INTRODUCTION
Mucoadhesive is a topic of current interest in the design of drug delivery systems to prolong the residence time of the dosage form at the site of application or absorption and to facilitate intimate contact of the dosage form with the underlying absorption surface to improve and enhance the bioavailability of drug [1]. Microparticulate delivery system includes many pellets, beads, microbeads, microspheres, lipospheres etc. Generally these micro particulate delivery systems are intended for oral and topical use [2]. This study describes the formulation and evaluation of gastric-mucoadhesive beads of Ramipril employing various mucoadhesive polymers designed for oral controlled release. Beads are the matrix system containing drug throughout the structure provide better for oral controlled release. The various substances (polymers) used as carriers in microspheres are human serum albumin, bovine albumin, egg albumin, gelatin, waxes, chitosan, sodium alginate, ethyl cellulose etc. Different types of coated particles can be obtained depending on the coating process used. The particles can be embedded within a polymeric or proteomic matrix network in either a solid aggregated state or a molecular
dispersion resulting in the formation of microspheres [3]. The ultimate objective of micro particulate with mucoadhesive delivery system is to control and extend the release of the active ingredient from the coated particles without attempting to modify the normal biofate of the active molecules in the body after administration along with the mucoadhesiveness.

Ramipril is an orally active inhibitor of angiotensin converting enzyme and it is widely used in the treatment of hypertension and congestive cardiac failure. The bioavailability of Ramipril is approximately 28-35 % have relatively short half-life of 2-4 hours and requires frequent administration of dose 5 – 15 mg, 2-3 times daily. Hence it is necessary to develop sustained release formulation to overcome this draw back. Studies showed that, prolonged inhibition of ACE activity of ramipril could be achieved by control release dosage form, using oily matrix formulation filled in gelatin capsules [4]. Because of oily vehicles gastric emptying time was delayed and decreased GI motility, thereby retaining the drug for longer period of time at the site of absorption. Ramipril is freely methanol soluble drug and has site specific absorption from GIT and on other hand, the drug is unstable in the alkaline pH of the intestine, whereas stable in acidic pH and specifically absorbed from stomach. Based on the above reasons there is a clear need to localize the developed formulation at the target area of the GIT.

Preparation of mucoadhesive systems of Ramipril will permit localization of the drug to GI mucosal membrane for a prolonged period of time, due to mucoadhesion. Thus increasing the bioavailability of drug leads to significant reduction in the dose and frequency of administration. Controlling the placement of a drug delivery system in a particular region of the GI tract often improves absorption of those drugs. Furthermore, it would be desirable to achieve a longer transit time, especially in the upper part of the GI tract, in order to maximize drug absorption and thus enhance the therapeutic effect [5].

The purpose of present investigation is to develop calcium-alginate mucoadhesive beads with different mucoadhesive polymers like hydroxy propyl methyl cellulose, carbopol 934P, chitosan and cellulose acetate phthalate with drug Ramipril by orifice ionic gelation process.

MATERIALS AND METHODS
Ramipril a gift sample obtained from Micro Laboratories, Bangalore Sodium alginate obtained from SSFLC, Mumbai, Hydroxy propyl methyl cellulose, Carbopol934P, Cellulose acetate Phthalate, Chitosan from SD fine –chem limited, Mumbai.

Preparation of Drug Encapsulated Beads
Beads containing Ramipril was prepared, by employing sodium alginate in combination with hydroxy propyl methyl cellulose, carbopol 934P, Chitosan and cellulose acetate phthalate. An orifice ionic gelation process was used to prepare large sized alginate beads [6].

Sodium alginate and the mucoadhesive polymer were dissolved in purified water to form a homogeneous polymer solution. The active substance, Ramipril was added to the polymer solution and mixed thoroughly with a stirrer to form a viscous dispersion. The resulting dispersion was then added manually drop wise into calcium chloride (10% Wt/Vol) solution (50 mL) through a syringe with a needle of size no. 18. The added droplets were retained in the calcium chloride solution for 15 minutes to complete the curing reaction and to produce spherical rigid micro beads. The micro beads were collected by decantation, and the product thus separated was washed repeatedly with water and dried at 45°C for 12 hours.

Characterization of Beads
The developed mucoadhesive beads were studied for compatibility studies by FTIR and subjected for various characterizations like Size and Shape analysis, Drug content, Microencapsulation efficiency, In vitro wash off test for mucoadhesion, Stability study.

FTIR Studies
IR spectroscopic studies were carried out for prepared beads, by using Shimadzu FT IR 8700 model to determine the integrity of the drug in the formulation.

Size and shape analysis
Microscopic analysis was performed to determine the average size of micro beads. The micro beads prepared were dispersed in liquid paraffin and a drop of above dispersion was put in to a glass slide and observed under a microscope. The diameter of 100 micro beads was determined using calibrated eyepiece micro meter and stage micro meter. The average diameter was calculated using the following formula.

\[ \text{Average diameter} = \frac{\sum nd}{n} \times c.f \]

Where,
- \( n \) = number of microbeads.
- \( d \) = diameter of the microbeads, C.F = calibration factors

Drug content estimation
Drug content estimation was done by a reported method by El-Kamel et al, 2003. 20 mg of the micro beads were stirred in 3 ml of sodium citrate solution (1 % w/v) until complete dissolution occurs. 1 ml of methanol was added to sodium citrate solution to gel the solubilized calcium alginate and further solubilize Ramipril. This solution was then filtered to obtain drug solution. The filtrate is suitably diluted with 0.1N hydrochloric acid and absorbance was taken at 210 nm [7].

Microencapsulation Efficiency
Microencapsulation efficiency was calculated using the reported formula (Chowdary and Srinivasa, 2003).

\[
\text{Microencapsulation efficiency} = \frac{\text{Estimated % drug content}}{\text{Theoretical % drug content}} \times 100
\]

**In vitro test for mucoadhesion**

The time taken for detachment of beads from sheep stomach mucosa was measured in 0.1N hydrochloric acid (pH 1.2). This was evaluated by an *in vitro* adhesion testing method, known as wash off method. The mucoadhesive property of beads was compared with that of a non-adhesive material, ethylene vinyl acetate beads. A piece of sheep stomach mucosa (2x2 cm) was mounted onto glass slide (3x1 inch) with cyanoacrylate glue and one more glass slide was connected with a support. The beads (50 no) were counted and spread over the wet rinsed tissue specimen and immediately thereafter the support was hung on the arm of a USP tablet disintegrating test machine as shown in photographs in Figure 1. By operating the disintegration machine the tissue specimen was given a slow regular up and down moment.

The slides move up and down in the test fluid at 37 ± 0.50 C. The number of beads adhering to the tissue was counted at 2-hour intervals up to 8 hours [8].

**In Vitro Drug Release Studies**

Dissolution studies were performed for beads containing quantity equivalent to 100 mg of drug filled in capsules by using USP 23 TDT-06T (Electrolab- paddle method) at 50 RPM. The media used were 900 ml of 0.1N hydrochloric acid (pH 1.2), maintained at 37 ± 0.50C. 5 ml of samples were withdrawn at different time intervals and replace with 5 ml of dissolution medium [9-11]. The samples were filtered and assayed spectrophotometrically at 210 nm after appropriate dilutions. Dissolution testing was also performed for 100 mg pure drug.

**RESULTS AND DISCUSSIONS**

Formulation of Ramipril control release gastric mucoadhesive beads was done by using alginate as core coating polymer, as alginate is easily gelled by the addition of Ca+2. An aqueous in-soluble calcium-alginate gel is formed by cation exchange between Na+ and Ca+2. The gelation and cross linking are due to the stacking of the glucoronic acid-Gblocks of alginate chains with the formation of egg-box junction. An orifice-ionic gelation process was used to prepare Ramipril encapsulated beads, employing sodium alginate in combination with four muco adhesive polymers like hydroxy propyl methylcellulose, carbopol 934P, chitosan and cellulose acetate phthalate in 1:1 ratios. Beads were found to be discrete, slight spherical, free flowing, monolithic matrix and had smooth surfaces, an exception of alginate-hydroxy propyl methyl cellulose beads.

The interaction between the drug and various mucoadhesive polymers was studied by using the FTIR spectroscopy wherein infrared spectra of Ramipril and various mucoadhesive polymers was taken individually first and then, were compared with the spectra of the formulations in which the drug was matrixes with various mucoadhesive polymers were taken and compared (Figure 2a to 2e). Which showed prominent peak at 1875.4 Cm-1 because of C = O stretching and vibration due to the presence of carboxylic group. Peak at 1332.8 Cm-1 because of C-N stretching due to the presence of tertiary amine group. Peak at 3434 Cm-1 because of N-H vibration due to the presence of amino-group, Peak at 3463 cm-1 because of O-H stretching due to the presence of hydroxyl group, peak at 1498 cm-1 because of the C-C Stretching due to presence of aromatic group. After comparing all the IR spectra, that there was no significant interaction between the ramipril and various polymers. Sodium alginate 600mg were employed in the preparation of 1:1. The amount of Ramipril taken was kept constant at ratio 1:1 based on the total polymer concentration in all the formulations. It was observed during preparation of beads that 1:1 polymer solution had slight viscosity and excellent spherical droplets were formed. Whereas 1:1 polymer solution was difficulty to retain their spherical form during the process of drying. The beads were sieved using a set of standard sieves with different apertures. The granulometric classes of particles smaller than 100μm were determined by a microscopic method. All beads were found to be uniform in size i.e 600 μm and 710μm for beads of 1:1 ratio respectively, except alginate-carbopol934P beads, which were larger in size and mean size was found to be 710 μm. Shape of beads (Figure 3) was found to be discrete, large, spherical, free flowing, monolithic matrix and had smooth surfaces.

The percentage yield was observed (Table 2) with of alginate and four different mucoadhesive polymer. In case of 1:1 ratio formulations (FA1, FA2, FA3, FA4) its shows, Alginate−hydroxy propyl methyl cellulose beads (FA1) demonstrated highest yield i.e 85.43, 8.10%. Alginate-carbopol 934P beads (FA2) demonstrated highest yield i.e. 77.13, 08.0 %, Alginate-chitosan beads (FA3) demonstrated highest yield i.e 63.03, 3.4%. Alginate-cellulose acetate phthalate beads (FA4) demonstrated highest yield i.e 59.43, 8.05%.

Drug loading was observed good (Table 2) from the alginate-Carbopol 934P beads. This is attributed to the very good inter-polymeric complex formation of alginate and Carbopol 934P. The complex formed between both polymers is produced by electrostatic attraction between amine group of Carbopol 934P and the carboxylic group of alginate. Microencapsulation efficiency was found to be (Table 2) different with alginate different mucoadhesive polymer. This is due to the alginate droplets forms gel spheres instantaneously and entrap the drug in a three dimensional lattice of ionically cross linked alginate.
Alginate the amount of polymer in aqueous solution increases the number of lattice with different mucoadhesive polymer therefore drug loading. In all formulations, alginate with different mucoadhesive polymer. In formulation Carbopol934P– alginate beads showed excellent microencapsulation efficiency was observed with 1:1 ratio beads with 83.44 % efficiency. Mucoadhesive property was studied on beads and all the formulated beads demonstrated good mucoadhesive property compare to non-mucoadhesive polymer (ethylene vinyl acetate). The following stages may have occurred during mucoadhesion.

Initially, an intimate contact i.e (wetting) between the mucus gel and the swelling of mucoadhesive polymer. Which makes the polymer strands to relax, this is followed by the penetration of the mucoadhesive polymer into the mucus gel network and finally the formation of secondary chemical bonds between the mucus and the mucoadhesive polymer. It was observed that the changing the different mucoadhesive polymer it shows the different mucoadhesive properties of beads (Table 3). Mucoadhesion of alginate-carbopol 934p was found to be significantly high, this may be due to significant mucus gel strengthening, which results information of stable mucoadhesive joint. Hence the large force required to detach mucoadhesive dosage form the mucosal surface. Mucoadhesion of alginate-cellulose acetate phthalate beads was found to be poor when compared to alginate carbopol934p; this is due to their low swelling and mucoadhesive capacity.

An in vitro dissolution study was carried out in USP dissolution apparatus by basket method. Since the stomach mucosal pH is between 1 and 3, an acidic medium pH 1.2 was used for the dissolution studies. The release of the drug from the dosage form follows diffusion or erosion mechanisms through the matrix.

Thus as long as there is sufficient drug solubility, these mechanisms control the drug release. The pure drug release was found to be 73.8 percent in first hour of dissolution test and complete drug release of total content of capsule was within 2 hours. The in vitro release studies of drug encapsulated beads in acidic medium are shown in figure 4. The alginate-cellulose acetate phthalate beads demonstrated a drug release of 100 percent in 7 hours for 1:1beads respectively. The release of Ramipril from alginate-chitosan beads was 100 percent in 8 hours for 1:1 beads respectively. The release of Ramipril from alginate-carbopol 934p beads was 99.96 percent in 8 hours for 1:1 beads respectively.

The release of Ramipril from alginate–hydroxy propyl methyl cellulose beads was 98.8 percent in 8 hours for 1:1 beads respectively.

The release pattern of Ramipril from beads of all the formulations is given in Figure 4. A perusal of Figure 4 indicated a slow release of Ramipril from 1:1 alginate-hydroxy propyl methyl cellulose beads at the end of 8 hours. It was observed that only 37.1 percent of drug released from 1:1 ratio beads at the end of 2 hours as compared to pure drug. 100 percent of drug was released at the end of 2 hours. While comparing 1:1 with pure drug the beads showed delayed release property. The results clearly indicate that the rate of drug release decreased with the different mucosal adhesive polymer, because the drug cannot diffuse through the pore of alginate gelmatrix, which has not swollen. The order of increasing release rate observed with beads was alginate cellulose acetate phthalate beads < alginate-chitosan beads < alginate-carbopol 934p beads<alginate-hydroxy propyl methyl cellulose beads in 0.1 N hydrochloric acid.

The precise determination of the mechanism of drug release from the matrix is complex, especially when there is more than one polymer as matrix. The performance of the hydrophilic swelled matrices as a prolonged drug release system is dependent on the hydration properties of the polymers, gel forming properties and relaxation of polymer chains when the fluid gets into the matrix. The release data of Ramipril was processed to understand the linear relationship i.e., kinetic principles. The parameters and equations, given in Table 4 indicated that the release kinetics of Ramipril from the alginate beads followed zero order (R2 value was above 0.99 for all formulations on an average).

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug</th>
<th>Ratio</th>
<th>Alginate</th>
<th>HPMC</th>
<th>Carbopol</th>
<th>Chitosan</th>
<th>CAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA1</td>
<td>200mg</td>
<td>1:1</td>
<td>600mg</td>
<td>600mg</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FA2</td>
<td>200mg</td>
<td>1:1</td>
<td>600mg</td>
<td>-</td>
<td>600mg</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>FA3</td>
<td>200mg</td>
<td>1:1</td>
<td>600mg</td>
<td>-</td>
<td>-</td>
<td>600mg</td>
<td>-</td>
</tr>
<tr>
<td>FA4</td>
<td>200mg</td>
<td>1:1</td>
<td>600mg</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>600mg</td>
</tr>
</tbody>
</table>

Table 2. Characterization, average diameter, drug content, and percentage yield and percentage microencapsulation efficiency

<table>
<thead>
<tr>
<th>SL No</th>
<th>Formulations</th>
<th>Percentage Yield (%)</th>
<th>Average Diameter *(μm)</th>
<th>Drug content * (mg)</th>
<th>Microencapsulation efficiency</th>
<th>Characterization</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FA1</td>
<td>85.43 ±8.10</td>
<td>600 ±05.3</td>
<td>115 ± 0.049</td>
<td>57.50</td>
<td>slight Spherical</td>
</tr>
<tr>
<td>2</td>
<td>FA2</td>
<td>77.13±8.00</td>
<td>710 ±04.6</td>
<td>167 ± 0.023</td>
<td>83.44</td>
<td>Slight Spherical</td>
</tr>
<tr>
<td>3</td>
<td>FA3</td>
<td>63.03 ±3.43</td>
<td>600 ±06.8</td>
<td>129 ±0.055</td>
<td>64.72</td>
<td>Slight Spherical</td>
</tr>
<tr>
<td>4</td>
<td>FA4</td>
<td>59.43 ±.05</td>
<td>600 ±07.2</td>
<td>132±0.062</td>
<td>65.90</td>
<td>Slight Spherical</td>
</tr>
</tbody>
</table>
Table 3. Results of in vitro wash off test in 0.1N hydrochloric acid

<table>
<thead>
<tr>
<th>Beads</th>
<th>Percentage beads adhering to stomach mucosa in hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>FA1</td>
<td>94</td>
</tr>
<tr>
<td>FA2</td>
<td>96</td>
</tr>
<tr>
<td>FA3</td>
<td>78</td>
</tr>
<tr>
<td>FA4</td>
<td>56</td>
</tr>
<tr>
<td>EVA1</td>
<td>46</td>
</tr>
</tbody>
</table>

Table 4. Comparison of order of in vitro release of ramipril from beads in 0.1 N hydrochloric acid

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi / Matrix</th>
<th>Peppas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R²</td>
</tr>
<tr>
<td>FA1</td>
<td>0.9963</td>
<td>0.9834</td>
<td>0.9652</td>
<td>0.9654</td>
</tr>
<tr>
<td>FA2</td>
<td>0.9984</td>
<td>0.9879</td>
<td>0.9848</td>
<td>0.9647</td>
</tr>
<tr>
<td>FA3</td>
<td>0.9959</td>
<td>0.9711</td>
<td>0.9928</td>
<td>0.9812</td>
</tr>
<tr>
<td>FA4</td>
<td>0.9602</td>
<td>0.9245</td>
<td>0.9910</td>
<td>0.9820</td>
</tr>
</tbody>
</table>

Figure 1. In vitro wash off test with beads on stomach membrane

(A)

(B)
CONCLUSION
An Orifice ionic gelation process was employed for the preparation of various alginate beads in 1:1 alginate polymer ratio. The techniques were simple, reproducible and produced beads of slight regular shape and size. The FTIR studies indicated that there was no interaction between the drug and polymer. Among all the formulations, Alginate-hydroxy propyl methyl cellulose beads (1:1) showed the highest percentage of yield. The prepared beads were slight spherical in shape, discrete and free flowing. The size of beads was found to be in range of 600 μm.

Based on the amount of drug loaded in beads, micro encapsulation efficiency was calculated. There was no significant difference among the beads. As the alginate with other polymer there was significant increase in the microencapsulation efficiency in beads was observed. Alginate-carbopol 934p combination exhibited greater mucoadhesion as compared with all other formulations. Ramipril encapsulated beads.

In vitro release studies were carried out in 0.1 N hydrochloric acid (pH 1.2) which indicated that there was a slow and controlled release of drug for all the formulations. Alginate-hydroxy propyl methyl cellulose shows sustained release compared to all other alginate polymer combinations. The order of drug release was found to be zero order for all the formulations. Drug release data was better fit to Higuchi’s diffusion model and the release of drug from all the formulations is diffusion rate limited. The objectives of the present work was achieved i.e., formulation, evaluation and usefulness of sodium alginate mucoadhesive beads of Ramipril with different mucoadhesive polymers. Certainly these findings can be applied for sustained delivery of drugs with mucoadhesion. Further these findings help the industry to scale up the commercial production.

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