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PREPARATION AND EVALUATION OF ALGINATE / CHITOSAN PARTICULATE SYSTEM FOR RIFAMPICIN RELEASE

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ABSTRACT

This work investigated the preparation of biodegradable beads with alginate polymer by ionotropic gelation method. Ionotropic gelation was applied to prepare beads using chitosan and calcium chloride ($CaCl_2$) as cationic components and alginate as anion. The concentration of sodium alginate was kept at the minimum level (3.5%w/v), required for the formation of beads and study the effect of addition of poly vinyl pyrrolidone (PVP) (2%w/v) and 0.5% chitosan to the $CaCl_2$ solution. The drug loading, encapsulation efficiency and in vitro release characteristics were studied the results showed that the addition of, chitosan significantly improved drug loading and encapsulation efficiency and release characteristics but addition of PVP does not alter the release characteristics but improves drug loading and encapsulation efficiency.

Key Words:- Rifampicin, Sodium alginate, PVP, Chitosan, Ionotropic gelation method.

INTRODUCTION

Mycobacterium tuberculosis (TB) is the world's largest cause of death from a single microorganism. Treatment of tuberculosis is generally successful, except in the case of multiple-drug-resistant strains of Mycobacterium tuberculosis. Rifampicin (RIF) is 'first-line' antibiotics for TB treatment (Farnaz *et al*., 2007).

Rifampicin appears to have the largest number of side effects, notably hepatotoxicity, enzyme induction and interactions with several drug classes. Other common adverse effects such as nausea and the 'flu-like' syndrome sometimes associated with thrombocytopenia and acute renal failure often results in discontinuation of the treatment (Garcia-Contreras *et al*., 2006). In the last several years, many different types of rifampicin controlled release formulations have been developed to improve clinical efficacy of drug and patient compliance. (Sreenivasa Rao *et al*., 2002).

In this study, we prepared sodium alginate beads using rifampicin as a model drug. We studied the addition of 0.5%w/v chitosan and 2%w/v PVP to the $CaCl_2$ solution on the drug loading and *in-vitro* release characteristics were studied. The main objectives of this present study to prevent drug leaching during preparation and to improve the drug release rate.

Beads loaded with antibiotics would be useful for oral delivery in the intestinal region offers interesting possibilities for the treatment of diseases susceptible to the

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diurnal rhythm, such as asthma, arthritis or inflammation (Anil K. Anal *et al*., 2005).

Beads are one of the multiparticulate delivery systems and are prepared to obtain prolonged or controlled drug delivery, to improve bioavailability, stability and target drug to specific sites. Beads can also offer advantages like limiting fluctuation within therapeutic range, reducing side effects, decreasing the dosing frequency and improving patient compliance (Oya *et al*., 2007)**.**

In view of biocompatibility, natural polymer like sodium alginate has been used in drug delivery applications. Sodium alginate (SA) is a sodium salt of alginic acid, a naturally occurring non-toxic polysaccharide found in brown algae. It contains two uronic acids, α -L-guluronic and β-D-mannuronic and is composed of homopolymeric blocks and blocks with an alternating sequence (Draget *et al*., 2000). Gelation occurs by cross-linking of the uronic acids with divalent cations, such as Ca^{2+}, this phenomenon has been used to prepare an alginate bead for drug delivery system. The formation of calcium-alginate beads by ionotropic gelation was achieved by dropping the drug containing sodium alginate dispersion into a $CaCl_2$ bath (Ostberg *et al*., 1994; Sugawara *et al*., 1994).

Drug released from calcium-alginate beads depends on the diffusion of the drug in the gel matrix (Sugawara *et al*., 1994) and the release characteristics of the entrapped substances could be improved by surface complexation of alginate with water-soluble polymers into the beads, such as chondroitin sulfate (Murata *et al*., 1996).

Chitosan has been proposed as a useful excipient for either sustained release of water-soluble drugs and for enhancing the bioavailability of poorly water-soluble compounds. Cationic chitosan can form gels with non-toxic multivalent anionic counter ions such as polyphosphate and sodium alginate by ionic cross-linking (Anil K. Anal *et al*., 2005). Chitosan with excellent biodegradable and biocompatible characteristics is a naturally occurring polysaccharide. Due to its unique polymeric cationic character and its gel and film forming properties, chitosan has been examined extensively in the pharmaceutical industry for its potential in the development of drug delivery systems recently, the use of complexation between oppositely charged macromolecules to prepare chitosan beads (or microspheres) as a drug controlled release formulation (X.Z. Shu *et al*., 2000). The choice of Poly vinyl Pyrrolidone (PVP) was due to its long-standing and safe record in biomedical/pharmaceutical application. Additionally it has been reported to enhance drug circulatory time in plasma when used as delivery system (Ding Guowei *et al*., 2006).

METHODS

Sodium alginate was purchased from Himedia laboratories, Mumbai, India. Rifampicin, chitosan and poly vinyl pyrrolidine (PVP) were obtained from Sigma Aldrich chemie GmbH, Germany. Calcium chloride ($CaCl_2$) was purchased from Rankem laboratories, Mumbai. India.

Preparation of Rifampicin- alginate beads

3.0% w/v of Sodium alginate solution was prepared in 25 ml of deionized water under gentle heat and mixing. 1% of rifampicin was added to this sodium alginate solution and stirred using an over head stirrer (Remi instruments, Mumbai) for 5-10 minutes at 1000 rpm to obtain a homogenous mixture. The mixture was kept aside until the air bubbles disappear completely and then it was extruded drop wise into 50 ml of 1% Calcium chloride solution through the 26 gauge needle (F-1 beads). Similarly the F-2 beads were prepared by drug - alginate mixture and extruding drop wise into 50 ml of 1% $CaCl_2$ and 0.5%w/v chitosan. F-3 beads were prepared by extruding the drug-alginate mixture drop wise in to the 50 ml of 1% $CaCl_2$ solution containing 2%w/v of PVP, F-4 beads were prepared by drug alginate mixture in to 50 ml of 1% $CaCl_2$ solution containing 2% of PVP and 0.5%w/v of Chitosan. The different formulations prepared were represented (Table-1). The gel beads were cured in gelation solution for 1hr, then filtered, and rinsed several times with distilled water and dried at 45^0C for 12–16hr. in hot air oven.

Evaluation of alginate beads

Fourier transforms infrared measurements (FTIR)

The drug and polymer interactions were studied by infrared spectroscopy. The IR spectra were recorded in the wavelength region 400-4000 cm^{-1} for pure rifampicin, sodium alginate, chitosan plain alginate beads and rifampicin loaded alginate beads using Thermo Nicolet, Avatar 320 (USA) instrument.

Table 1. Formulation design of Rifampicin loaded sodium alginate beads

Formulation Code	Sodium Alginate (%)	Drug (mg)	$Cacl_2$ (%)	PVP (%)	Chitosan (%)
F-1	3.0	250	1	---	---
F-2	3.0	250	1	---	0.5
F-3	3.0	250	1	2	---
F-4	3.0	250	1	2	0.5

Scanning Electron Microscope (SEM)

The morphology and surface structure of the beads were observed using SEM photographs taken with Jeol JSM-6360 instrument. The beads were made conductive by sputtering thin coat of platinum under vaccum using Jeol JFC-1600 auto fine coater and then the images were recorded at magnification of 80x.

Determination of drug content of the beads

The known amount of beads was crushed in a mortar with a pestle and transferred into beaker containing 100 ml phosphate buffer pH 7.4 and stirred using overhead stirrer for the complete swelling and bursting of the beads, then the solution was filtered through 0.45 µm membrane filter and the concentration of drug in the solution was determined after appropriate dilution with phosphate buffer pH 7.4, using UV spectrophotometer (Perkin Elmer) at 475 nm. The drug loading and percentage of entrapment efficiency was then calculated as formulas.

$$\text{Percentage drug loading} = \frac{\text{Amount of drug in bead}}{\text{Amount of bead taken}} X \quad 100$$

$$\text{Percentage entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} X \quad 100$$

***In vitro* drug release**

The in vitro drug release from the beads was studied in buffer pH 1.2 and 7.4 using shaking incubator. The known quantity of alginate beads (100 mg) from each formulation was transferred into conical flasks containing 100 ml of buffer solution and the flasks were shaken with a rate of 50 rpm at 37^0C ±0.5^0C. The samples of 5 ml aliquots was withdrawn at predetermined time intervals and the same volume of fresh preheated buffer medium was replaced into the conical flasks to maintain sink condition throughout the experiment. The withdrawn aliquots was filtered and analyzed for drug content using UV spectrophotometer at 475 nm after suitable dilution with respective buffer solutions.

RESULTS

FTIR studies

The IR spectra of the substances used in the formulation and the prepared beads were shown in the figure 1-5. The IR spectra of the Rifampicin showed the peak at, 3425, 1618, 1429.65 and 1090.66cm^{-1}, its corresponding to rifampicin loaded alginate beads, and peaks at 3440.85 2371.96, 1628.90 and 1102.11cm^{-1} corresponding to the drug. This confirms that there is no interaction between the drug and the substances used in the formulation.

Fig. 1. IR spectra of sodium alginate

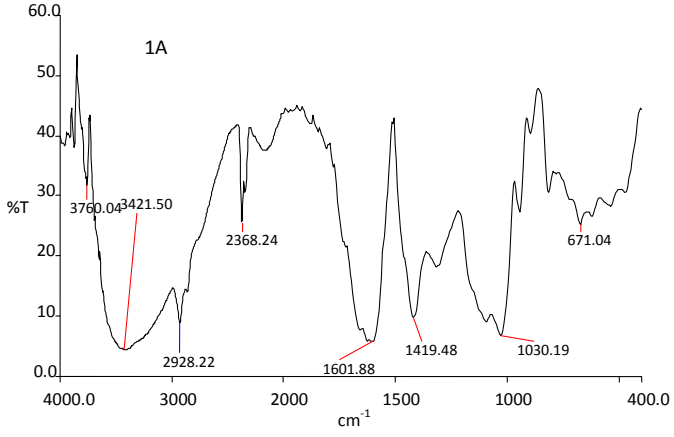


Fig. 2. IR spectra of Rifampicin

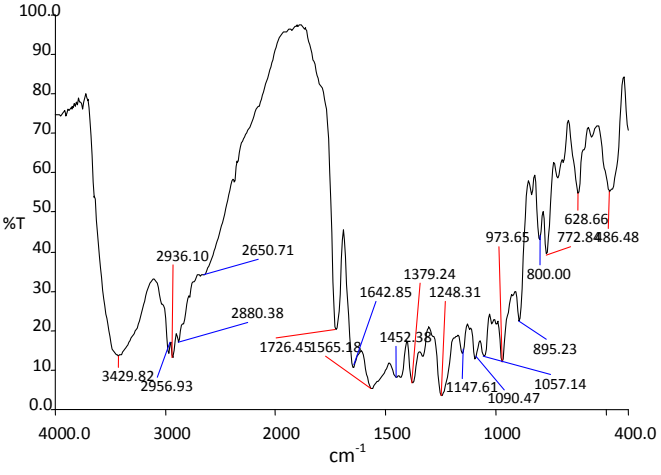


Fig. 3. IR spectra of Chitosan

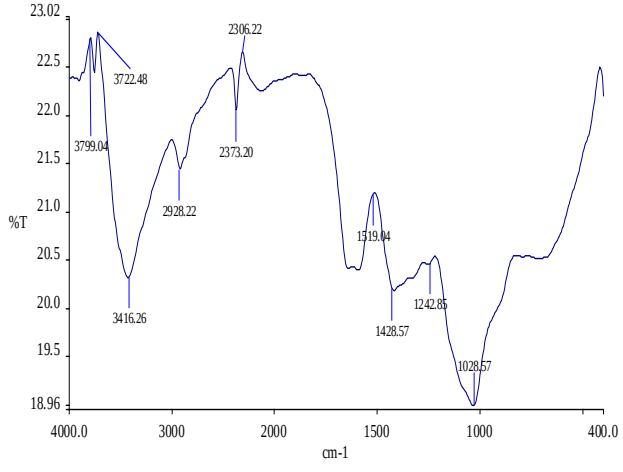


Fig. 4. IR spectra of plain beads

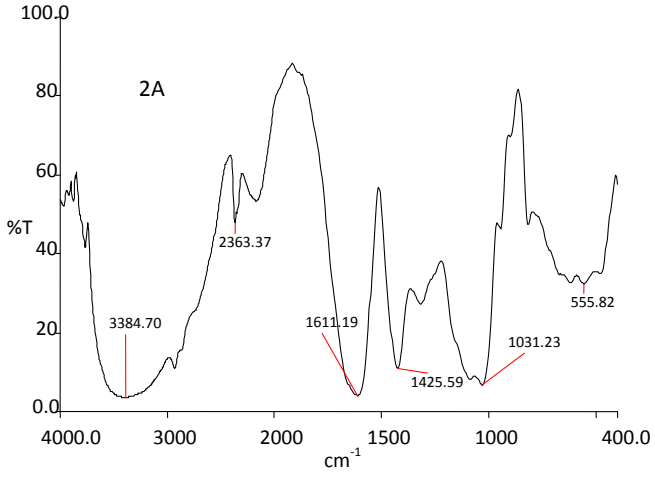


Fig. 5. IR spectra of Rifampicin loaded sodium alginate beads

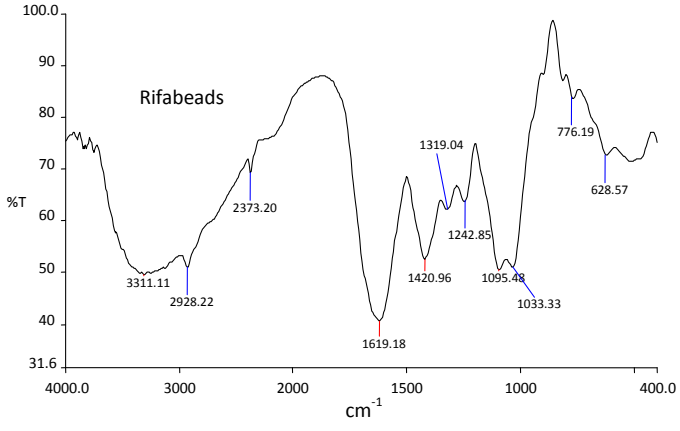
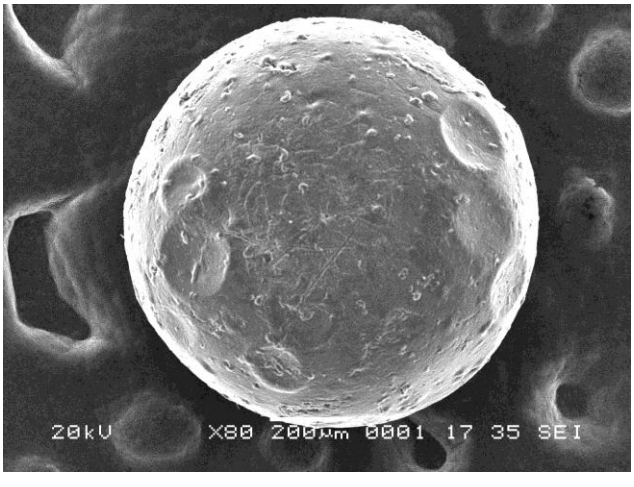


Fig. 6: SEM photograph of drug loaded bead (F-4) at 80x



Scanning Electron Microscopy analysis

A SEM photograph of formulation (F-4) a single bead taken at 80x magnification was shown in Fig.6. As seen from the figure; the drug loaded beads were almost of spherical in shape and have a rough surface.

Table 2. Drug loading and Encapsulation efficiency of formulations

Formulation Code	Drug loading (%)	Encapsulation efficiency (%)
F-1	8.6 ± 0.26	30.98 ± 2.81
F-2	42.47± 0.91	44.92 ± 0.40
F-3	9.39 ± 0.60	33.82 ± 3.16
F-4	13.25 ± 1.16	47.73 ± 0.32

*All values are expressed as mean ± S.E; n= 3

Drug loading and entrapment efficiency

The drug loading and entrapment efficiency of different formulations were shown in table 1. The formulation (F-4) with 0.5%w/v chitosan and 2%w/v PVP shows better drug loading efficiency compared to the other formulations.

Effect of Chitosan on drug loading and entrapment efficiency

The addition of chitosan (F-2 & F-4) had a significant effect on the loading and entrapment of rifampicin in alginate beads in the absence of chitosan, entrapment of rifampicin was very low (see table 2). This may be due to insufficient cross-linking and large pore size permitting the rifampicin to diffuse out during and after gelation. This is probably due to more firmness in the alginate–chitosan complex during gelation caused by increased ionic interactions between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. As a result less rifampicin is lost during gelation (Anil K. Anal *et al*., 2005).

Effect of PVP on drug loading and entrapment efficiency

The addition of PVP to the cross linking solution (F-3) has shown significant increase in the drug loading and entrapment efficiency. This may be due to the increase in viscosity of the cross linking solution by PVP, so that it might have been blocked the pores of the alginate beads and hence it might have been prevented the drug leaching to the cross linking solution.

***In vitro* drug release**

The drug release from the alginate beads were studied in buffer solutions pH 1.2 and 7.4 at 37^0C ±0.5^0C.

Figures 7 & 8 have shown the drug release in respective buffer solutions from different formulation. A pronounced difference was observed in the release data between pH 1.2, and 7.4. The formulation (F-4) shows that the sustained drug release was achieved compared to the other formulations

Fig. 7. *In-vitro* Drug release at pH 1.2

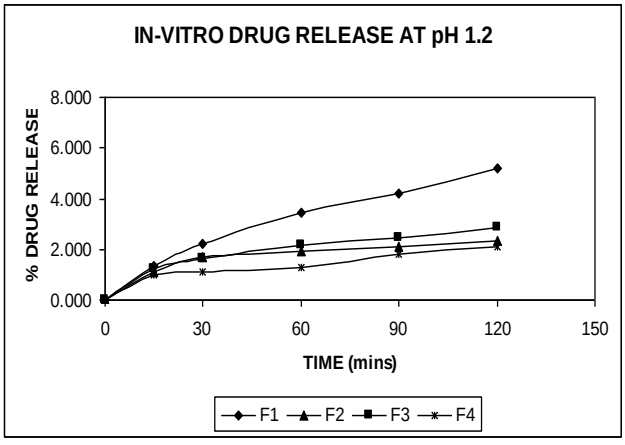
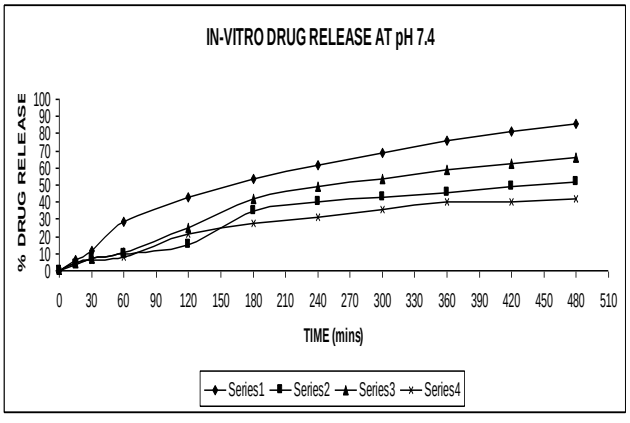


Fig. 8. *In-vitro* Drug release at pH 7.4



At acidic pH, rifampicin release from gel beads was low (1-5%) in all formulations for 2 h. At low pH values the less swelling should reduce the matrix permeability and limit the drug diffusion (Oya Sanli *et al*. 2007). At this pH, alginate is protonated into insoluble form of alginic acid this displays properties of swelling that explains low amount of the release. At pH 7.4 an increase in the release rate (40-90%) in 8 h was observed. At pH 7.4 the deprotonation of alginic acid causes disintegration of the bead systems and nearly completes the release of rifampicin as soluble ions.

When chitosan was added to the cross-linking solution (F-2), rifampicin release rate was decreased as it is seen from the figures 6 & 7. The release mechanism of the chitosan: alginate beads are related to the solubility of chitosan, which is poorly soluble in water. In acidic medium, protonation of the amine groups improves the solubility. The presence of negative charges due to the anionic nature of the alginate forms an interpolymeric complex which reduces drug release. At low pH, the interpolymeric complex exists in a gel form. When the beads were transferred to pH 7.4, the viscous complex previously formed swelled and the gel was slowly disintegrated, releasing the drug (M.J. Ferna´ndez-Herva´s *et al*., 1998).

The carbonyl group of PVP has a high molecular weight of approximately 1,000,000dalton and it is hypothesized that diffusion of the PVP within the bead is inhibited by its high molecular weight. The presence of PVP in the formulation leads to a modulation of the drug release from the 'pseudo-gel layer' surrounding the bead, which controls the drug release rate (Ian J. Hardy *et al*., 2007).

CONCLUSION

The rifampicin loaded sodium alginate beads were prepared by ionic gelation method. In this method various formulation variables such as the addition of chitosan and PVP to cross linking solution were studied. High drug loading, encapsulation efficiency and sustained drug release were obtained in F-4 formulation. Previously no substantial work have been reported by employing PVP in the cross linking solution. Hence chitosan and PVP can be used in the formulation to improve in vitro characteristics of rifampicin loaded alginate beads.

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