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DESIGN AND EVALUATION OF CONTROLLED RELEASE CHITOSAN-CALCIUM ALGINATE MICROCAPSULES OF ANTI TUBERCULAR DRUGS FOR ORAL USE

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ABSTRACT: Chitosan coated alginate microcapsules were developed as oral sustained delivery carriers for anti tubercular drugs in order to improve patient compliance, to reduce dose/dosing frequency in the management of tuberculosis (TB), which otherwise demands prolonged chemotherapy. Alginate–chitosan microparticles encapsulating three frontline anti-tuberculosis drugs (ATDs), rifampicin, isoniazide and pyrazinamide, were formulated by using ionotropic/external gelation method. Three different formulations containing rifampicin, isoniazide and pyrazinamide separately were prepared in the ratio of 1:2:2 (drug: sodium alginate: chitosan). The prepared microcapsules were evaluated by SEM analysis, size analysis, sphericity, drug content, encapsulation efficiency, swelling studies and mucoadhesion which were compared with that of pure drug. The microcapsules exhibited a slow and sustained release over a period of 72 hours. *In-vitro* release studies were carried out in pH 1.2 for 2 hours and then in pH 7.4 for 72 hours and the amount of drug released were 96.46, 95.33 and 97.27 from rifampicin, isoniazide and pyrazinamide microcapsules respectively where as the release rate for pure drugs was 95.46% (3hrs) for rifampicin, 98.99 %(30mins) for isoniazide and 96.44% (30mins) for pyrazinamide. Alginate–chitosan microcapsules hold promise as a potential natural biodegradable polymer-based oral ATD carrier for better management of Tuberculosis.
KEYWORDS: Controlled release; bioavailability; sodium alginate; chitosan; microcapsules.

1. INTRODUCTION

The failure of antitubercular chemotherapy is mainly due to patient non compliance [1], which is attributed to the requirement of multi drug administration daily or several times a week for at least 6 months. Although attempts to improve patient non compliance by applying modified drug delivery systems for antimycobacterial agents are encouraging [2], higher polymer consumption [3], lower drug entrapment[4], higher cost, use of toxic substances and organic solvents during preparation [5,6] and surgical requirements [7] are all drawbacks associated with these drug delivery systems. Moreover, these particulate systems (liposomes, microparticles and nanoparticles) used as drug carriers are rapidly taken up from the blood by mononuclear phagocytes, especially by the Kupffer cells in the liver, which is a drawback to gaining access to other target sites in the body, e.g. the lungs. In addition, the high frequency of pulmonary tuberculosis demands the development of novel drug delivery approaches that enhance the bioavailability of drugs. In recent years, one of the best ways

to achieve higher drug levels in the plasma has been the development of new formulations (nanoparticles/microparticles-based) that are directly delivered to the desired site[8].The controlled delivery of antimycobacterial agents may be accomplished by employing various polymeric drug carriers. Although experience with synthetic polymers is extensive and encouraging [9,10,11], the recent trend has been to shift towards natural polymers [12]. The major advantage of natural polymers (e.g. alginate and chitosan) 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 the US FDA are additional advantages [13]. The encapsulation of three frontline anti-tuberculosis drugs (ATDs), rifampicin, isoniazide and pyrazinamide, in alginate-chitosan microparticles demonstrated promising chemotherapeutic potential. The incorporation of chitosan to alginate**,** was not only capable of improving the drug(s) encapsulation efficiency and bioavailability, but also of reducing the dose and dosing frequency when compared to alginate alone microparticles. The present study was planned with an aim of developing a natural biodegradable polymer-based drug delivery system to overcome the limitations associated with various drug delivery systems. Hence alginate–chitosan microparticles containing isoniazide (INH), pyrazinamide (PZA) and rifampicin (RIF) were developed and characterized.

Sustained release (SR) dosage forms are gaining increased acceptance over conventional dosage forms in the treatment of several acute and chronic conditions. So, SR dosage forms are designed to achieve a prolonged therapeutic action by continuously releasing medication over an extended period of time after the administration of a single dose. In addition to better patient compliance they reduce the incidence and severity of side effects [14]. They have proved particularly valuable in ensuring continuous therapeutic effect in conditions such as arthritis, angina pectoris, tuberculosis and asthma.

Microencapsulation is a process for coating small particles of solids/droplets of liquids and dispersion using polymeric material to produce small particles of 1-5000µm in size. It is a rapidly expanding technology for achieving sustained release dosage forms. Microencapsulation is used to modify and retard drug release. Microencapsulation is receiving considerable attention fundamentally, developmentally and commercially [15].

The ionotropic /external gelation process did not include emulsification step and stage of organic solvents, thereby minimizing inactivation of encapsulated drugs[16]. The gelation and cross-linking of the polymers are mainly achieved by the exchange of sodium ions from the guluronic acids with the cations (calcium ions and chitosan), and the stacking of these guluronic groups to form the characteristic egg-box structure. The cations bind to the L-guluronic acid blocks in a highly cooperative manner and the size of the cooperative unit is more than 20 monomers. Each alginate chain can dimerize to form junctions with many other chains, and as a result, gel networks are formed rather than insoluble precipitates. The both positively charged chitosan molecules and the positively charged calcium ions are competing with the negative charges on the surface of the alginate core .The mutual neutralization between oppositely charged alginate and chitosan decreases the solubility of the whole system. Therefore the driving force for the microparticle formation is a decrease of solubility. This mechanism explains the high efficiency of the microparticle formation, irrespective of the different experimental conditions.

To reduce the porosity and increase stability, alginate microparticles have been coated with chitosan by electrostatic interaction [17]. The negatively charged carboxylic acid groups of alginate bind ionically with positively charged amino groups of chitosan, a cationic polymer, to form a polyelectrolyte complex on the basis of their opposite charges [18]. Moreover, chitosan has been reported to enhance absorption of various compounds across the mucosal barrier owing to its properties of mucoadhesion, which increase the contact time of the drug with the mucosa, and its ability to induce a transient opening of epithelial cell tight junctions [19,20,21].

Anti tubercular drugs has been selected as model drugs because they exhibit all the pharmacokinetic and physico-chemical properties required for sustained release. Rifampicin, isoniazide and pyrazinamide are well absorbed from GIT after oral administration and have a short biological half life of 2-5 hours, 3.1 $\pm$ 1.1hours and 9-10 hours respectively.

The anti tubercular drugs are usually administrated in the form of tablets and capsules containing 100-300mg (isoniazide), 300-450mg (rifampicin) and 1500mg (pyrazinamide). The effect of the drugs that lasts for a few hours due to their short biological half life, necessitates that it be administrated daily once in the treatment of tuberculosis for a six months period. Thus, the development of controlled release dosage forms would clearly be advantageous. Hence to improve therapeutic efficacy and patient compliance and to reduce adverse effects, fluctuations in plasma concentration and to decrease the dose and dosing frequency controlled release preparations of anti tubercular drugs are essential.

2. MATERIALS AND METHODS

2.1 MATERIALS

Rifampicin, isoniazide, pyrazinamide, sodium alginate and chitosan were gift samples from Alkem laboratories limited, Mumbai and calcium chloride was from SD Fine Chem. Limited, Mumbai. All other chemicals and solvents used were of analytical grade. Paddle stirrer (Remi Motors), dissolution apparatus (Campbell Electronics, Mumbai) and UV-visible spectrophotometer (Systronics) were the equipments used in this study.

2.2 PREPARATION OF MICROCAPSULES [22]

Microcapsules containing rifampicin/isoniazide/pyrazinamide were prepared using chitosan/sodium alginate as coat material employing ionotropic/external gelation technique similar to that described by Gonza'lez-Rodri'guez M.L., (2002) with slight modification. Ionotropic gelation was applied to prepare microparticles using combinations of chitosan and Ca^{2+} as cationic components and alginate as anion. The required quantity of sodium alginate (500mg) was soaked in distilled water (12ml) for 12 hours. The active substance, rifampicin/isoniazide/pyrazinamide (250mg) was dissolved in 5 ml of methanol/distilled water and was added to aqueous solution of sodium alginate and stirred up to complete dissolution. After thorough mixing, 20–30 min was allowed to elapse in order to make the solution bubble-free. The mixture was dropped using an hypodermic syringe drop wise at 60 drops/min into 500 mL of 0.1 *M* calcium chloride solution containing 500 mg chitosan, previously dissolved in acetic acid solution (0.5% v/v), (i.e. drug/alginate/chitosan 1:2:2) at pH 4.5. The beads formed instantaneously and were left as such for 2-3 hrs at room temperature. Subsequently, the beads were recovered by filtration, washed twice with distilled water and dried at room temperature. The whole preparation was carried out at room temperature.

3. CHARACTERIZATION OF MICROCAPSULES

The microcapsules were found to be discrete and free flowing. The prepared microcapsules were evaluated by SEM analysis, size analysis, sphericity, drug content, encapsulation efficiency, swelling studies, mucoadhesion, IR studies and *in-vitro* release studies.

3.1 SEM ANALYSIS [23]

The particle size, shape and surface morphology of the microcapsules were examined by scanning electron microscopy. A small amount of microcapsules was spread on glass stub. After the stub containing the sample was placed in the scanning electron microscope chamber and coated with gold using a sputter coater SC 502, the scanning electron photomicrographs were taken at the acceleration voltage of 20 Kv, chamber pressure of 0.01mm Hg and original magnification ×600. The microcapsules were then analyzed by SEM [model LEICA S-430, London, U.K.]. The photomicrographs were depicted in fig.1, 2 and 3.

The following parameters were selected to characterize the ATDs microcapsules {Gonza'lez-Rodri'guez M.L., (2002)}

- Area (*A*), is the selected surface;
- Perimeter/length (*l*), is the length around the outside of the selection, or line length for line selections;
- Diameter of the equivalent area circle (ECD), is an equivalent diameter that is calculated as follows
  $Dcirl = 2\sqrt{A/\pi}$
  Where, *A* is the area of the closed boundary of the particle.
- Shape factor (*s*), this parameter is used to measure object complexity, namely contour complexity. The more the variation of the contour, the more the shape factor parameter is elevated. The value of *s* is determined as follows

$$s = \frac{P^2}{4\pi A}$$

Where, *P* and *A* are the perimeter and the area of the closed boundary of the particle, respectively.

The approximate shape of the prepared microcapsule can also calculated by the equation [24].

$$S = P^2 / 12.56 \times A$$

Where, *P* and *A* are the perimeter and the area of the closed boundary of the particle, respectively.

3.2 DETERMINATION OF PARTICLE SIZE BY OPTICAL MICROSCOPY METHOD [25]

The size of the prepared microcapsules was measured by the optical microscopy method using a calibrated stage micrometer. (Labomed CX RIII, Ambala, India). Fifty randomly chosen microparticles were taken to measure their individual shape and size. Microparticles were visualized under 10× magnification. Optical microscope was used to determine the size of the particle that lies within a range from 0.2 µm to equal divisions and hence each division is equal to 10 µm and the particles are measured along an arbitrarily chosen fixed line across the center of the particle. The particle size is a factor to be considered important in formulation of microcapsules. Particle size was calculated by using formula [26],

Average particle size = $\varepsilon nd / \varepsilon n$

Where, n= total number of microcapsules.
d=mid point of the size range.

3.3 ESTIMATION OF DRUG CONTENT AND ENCAPSULATION EFFICIENCY IN MICROCAPSULES.

The rifampicin/ isoniazide/ pyrazinamide content in the microcapsules was determined by a digestion method. The ATDs-loaded microcapsules (10 mg) were pulverized and incubated in 10 ml methanol/phosphate buffer (pH = 7.4) phosphate buffer (pH = 7.4) at room temperature for 24 h. The suspension was then centrifuged at 6000 rpm for 30 min. The supernatant was assayed spectro- photometrically for rifampicin/isoniazide/pyrazinamide content at the wavelength of 475 nm/263nm/268nm. Supernatant from the empty microcapsules (without ATDs) were taken as blank. All samples were analyzed in triplicate.

The encapsulation efficiency was calculated using the formula [27],

$$\%\text{Encapsulation Efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}}$$

3.4 THE ENCAPSULATION YIELD (EY) [28]

The EY was calculated as the ratio of the mass of microcapsules obtained at the end of the process and the mass of initial substances added including drug, chitosan and sodium alginate.

$$\%\text{Percentage Yield} = \frac{\text{Weight of microcapsules}}{\text{Total expected weight of drug and polymer}} \times 100$$

3.5 SWELLING STUDIES [29]

The swelling properties of the chitosan–alginate microcapsules were determined in SGF (pH 1.2) and in SIF (pH 1.2). Samples of microparticles of known weight (50 mg) were placed in a glass vial containing 10 ml of swelling solution and allowed to swell at 37^0 C. The swollen beads were periodically removed and weighed. The wet weight of the swollen beads was determined by blotting them with filter paper to remove moisture adhering to the surface, immediately followed by weighing on an electronic balance. All experiments were done in triplicate. The percentage of swelling of the beads was calculated from the formula [30]:

$$\text{Swelling index} = \frac{\text{Final weight of microcapsules } (Wt) - \text{Initial weight of microcapsules } (W0)}{\text{Initial weight of microcapsules (W0)}}$$

3.6 MUCOADHESION TESTING BY IN VITRO WASH-OFF TEST

The mucoadhesive property of the microcapsules was evaluated by an *in vitro* adhesion testing method known as the wash-off method [31]. Freshly excised pieces of intestinal mucosa (2 × 2 cm) from sheep were mounted onto glass slides (3 × 1 inch) with cyanoacrylate glue. Two glass slides were connected with a suitable support. About 50 microcapsules were spread onto each wet rinsed tissue specimen, and immediately thereafter the support was hung onto the arm of a USP tablet disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given a slow, regular up-and-down movement in the test fluid at 37°C contained in a 1 L vessel of the machine. At the end of 30 minutes, at the end of 1 hour, and at hourly intervals up to 12 hours, the machine was stopped and the number of microcapsules still adhering to the tissue was counted. The test was performed at both gastric pH (0.1N HCl, pH 1.2) and intestinal pH (phosphate buffer, pH 7.4).

3.7 IN-VITRO DISSOLUTION STUDIES

The *in vitro* dissolution studies were performed at two different pH values:(i)1.2 pH, i.e., simulated gastric fluid pH and (ii) 7.4 pH, which is simulated intestinal fluid pH. An accurately weighed sample was responded in dissolution media consisting 900 ml of 0.1 N (pH 1.2) HCl by using rotating basket method specified in USP XXIV. 200 µg/ml ascorbic acid was added as an antioxidant [32] to the dissolution media containing rifampicin in order to avoid the decomposition of rifampicin. An amount of microcapsules equivalent to 100mg each of rifampicin, isoniazide and pyrazinamide were taken in the basket. A speed of 75 rpm and temperature of 37±0.5°C was maintained throughout the experiment and the dissolution was done for 2 hours. Microcapsules were then transferred into simulated intestinal fluid pH. (7.4 pH) and dissolution was performed up to 72 hours. At fixed time intervals, aliquots (5ml) of sample was withdrawn and replaced with fresh dissolution media and the withdrawn samples were diluted if required and then estimated for rifampicin/isoniazide/pyrazinamide concentration at 475 nm/263nm/268nm spectrophotometrically (Shimadzu Pharmspec UV-1700 series, Japan) 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.

3.8 DRUG RELEASE PATTERN FROM MICROCAPSULES

In order to understand the mechanism and kinetics of drug release, the results of the in vitro drug release study were fitted with various kinetic equation like zero order (% release *vs* t), first order

(log % release *vs* t)and Higuchi model [33] (Mt/M¥ *vs* t). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Peppas equation [34], Mt/M¥ = ktn, where Mt is the amount of drug released at time t and M¥ is the amount released at time ¥,thus the Mt/ M¥ is the fraction of drug released at time t, k is the kinetic constant and n is the diffusional exponent, a measure of the primary mechanism of drug release. R^2 values were calculated for the linear curves obtained by regression analysis of the above plots.

4. RESULTS AND DISCUSSION

Alginate-based systems are known to work better when used in conjunction with polycationic stabilizers such as chitosan [35].This study describes the formulation of alginate–chitosan microcapsules for the controlled release of ATDs with the aim of reducing the dosing frequency as well as the dose in TB chemotherapy.

In the ionotropic/external gelation method, the alginate network is formed by the reaction with both chitosan and calcium ions, allowing also the diffusion of chitosan molecules into the alginate gel core. The concentration of calcium ions in the chitosan solution during the particle preparation had a large effect on the ability of the microparticles to bind chitosan [36]. The results suggested a higher diffusion rate of chitosan molecules into the alginate core in the presence of calcium chloride concentrations resulting in a higher porosity of alginate core.

The results shown in table 1 demonstrated that the microcapsules obtained under these conditions were found to be spherical and without aggregation. The SEM analysis revealed that the microparticles prepared in this study were spherical with smooth surfaces. Fig. 1 , 2 and 3 show that microcapsules prepared by adding Ca2+, exhibit acceptable sphericity and notable surface porosity, with a shape factor greater than 0.999 in all the cases(Table 1). The "circularity" of particle was within the value between 0 and 1, with 1 representing a perfect circle [37]. This morphology was found independent of the starting composition, provided that Ca^{2+} ions were the gellifying agent. Due to the adhesive properties of chitosan, microcapsules tend to agglomerate [38].

Table 1.Evaluation parameters of various microcapsules

Parameters	**Rifampicin**	**Isoniazide**	**Pyrazinamide**
Particle size(µm±S.D)	832±12	674±15	680±22
Sphericity of microcapsules	Spherical and free flowing	Spherical and free flowing	Spherical and free flowing
Drug content (mg) ±S.D	11.44±0.1 (Theoretical) 9.77± 0.5(Practical)	13.35±0.3 (Theoretical) 8.56± 0.4(Practical)	14.5±0.7 (Theoretical) 10.13± 0.9(Practical)
Encapsulation efficiency(%±S.D)	85.42±0.38	64.17±1.79	69.87±1.27
Percentage yield (%)	55±1.45	60±0.05	68±0.03
Area (mm^2)	15.89±2.33	18.08±4.10	17.34±2.10
Perimeter (mm)	14.13±1.48	15.07±3.21	14.75±2.45
Shape factor	1.040±0.05	1.000±0.04	0.998±0.01
ECD (mm)	4.49±0.24	4.790±0.65	4.699±0.54

Table 2.Results of *in vitro* wash –off test to assess mucoadhesive properties of microcapsules prepared

Percentage of microcapsules adhering to the tissue										
Microcapsules	In 0.1 N HCl, pH 1.2					In phosphate buffer, pH 7.4				
Time(hours)	1	2	4	6	8	1	2	4	6	8
Rifampicin	50 (1.5)	47 (2.0)	43 (1.5)	42 (1.2)	41 (1.0)	50 (1.5)	32 (2.0)	26 (2.0)	24 (2.0)	23 (0.6)
Isoniazide	50 (1.5)	34 (1.4)	30 (0.7)	28 (0.1)	25 (0.3)	50 (1.0)	33 (1.2)	28 (1.9)	23 (0.6)	18 (1.9)
Pyrazinamide	50 (1.0)	40 (0.5)	25 (0.8)	23 (0.5)	22 (0.4)	50 (2.2)	28 (1.1)	21 (1.9)	20 (1.5)	20 (1.5)

†Figs in parentheses are coefficient of variation values.

Table 3.Kinetic values obtained for chitosan-alginate microcapsules containing ATDs in pH 1.2 and 7.4

Microcapsule	Zero order kinetics	First order kinetics	Higuchi equation	Korsemeyer equation
RIFAMPICIN	n=1.3061 r=0.9994	n=0.0178 r=0.9584	n=12.922 r0.9226	n=0.7894 r=0.7649
ISONIAZID	n=1.4520 r=0.9590	n=0.0159 r=0.9773	n=12.582 r0.9693	n=1.0089 r=0.8942
PYRAZINAMIDE	n=1.3958 r=0.9507	n=0.0170 r=0.9699	n=12.107 r=0.9812	n=1.0799 r=0.8583

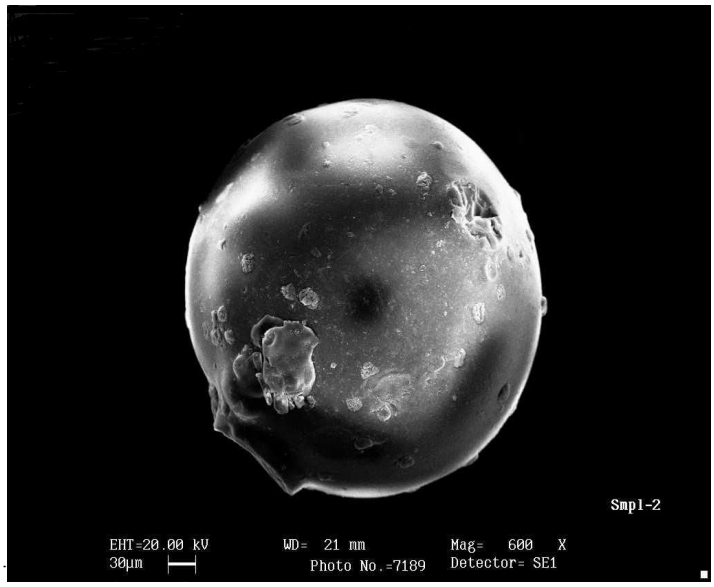

Figure 1. SEM photograph of chitosan alginate microcapsules containing rifampicin

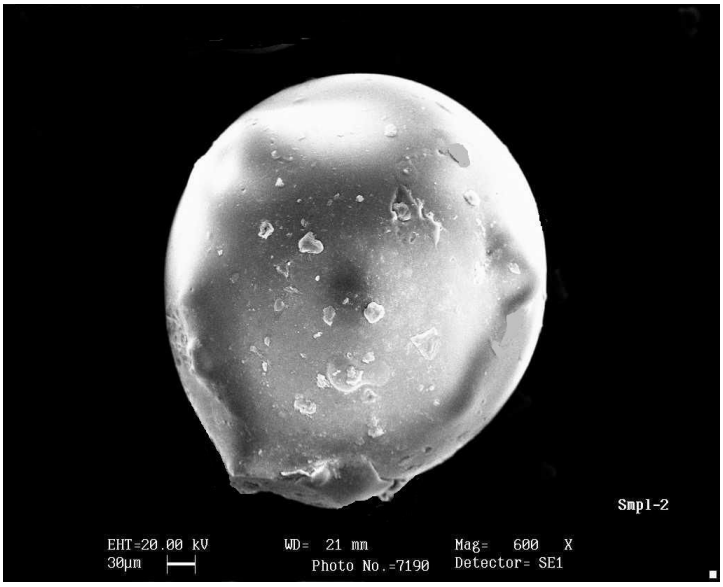

Figure 2. SEM photographs of chitosan alginate microcapsules containing isoniazid.

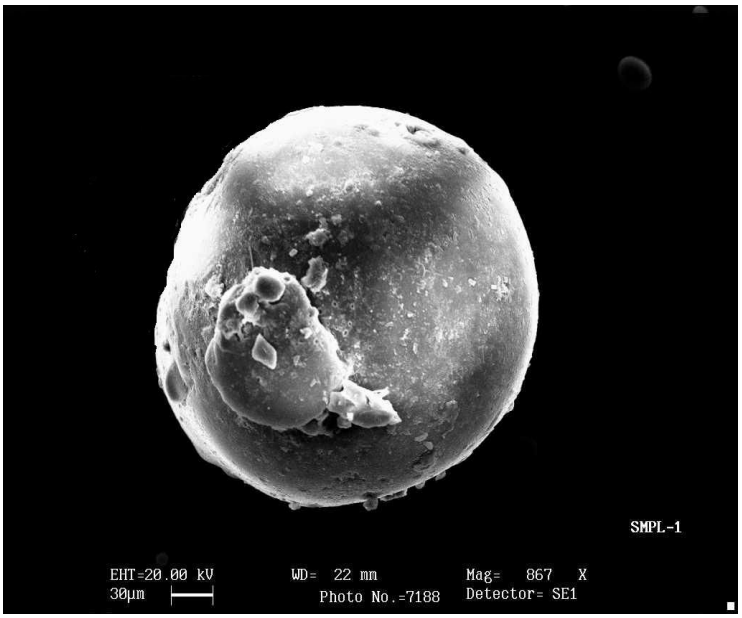

Figure 3.SEM photographs of chitosan alginate microcapsules containing Pyrazinamide

The particle size distribution of all the formulation was presented in fig. 4, and mean average particle size was found to be in the range of 680 to 832µm. Earlier reports also suggested that the size of the beads increase with the use of chitosan in the cross linking fluid [39,40]. The encapsulation efficiency, drug content and percentage yield of all the formulations was to be satisfactory and each formulation demonstrated high values, as summarized in table1. The rifampicin microcapsules showed high encapsulation efficiency than isoniazide and pyrazinamide due to their water insoluble in nature. In the absence of chitosan, entrapment of drugs was very low (values not shown). This may be due to insufficient cross-linking and large pore size permitting the drug to diffuse out during and after gelation. Addition of chitosan to the cross linking fluid resulted in a large increase of the entrapment. This is probably due to more firmness in the alginate–chitosan complex during gelation caused by increased ionic interactions at pH 4.5 between the carboxylate groups in the alginate and the protonated amine groups in the chitosan. As a result less drug is lost during gelation. Results were shown in table 1. Percentage yield of all formulations varied from 55% to 68%.

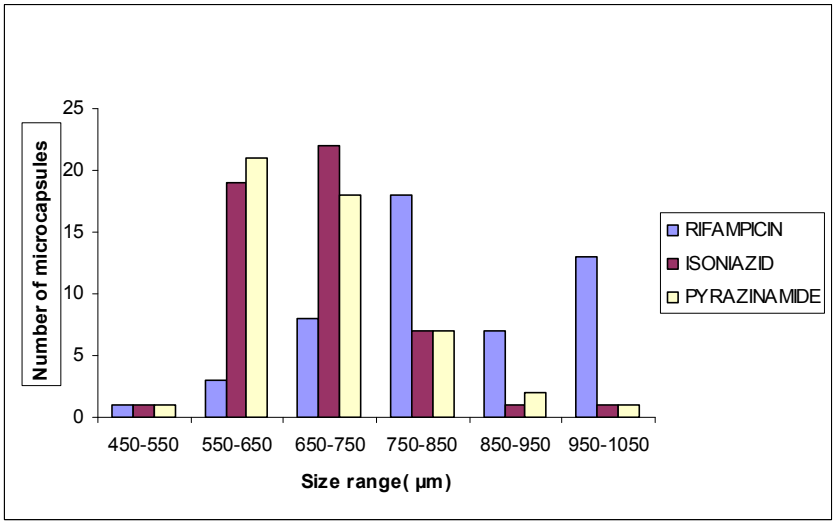

Figure 4. Size analysis of chitosan–alginate microcapsules containing anti tubercular drugs

Swelling studies of the prepared microcapsules are given in fig. 5.The chitosan alginate microcapsules showed swelling index value 1.6 at the end of 6 hours in the pH 1.2 where as the swelling index value was increased to 2.8 at the end of 6 hours in pH 7.4 medium in case of rifampicin microcapsules, where as in isoniazide it was 0.8 in pH 1.2 and 2.4 in pH 7.4, and in the pyrazinamide microcapsules 1.4 in pH 1.2 and 3.6 in pH 7.4. The results showed that swelling ratio of chitosan microcapsules was higher in pH 7.4 than pH 1.2. When ATDs were placed into SGF, the microcapsules swelled slightly, this is due to the fact that, in acidic medium, chitosan in the outer layer gets dissolved out from the microcapsules and swelling index was resulted from the cores made of calcium alginate. It has been suggested that, in acidic medium, the calcium alginate beads were converted to the insoluble alginic acid beads which had a low swelling index [41]. In SIF (pH 7.4) also, the ATDs showed lower swelling indices. This may be due to the less solubility of chitosan (in the outer layer) at higher pH that restricted them from further expansion. Swelling index was high in case of rifampicin than isoniazide and pyrazinamide due to its zwitter ionic nature.

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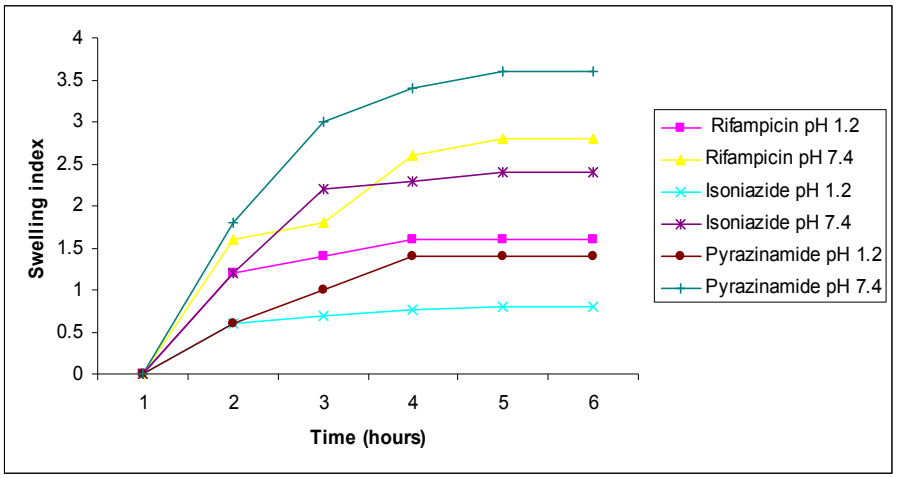

Figure 5. Comparison plots of swelling indices of chitosan-alginate microcapsules containing ATDs in pH 1.2 and pH 7.4.

Results of *In-vitro* mucoadhesion test of prepared microcapsules are given in table 2. Mucoadhesion test was carried out with everted sheep intestinal sac in the presence of pH 1.2 and pH 7.4. Both chitosan molecules and calcium ions are competing which each other at the same time with the negatively charged groups of the alginate molecules and this competition may result in that chitosan molecules are only slightly bound and hence keep their flexibility when the particles are suspended in aqueous milieu. As a result of this, they are able to interact with the mucus chains and show good mucoadhesiveness. This is supported by a study of Huang et al. (2000)[42] demonstrating that the adhesive capabilities of hydrogels can be improved by tethering of long flexible chains to a particle surface. The resulting hydrogels exhibit increased mucoadhesive properties due to enhanced anchoring of the flexible chains with the mucosa.

The wash–off was faster at intestinal pH than at gastric pH. The rapid wash-off observed at intestinal pH was due to ionization of carboxyl and other functional groups in the polymers at this pH. The results of the wash-off test indicated that the microcapsules had fairly good mucoadhesive property.

In-vitro drug release profiles of various formulations of rifampicin-chitosan microcapsules are shown in fig.6. The release of the drug from the microcapsules exhibited a sustained release over a period of 72 hrs. The release rate of microcapsules rifampicin, isoniazide and pyrazinamide were found to be 96.46%, 95.33% and 97.27% respectively where as the release rate for pure drugs was 95.46% (3hrs) for rifampicin, 98.99 (30mins) for isoniazide and 96.44 (30mins) for pyrazinamide. Sustained release of all the formulation may be in acidic medium, calcium alginate matrices are depleted of calcium ions and converted to insoluble alginic acid within 30 minutes without any visible changes in the morphology [43].This may reduce the gel strength favoring drug release by diffusion. In the case of chitosan-coated microcapsules, ATDs need to pass through a second layer of alginate-chitosan complex membrane to be released. Chitosan coating decreases drug release rate, and an incomplete release of ATDs were obtained owing to suppression of erosion of alginate gel microcapsules [44]. No burst effect was observed for all the formulations after changing dissolution media, and only a small fraction of remaining drug was released. One possible explanation is that the aggregation occurring between alginate microcapsules during the coating process may protect more from the pH influence in the alginate network due to a decreased surface area of exposition. The plots of amount of drug released *vs* square root of time were found to be linear in case of isoniazide and pyrazinamide indicating that the drug release mechanism is diffusion controlled. The fraction release (Mt/M¥) at time t is fitted to Peppas equation. In the present systems, rifampicin follows non-fickian transport and zero order kinetics where as isoniazide and pyrazinamide follows super case II transport mechanism and first order kinetics.

5. CONCLUSION

The results of our study clearly indicate that there is great potential in delivery of ATDs as an alternative to the conventional dosage form. However, more sustained drug release can be obtained by coating with lectin polymer. Sodium alginate, chitosan are biocompatible polymers; we expect it to cause no harmful effects if used for prolonged periods.

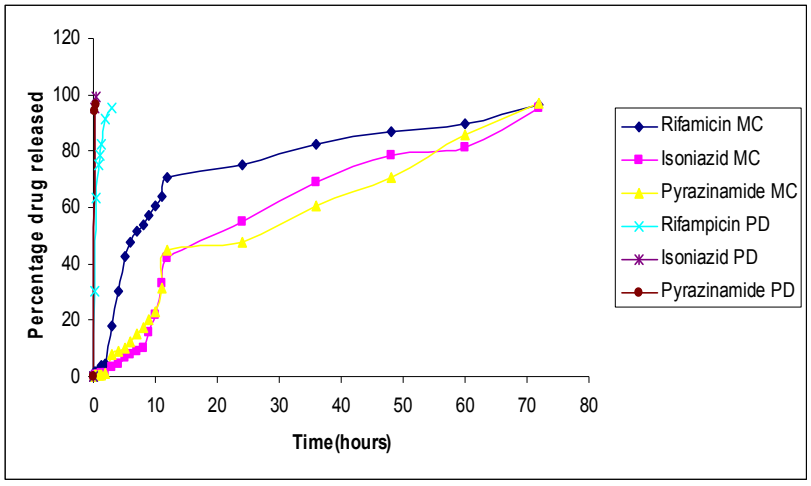

Figure 6. *In vitro* release profile of anti tubercular drugs in simulated gastric fluid for 2 hours and then in simulated intestinal fluid for formulations (MC=microcapsules) and pure drugs (PD)

6. ACKNOWLEDGEMENT

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