spectra of Chitosan microcapsules containing Rifampicin (1:3 ratio)

Swelling studies
Swelling studies of the prepared microcapsules are given in Fig.6. The chitosan microcapsules showed higher equilibrium swelling ratio value 2.4 at the end of 4 hrs in the pH 1.2 where as the swelling ratio value was reduced to 1.00 at the end of 4 hrs in pH 7.4 medium. The results showed that swelling ratio of chitosan microcapsules was higher at pH 1.2 than, at pH 7.4. This may be due to the lower molecular weight polymer chitosan forming weaker, more easily fractured gel in the test system with restricted water availability, and also chitosan chains possess glucosamine groups that can be deprotonated if the pH increases, therefore modification of pH from acid to base caused a deswelling process based on a reduction of the intermolecular electrical repulsions inside the particle mesh.
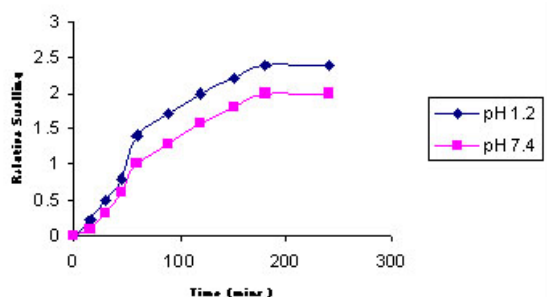
Fig.6 : Comparison plots of swelling ratios of rifampicin-chitosan microcapsules of core:coat ratio (1:3) in pH 1.2 and pH 7.4

In-vitro mucoadhesion test

Results of *In-vitro* mucoadhesion test of prepared microcapsules are given in Table 1. Mucoadhesion test was carried out with everted rat intestinal sac at pH 1.2 and at pH 7.4. The test revealed that percent adhesion of microcapsules was in the range of 38-56% at pH 7.4 where as 42-60% at pH 1.2. Percent of adhesion was decreased with increased proportion of coat composition. Percent of adhesion was high at pH 1.2 than at pH 7.4 media. This may be due to the ionization of glucosamine and other functional groups in the polymer, at pH 7.4 which increases their solubility and reduces their adhesive strength.

Table 1 : In-vitro mucoadhesive properties of rifampicin-chitosan microcapsules of various core :coat ratios in pH 1.2 and pH 7.4

Formulation	Percent adhering to tissue in pH 7.4 medium	Percent unbound	Percent adhering to tissue in pH 1.2 medium	Percent unbound
1:1	56	44	60	40
1:2	42	58	52	48
1:3	38	62	42	58

In-vitro dissolution studies

In-vitro drug release profiles of various formulations of rifampicin-chitosan microcapsules are shown in Fig.7. The release of the drug from the microcapsules exhibited a sustained release over a period of 12 hrs. The release rate was increased with the decreasing size of microcapsules. This may be due to the increased surface area with increase in the coat composition. The release rate decreased as the proportion of coat material increased. The percentage drug release from the microcapsules 1:1, 1:2 and 1:3 were found to be 60.20%, 43.25% and 39.63% respectively. The plots of amount of drug released *Vs* square root of time were found to be linear indicating that the drug release mechanism is diffusion controlled and followed first order kinetics.

Stability studies

Stability study of formulation (1:3 ratio) confirms that the microcapsules are stable and there was no significant change in drug content and dissolution profile. The data is shown in Table 2.
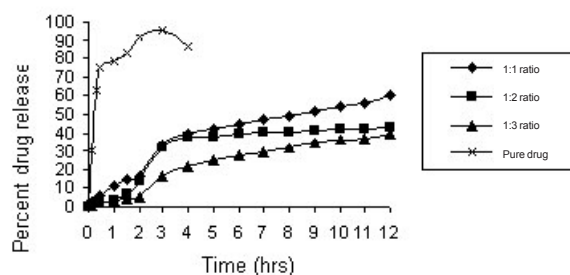

Fig.7: In-vitro release profiles of rifampicin- chitosan microcapsules of core:coat ratio of 1:1, 1:2 ,1:3 and pure drug in pH 1.2 and 7.4.

Table 2: *Effect of storage time on the characteristics of microcapsules at 40 ^{0}C (Mean ±SD, n=3)*

Formulation	Storage time	Drug content(mg)	Encapsulation efficiency (%)	% Drug release over the period of 12 hrs.
1:3 Ratio	0 day	5.94±0.239	98.62±0.264	39.63±0.249
	1 month	5.48±0.106	97.26±0.207	40.00±0.308
	2 months	5.19±0.020	97.05±0.362	38.22±0.785
	3 months	5.04±0.102	97.00±0.413	38.00±0.424

CONCLUSION

This study differs from the some earlier work in the following manner. In this work rifampicin microcapsules were formulated using chitosan alone i.e, without addition of any other polymer. Ming-Yue Mo[26] et al formulated rifampicin / chitosan microcapsules by solvent evaporation and emulsifying cross-linking method which shows encapsulation efficiency of 42.67%. In the present study the % encapsulation efficiency achieved were, for 1:1 microcapsules 41%, 1:2 microcapsules 62.8% and 1:3 microcapsules 98.62%.

The method of preparation of microcapsules of rifampicin was found to be simple and reproducible. The sustained release of rifampicin from microcapsules will help to improve the therapeutic efficiency and patient compliance by reducing the dose and dosing frequency of rifampicin. This study shows that chitosan microcapsules could be a carrier for rifampicin.

ACKNOWLEDGEMENT

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