# Zinc-Alginate Beads for the Controlled Release of Rifampicin

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## ABSTRACT

The present study describes the preparation of a carrier system based on zinc-alginate beads and to evaluate its potential for the controlled release of rifampicin, the poorly water soluble drug using ionic gelation technique. The surface topology of the developed beads were analyzed by Scanning Electron Microscopy and the crosslinking process was investigated by Fourier Transform. Infrared spectroscopy. The pH dependent swelling properties of the beads were analyzed. Drug entrapment efficiency and *in vitro* drug release studies were also investigated. The *in vitro* cytotoxicity and antibacterial activity were evaluated. Results indicated that the entrapment efficiency improves as the amount of polymer incresed. The developed beads showed a pH dependent swelling behaviour. The sustained release of drug was more prominent in pH 7.4 than 1.2. The MTT assay results proved the cell biocompatibility of the developed beads. The beads showed good antibacterial activity.

**Keywords**: Alginate, Drug delivery, Rifampicin, Cytotoxicity, Tuberculosis.

## INTRODUCTION

Tuberculosis(TB) is a chronic pulmonary disease caused by mycobacterium tuberculosis. Rifampicin (RIF) is one of the effective first line drug for TB and isthe semi synthetic hydrazine derivative of rifampicin B.Major limitations of conventional tuberculosis therapy using RIF tablets are the long duration of treatment with frequent multiple dosage due to its short biological half-life, low stability and low water solubility. The continuous presence of RIF at high concentration above therapeutic range for prolonged period of time is associated with for prolonged effects such as renal failure, hepatotoxicity, fever, thrombocytopenia and antibody formation[1,2]. Development of drug delivery system that release the antimicrobial agent in a slow and sustained manner, could provide maximum therapeutic benefit while minimizing the side effects.

Several studies have been made towards developing controlled drug delivery systems for antitubercular drugs to improve the therapeutic efficacy and minimize the side effects[3]. Many of these systems suffer from problems like lower drug entrapment, higher cost, safety issues, use of toxic organic solvents and complicating preparation steps[4,5]. Alginate based formulations attracted considerable attention due to its biocompatible nature[6]. Most of them use calcium as the cross linking metal. But calcium–alginate complexes were reported to show some disadvantages such as sensitivity towards the physiological environment and rapid release of encapsulated drug[7]. It was found that zinc ions possess a superior binding capacity towards alginate than calcium and which are more resistant to hydration[8]. Metal ions also have specific physiological functions in the body. Zinc is an important trace element, and possess key role in biomineralization, maintenance of membrane structure and its function and nucleic acid metabolism. It also possess antibacterial activity[9]. The antibacterial activity of zinc may also offer unique therapeutic applications.

The purpose of the present study is to prepare zinc alginate (Zn-ALG) beads as a carrier for poorly water insoluble drug RIF. The drug encapsulation efficiency and drug release profile were analyzed. The interaction between zinc alginate and RIF was analyzed. Antimicrobial activity of the obtained zinc alginate beads were studied against *Staphylococcus aureus* and *Escherichia coli .* The *in vitro* cytotoxicity of the beads was tested using MTT assay.

## MATERIAL AND METHODS

### Material

Sodium alginate of medium viscosity (viscosity of 2% solution, 25 °C ≈ 500 cps, Sigma-Aldrich, London), Rifampicin (Himedia Laboratories, Nasik), Zinc chloride (Merck, Germany).

### Preparation of Zn-ALG and RIF loaded Zn-ALG beads

Known amount of sodium alginate was dissolved in deionized water and stirred for 30 min. to obtain homogenous solution. A volume of 10 mL of this solution was dropped into zinc chloride solution of definite concentration using a 25-G needle from 5 cm distance from the surface at room temperature. The droplets were kept in solution for 20 hours. Then, Zn-ALG beads were separated from the solution, washed several times with distilled water and dried.

For preparing RIF loaded Zn-ALG beads, required weight of RIF was dispersed into sodium alginate solution and the same procedure was repeated.

### Characterization Zn-ALG beads

The morphology of Zn-ALG bead was characterized using scanning electron microscope (JEOL JSM-6390LA). Zn-ALG crosslinking and RIF-ALG interactions were analyzed by recording the Fourier transform infrared (FTIR) spectra of RIF, sodium alginate, Zn-ALG beads and RIF loaded Zn-ALG beads using Schimadzu FTIR model 1801 between 400 and 4000 cm$^{-1}$ wavelength range.

### Swelling

The degree of swelling was measured by suspending accurately weighed 100 mg of Zn-ALG beads in 10 ml solutions of pH 1.2 and 7.4 for 6 h. The increase in the weight of beads at predetermined time intervals were noted. The swelling degree was calculated using equation (1). Data were presented as mean ± standard deviation (SD) based on 3 independent measurements.

$$S = \left(\frac{W_s - W_d}{W_d}\right) \times 100 \qquad (1)$$

Where $W_s$ is the weight of swollen samples and $W_d$ is the initial weight of the beads.

### Entrapment efficiency of Zn-ALG beads

About 100 mg of RIF loaded Zn-ALG beads were dispersed in  phosphate buffer solution (100 mL) of pH 7.4 and sonicated for 30 min. to extract RIF completely from the beads. The concentration of extracted drug was analysed  by measuring the absorbance at 475 nm, on Perkin Elmer Lambda bio 40 UV–VIS spectrophotometer by using the calibration curve constructed from a series of solutions with known drug concentration. The entrapment efficiency percentage (% EE) was calculated using equation (2)

$$\% \, EE = \left( \frac{Actual \, drug \, loading}{Theoretical \, drug \, loading} \right) \times 100 \qquad (2)$$

### *In vitro* RIF release study

In order to understand the RIF release pattern, *in vitro* release studies were carried out in simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.4) as per United States Pharmacopoeia standard[10]. About 30 mg of dried drug loaded beads were introduced into a dialysis bag of molecular weight cut off 12,000 Da and is incubated with 100 ml of the release medium. Release studies were conducted at 37 °C in an incubated shaker at 100 rpm. About 5 ml of solution was taken at predetermined time intervals, and add equal amount of release medium at the same time. The RIF content in the solution was found by measuring the absorbance at 475 nm, using Perkin Elmer Lambda bio 40 UV–VIS spectrophotometer. Data were presented as mean ± standard deviation (SD) based on 3 independent measurements.

### *In vitro* cytotoxicity

The *in vitro* cytotoxicity of the Zn-ALG beads were analyzed by MTT assay using L929 fibroblast cell lines. The cells were cultured with DMEM containing 10% FBS and the cultured cell lines were remained at 37 °C in a humidified 5% $CO_2$ incubator for 24 hours. The medium in all the wells except the control, was then replaced with test solution and incubated at 37 °C in a humidified 5% $CO_2$ incubator for specified time. There after 30 µl of MTT (5mg/ml) was introduced in to the wells and incubated at 37 °C in a humidified 5% $CO_2$ incubator for 4 hours. Then the medium was removed and 100 µl DMSO was added to dissolve the formazan crystals. The absorbance of the solution were measured at 570 nm using micro plate reader to determine the optical density (OD). OD of control was obtained from the cells without sample dosing. Cell viability of the sample (%) was calculated using equation (3)

$$Cell \, Viability \, (\%) = \left( \frac{OD \, of \, sample}{OD \, of \, control} \right) \times 100 \qquad (3)$$

### Evaluation of antibacterial activity

The antibacterial activity of Zn-ALG beads were studied against *S. aureus* and *E.coli.* Petriplates containing 20 ml Muller Hinton agar medium were seeded with bacterial culture of *E.coli*

and (S.aureus) (growth of culture adjusted according to McFards Standard, 0.5%). Wells of around 10 mm was bored by means of a well cutter and add the sample. The plates were incubated for 24 h at 37 °C. The activity was obtained by measuring the diameter of the zone of inhibition (in mm) formed around the well (NCCLS, 1993).

### Statistical analysis

All the experiments were carried out in triplicate and the data was exhibited as mean ± standard deviation. The error bars have been inserted in all the data points in the figures.

### RESULTS AND DISCUSSION

### Preparation and characterization

The Zn-ALG beads were prepared by ionotropic gelation technique. Spherical beads were formed due to the ionic interaction between the negatively charged carboxyl group of alginate and the positively charged zinc ions. The effects of process variables like drug-polymer ratio and concentration of the cross linking solution onthe drug entrapment efficiency was evaluated. It was noted that the entrapment efficiency improves with increase in the concentration of polymer. Concentration of metal ions is very critical in determining the shape of the bead. At low-concentration below 5% metal chloride solution, spherical beads were not formed and only flakes were observed. At high concentration of metal ions irregular lumps were formed. This may due to the extensive cross linking of zinc ion with the guluronic acid component of ALG. Spherical beads with good entrapment efficiency were obtained at 1:4 drug-polymer ratio and 5% $ZnCl_2$ solution.

The morphology of Zn –ALG beads were studied by recording their SEM images as shown in Fig.1. In order to get a clear picture about the surface topography of the beads, the images were recorded at low and high magnifications.It can be seen that beads were spherical in shape with rough surface (Fig. 1 a). Some minor cracks can be seen on the surface of bead. This may due to the rupture during the electron beam irradiation.

**Table. 1:  Optimization parameters for the preparation of Zn-ALG beads**

| Formulation codes | $Zn^{2+}$ Concentration (%, v/v) | RIF:ALG | Drug entrapment efficiency (EE)% |
|---|---|---|---|
| F1 | 2 | 1:4 | - |
| F2 | 5 | 1:4 | 35.48±2.85 |
| F3 | 10 | 1:4 | - |
| F4 | 2 | 1:2 | - |
| F5 | 5 | 1:2 | 26.98±2.78 |
| F6 | 10 | 1:2 | - |
| F7 | 2 | 1:1 | - |
| F8 | 5 | 1:1 | 27.5±2.05 |
| F9 | 10 | 1:1 | - |
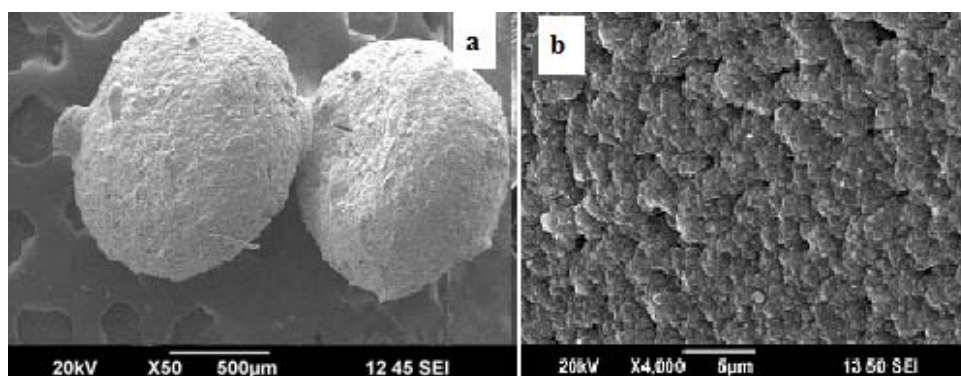


**Fig. 1. SEM image of Zn-ALG bead**

The interaction between drug and polymer were studied by FTIR spectroscopy. Spectra of the RIF, Na ALG, Zn -ALG and RIF loaded Zn-ALG beads were given in Fig. 2. The characteristic peak at 3445 cm$^{-1}$ in the spectra of the sodium alginate is indicative of stretching vibrations of OH bond. The bands around 1649 and 1446 cm$^{-1}$ were assigned to asymmetric and symmetric stretching vibrations of carboxylic carbonyl groups. During zinc cross linking process there is a remarkable shift of this carbonyl stretching vibration to lower wave number(1649 to 1635 cm$^{-1}$). This is due to the coordinate bond formation between ALG and zinc ions[11]. RIF gives a unique band at 1736 cm$^{-1}$ due to furanone ring structure. This band is also present in the spectra of RIF loaded Zn –ALG showing the successful encapsulation of RIF in Zn-ALG without any change in its chemical nature. This also indicate that there is no chemical interaction between the drug and polymer during encapsulation.
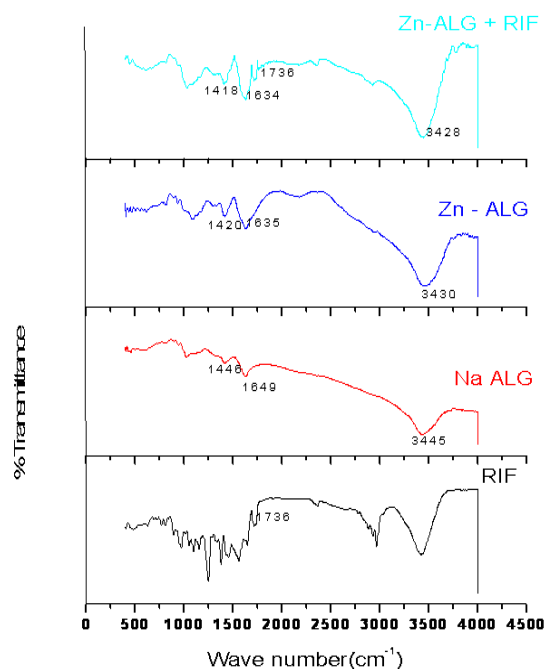


**Fig. 2. FTIR of a) RIF b) Na ALG c) Zn-ALG d)RIF loaded Zn-ALG**

**Swelling Percentage**

The swelling behaviour of Zn-ALG beads were investigated in simulated gastric fluid and intestinal fluid at room temperature as shown in Fig. 3. Swelling behaviour of drug carrier has a profound effect on the mechanism and kinetics of drug release. The swelling percentage was low at pH 1.2. This is due to the formation insoluble alginic acid as a result of protonation of carboxylate group. This reduces electrostatic repulsion among the carboxylate groups and minimize the degree of swelling. The strong hydrogen bond interactions also help to maintain the three dimensional network structure and hence reduce the water uptake. At this pH the system was stable and does not undergo any type of disintegration.
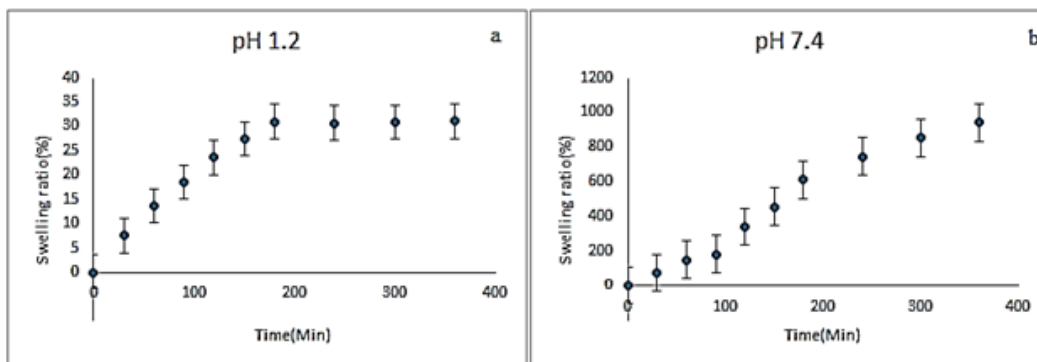


**Fig. 3. Swelling behaviour of Zn-ALG beads in (a) pH 1.2 (b) pH 7.4(Data presented as mean ± SD, n = 3)**

At pH 7.4 a profound swelling is observed. This is due to the anionic nature of ALG. At this pH the extent of ionization of carboxylate group of ALG increases, thus produces more carboxylate ions and these negatively charged ions repel each other and causes a rapid relaxation in ALG network. This relaxation enhances the inclusion of water molecule in to the bead network structure.

**In vitro drug release study**

Figure. 4 showed the release behaviour of RIF from Zn-ALG beads in SGF (pH 1.2) and SIF (pH 7.4).Approximately 10% of the drug was released in SGF over a period of 6 h. The pKa value of ALG is 3.6. Below this value, it is converted into alginic acid and form a tight three dimensional network. Zinc is also less selective towards alginate and can form a strong three dimensional network and retain the drug inside the bead.

At alkaline conditions, the pKa value is higher than 3.6, then the negatively charged carboxylate ions repel each other. As a result the three dimensional network relaxes and fluid can penetrate in to the Zn-ALG network. So RIF release is higher and almost 80% of the entrapped RIF was released in 6 h. The formation of gel diffusion layer help to hinder the outward transport of RIF, and maintain sustained release.This in vitro drug release study showed that the Zn-ALG system give a pH dependent drug release.
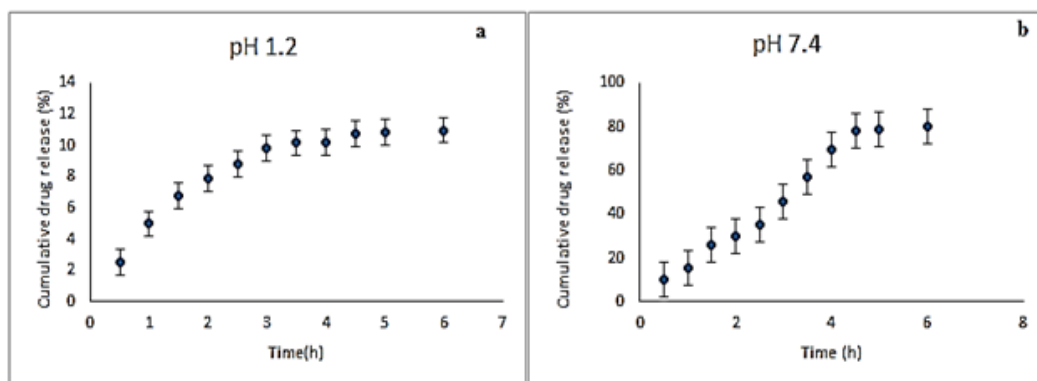


**Fig. 4. Cumulative RIF release at a) pH 1.2 and b) 7.4 (Data presented as mean ± SD, n = 3)**

### *In vitro* cytotoxicity

Cytotoxicity evaluation is very important for a drug carrier since it is applied inside the body. MTT is usually used to measure the cytotoxicity. In this method the cytotoxicity is expressed in terms of cell viability. The cell viability of control is 100%. As seen in Fig. 5, the L929 cell viability after being treated with the Zn -ALG was more than 80% even when the concentration was up to 100 µg/ml, indicating the drug carrier did not have significant cytotoxicity and possess excellent biocompatibility.
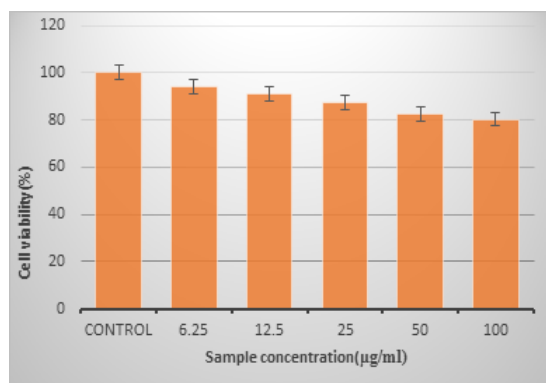


**Fig. 5. The cell viability of L929 cells treated with Zn-ALG**

### Evaluation of antibacterial activity

The antibacterial activity of Zn-ALG bead was analyzed by measuring the bacterial growth and *E.coli* (*Gram negative* bacterium). The zone of inhibition (100 µg/ml) for *S.aureus and E.coli* was 29 mm and 32 mm respectively. These observations clearly indicate that the obtained bead shows antibacterial activity against both *Gram positive* and *Gram negative.* The antibacterial activity of $Zn^{2+}$ can be explained on the basis of its ability to form bond with imidazole, thiol, carboxyl and amino group present in the bacterial protein. As a result some structural changes may occur and this hinders the transport through the plasma membrane and results in cell death[12].



**Fig. 6. Photograph of antimicrobial test results of Zn-ALG against a)** *S.aureus* **and b)** *E.coli*

The prepared Zn-ALG beads showed sustained RIF release, biocompatible in nature and can effectively inhibit the growth of both S.aureus and *E.coli.* The *in vitro* studies suggest that the biocompatible drug delivery systems can be used for sustained release of other drugs, enzymes, genomic materials and proteins. Further investigation using in vivo models should be performed to substantiate the results obtained from the *in vitro* studies.

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