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Preparation and characterization of sodium alginate-carbopol-934P based mucoadhesive microbeads

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ABSTRACT

The purpose of the research work was to formulate and evaluate oral sustained release microspheres of clarithromycin for the treatment of Helicobacter pylori. The stomach specific alginate carbopol microbeads were selected in order to improve patient compliance by simplifying its administration, improving its therapeutic effect and reducing its dose related side effects. To eradicate the H. pylori, stomach specific alginate carbopol microbeads were prepared, by orifice ion-gelation process using different ratio of polymers. The microbeads were characterized by particle size, percentage drug entrapment efficiency, % adhering, antibacterial activity in terms of % Growth Inhibition [GI] and stability study. Sizes of microbeads were found in the range of 800-980 µm, SEM photomicrograph showed microbeads with smooth surface and spherical shapes. The percentage drug entrapment was up to 80 %. In vitro drug release studies were performed in phosphate buffer saline [PBS, pH 7.4] and simulated gastric fluid [SGF, pH 1.2] at 37±2^{0}C. In vitro and in vivo mucoadhesive study was performed on albino rats, which showed that microbeads were remained adhered to mucus membrane of stomach for longer period of time. The efficacy of formulation was determined in term of its percentage growth of inhibition on H. pylori. The Stability studies were performed at different storage conditions. The site specific as well as sustained release of antibiotic could decrease the likelihood of significant systemic toxicity and may provide selective delivery of biologically active molecules on the site of action. The developed systems could possibly improve site specific release of drug at bacterial site and selectively kill the micro organism.

Keywords*:* site specific delivery, sodium alginate-carbopol 934 P; microbeads; mucoadhesive; *H. pylori.*

INTRODUCTION

In 1982 Warren and Marshall isolated a gram negative flagellated, spiral, urease producing

microorganism from patient with peptic ulcer. Microorganism known as *Helicobacter pylori*, the main etiological factor in development of gastritis, gastric ulcer and gastric carcinoma[1,2]. *H. pylori* reside mainly in the gastric mucosa or at the interface between the mucus layer and epithelial cells of the antral region of the stomach[3]. Most of the antibacterial agents have low minimum inhibitory concentrations [MIC] against *H. pylori* in culture. However, single antibiotic therapy is not effective for the eradication of *H. pylori* infection *in vivo*. This is because of the low availability of the drug under mucosa, instability of the drug at lower pH of the gastric fluid and short residence time of the drug in stomach. Therefore, combination of more than one antibiotics and anti-secretary agent are required for complete eradication of *H. pylori*[4]. Other than the multi therapy some other approaches were used, one of them was the use of an oligosaccharide, sodium salt of 3'-sialyllactose to inhibit the adhesion of the *H. pylori* to human epithelial cells[5]. A sustained release liquid formulation of ampicillin was prepared using sodium alginate for eradication of *H. pylori*[6]. The alginate formulation gelled in the stomach and released the drug locally on infection site. A combination of the metronidazole, amoxicillin and bismuth subnitrate for eradication of *H. pylori* was used[7]. The non antibiotic therapies were also able to inhibit the growth of *H. pylori in vitro* as well as *in vivo.* However, one of the best method to eradicate the *H. pylori* is to provide the drug locally in stomach[4,8]. Better stability and longer residence time will allow more antibiotic to penetrate through the gastric mucus layer to act on the *H. pylori*. Hence in order to develop stomach specific formulation of clarithromycin for eradication of *H. pylori*, drug containing alginate-carbopol microbeads were prepared.

Alginates are random anionic, linear, polymers consisting of varying ratio of glucuronic and manuronic acid unit. Salts of alginate are formed when metal ion react with glucuronic or manuronic acid residue. Alginate has been used in many biomedical applications, including drug delivery systems, as they are biodegradable, biocompatible and mucoadhesive. These delivery systems are formed when they are in monovalent, water soluble, alginate salts under goes an aqueous sol-gel transformation to water-insoluble salts due to the addition of divalent ions such as calcium, strontium and barium[9]. Mainly calcium alginate matrix is used for drug delivery system including beads, gel, films, microparticles and sponges[10,11,12,13]. Calcium ions have unequal affinity for glucuronic and manuronic acid unit of alginate. As a result, calcium ions initially react with repeating unit of glucuronic acid unit to form egg-box gel shaped structure that stack upon each other [10,11,12,13,14]. Additional calcium ions then interact with untreated glucuronic and manuronic acid unit to form a calcium alginate complex [15]. Alginates with a high glucuronic acid contents form more rigid, porous gel due to their orientation with in the egg- box structure and conversely gel with low glucuronic content are more randomly packed and less porous [16].

Carbomers are synthetic high-molecular-weight polymers of acrylic acid that are crosslinked with either allyl sucrose or allyl ethers of pentaerythritol. They contain between 56% and 68% of carboxylic acid [COOH] groups calculated on the dry basis. The BP 2004 and PhEur 2005 have a single monograph describing carbomer; the USPNF 23 contains several monographs describing individual carbomer grades that vary in aqueous viscosity and in labeling for oral or non-oral use.

Carbopol are also called as carboxy poly methylene, poly acrylic acid. They are high molecular weight polymers of acrylic acid that are cross-linked with either allyl sucrose or allyl ethers of penta erythritol. They contain 56% and 68%of carboxylic acid groups calculated on the dry bases[17,18,19]. They are used as suspending agent or viscosity increasing agent, dry and wet binder and as rate controlling agent in tablets and enzyme inhibitor of intestinal protease in peptide containing dosage form etc.

The carbomer resins have also been investigated in the preparation of sustained-release matrix beads[20] as a bioadhesive for a cervical patch[21] and for intranasally administered microspheres[22] in oral mucoadhesive controlled drug delivery systems [23,24].

The purpose of this study was to investigate [i] the effect of different ratio of the polymer used, on the % entrapment of drug, size of microbeads, % yield of microbeads, [ii] *in vitro* and *in vivo* drug release profile, [iii] *in vitro* and *in vivo* mucoadhesion study of microbeads, [iv] *in vitro* cell-line anti bacterial interaction of microbeads on *H. pylori* bacteria and [v] stability study of the formulation.

MATERIALS AND METHODS

2.1. Materials

Clarithromycin was obtained as gift sample from IND SWIFT Ltd. [Panchkula, India]. Sodium alginate was obtained from Loba Chemie Pvt Ltd. [Mumbai, India], Carbopol 394P [0.2 % solution having viscosity 2050- 5450cps] from CDH, [Delhi, India], Calcium chloride di-hydrate from E. Merck [India Limited, Mumbai, India] was purchased. Other used reagents were of laboratory grade.

2.2. Method

2.2.1. Preparation of microbeads

The mucoadhesive microbeads of sodium alginate-carbopol 934P were prepared by orifice-ionic gelation process, with some modification, which has been extensively used to prepare the large size alginate-carbopol beads. In brief: -

The quantity of the both polymers as shown in the table-1 were taken and dissolved in the purified water to make 2% solution of homogenized polymer mixture. The drug was added to polymer solution as per table-1, mixed well to get smooth viscous dispersion. The smooth dispersion was added to 40 ml of calcium chloride[10%w/v] solution, drop wise through needle size #21, which was retained in calcium chloride solution for 20 minutes for complete curing reaction and to produce the spherical rigid beads. The micro beads were washed with purified water twice. The collected beads were dried at room temperature for 24 hours and collected.

2.2.2. Size determination of microbeads

Particle size was determined by using a laser diffraction particle size analyzer [Cilas 1064L, Orleans, France]. Microbeads were suspended in the chamber of the particle size analyzer containing distilled water, and the particle size was determined using the software provided by the manufacturer.

2.2.3. Determination of % yield of microbeads

The % yield of different formulations of micro beads has been calculated by using the given formula [Table1].

$$\%\text{Yield of micro beads} = \frac{\text{Total yield of micro beads}}{\text{Total wt. of polymers +drug}} \times 100$$

2.2.4. Drug entrapment efficiency

It is the quantity of the drug retained by the system. There are two types of methods to calculate this, one is direct method and other is indirect. Here the direct method was used to calculate the entrapment efficiency. The calculated amount of the microbeads were crushed and digested with acetone for 2 hours with continuously shaking. Fixed amount of above solution was taken and after making proper dilution with SGF [pH-1.2], assayed spectrophotometrically. The % entrapped drug was calculated by using following formula:

$$\%\text{ entrapped drug} = \frac{\text{Total amount of drug found in formulation}}{\text{Total amount of drug used in formulation}} \times 100$$

2.2.5. *In vitro* drug release studies

Different formulations prepared by changing the drug polymer ratio were subjected to *in vitro* drug release study for clarithromycin in PBS [pH-7.4] and SGF [pH-1.2] solutions, respectively. Drug release from various mucoadhesive microbeads formulations was performed in different mediums [i] SGF [pH-1.2] [ii] PBS [pH-7.4]. These studies show the effect of different fluid environment of the body on the drug release pattern from prepared microbeads. The release of drug was determined by using the USP XXII basket method, containing 900 ml of SGF [pH-1.2] and the microbeads containing clarithromycin equivalent to 100 mg was kept in basket of dissolution apparatus. This basket was rotated at the speed of 50 rpm and the temperature of medium was maintained at 37 ± 2^0C. Perfect sink conditions were maintained during the drug dissolution testing, 5 ml of sample was withdrawn at suitable time interval of 1 hour. The dissolution medium was replaced with same amount of SGF [pH-1.2] solution. The sample withdrawn was assayed for drug using UV/visible spectrophotometer after making proper dilutions., Similarly, above procedure was applied on other formulations, which were prepared by changing the polymers ratio. The above procedure was similarly repeated with PBS [pH-7.4] as dissolution media. The percent release of drug was determined and the cumulative % drug release was plotted against the time.

2.2.6. *In vitro* mucoadhesive potential of sodium alginate-carbopol 934P microbeads

This study was performed to check the mucoadhesive property of drug loaded microbeads so that it can release the drug locally where it may bound and retained in stomach for the extended time period of time. Mucoadhesive study was carried out as per procedure followed by Yasunori Miyazaki et al.[25] with some modification. For this study male albino rat of weight 300-350 gm were taken, anaesthetized by using chloroform. The stomach was removed from animal and flushed with PBS pH-7.4 solution properly. The stomach was cut longitudinally and mounted around the glass rod, exposing the mucus layer out ward. It was again flushed with PBS pH-7.4 solution and a counted number of microbeads were spread uniformly to the mucus surface. This rod was then hanged in the beaker in proper manner [containing 50 ml of the PBS [pH -7.4]

solution], the mucus/ tissue remains 1cm above from the bottom of beaker, but whole tissue should be remain in PBS [pH-7.4] solution. This was placed in orbital shaker incubator and horizontally shaking was made at rate of 50-60 rpm at temp 37° ± 2°C. After the specified time [1 hour], tissue was placed in other beaker containing the similar quantity of PBS [pH-7.4] solution. In the first beaker the unbound microbeads were counted. This procedure was carried out for 9 hours. From the unbound microbeads the number of adhered particles was calculated and adhering % was calculated after each interval of time, where N_t is the no. of un-adhered particles.

Formula:

$$\text{Adhering \%} = \frac{100 - \sum_{t=0} N_t}{100} \times 100$$

2.2.7 *In vivo* mucoadhesive potential of sodium alginate-carbopol 934P microbeads

In vivo mucoadhesion test was performed on male albino rats weighing 300-350 gm and the rats were fasted for 24 hours before experiments. The one hundred microbeads were orally administered with the help of 0.2ml water using polyethylene tube attached to syringe. After one hour the first group of rats was sacrificed with chloroform, stomach was removed, cut longitudinally and opened. The microbeads were counted and adhering percent was concluded. Similar procedure was followed at interval of two hours and experiment was carried out for 7 hours, with remaining group of rats.

2.2.8. *In vitro* antibacterial activity of formulations

To study the effect of microbeads of clarithromycin on pure *H. pylori* strain, 5 ml of Brucella broth was inoculated with 10µl of stock culture of *H. pylori* with the help of micro pipette and appropriate amount of the formulation was added into the culture vials containing the *H. pylori* strain. All vials were incubated in the candle jar under micro-aerophilic conditions with humidity at 37^0C. After incubation of all vials for a stated period of time, the growth inhibition was determined for each time interval using the UV/visible spectrophotometer.

Growth inhibition was defined as the ratio of optical density of the given test mixture against that of the culture vial containing the *H. pylori* alone and % growth inhibition [GI] was determined for each time interval, using the following formula:

$$\%GI = \frac{\text{OD of test organism at a particular time interval} - \text{OD of test mixture at the same time interval}}{\text{OD of test organism at a particular time interval}} \times 100$$

2.2.9. *In vitro* stability study

The stability study was performed on the optimized formulation. For this microbeads containing equivalent to 50 mg of drug were taken and stored in amber colour bottles at different temperature [4-8^0, RT, 45^0 and 60^0C]. After 10 days, microbeads [SACM-4] containing equivalent to 10 mg of drug were taken from different temperature conditions, crushed and placed with acetone for digestion for 2 hours with shaking. After making proper dilutions the samples were analysed for drug content. For calculations, the initial amount present in the formulation was assumed as 100% before placing them at different temperature.

In the similar way the formulation was again analyzed for the drug content placed at different temperature after 20 and 30 days interval.

RESULT AND DISCUSSION

The surface morphology was examined by SEM. SEM micrograph of alginate-carbopol 934P microbeads showed that microbeads have a spherical shape [Fig.1]. It was found that on increasing the ratio of the carbopol to alginate, % entrapment of drug increases. It was maximum [78%] with SACM-4 and minimum [68.2%] with SACM-1. This may be due to increased concentration of carbopol in microbeads, which imparts increase in the viscosity to the homogenous gel mixture. Thus increase in viscosity is responsible for increase in entrapment of drug.

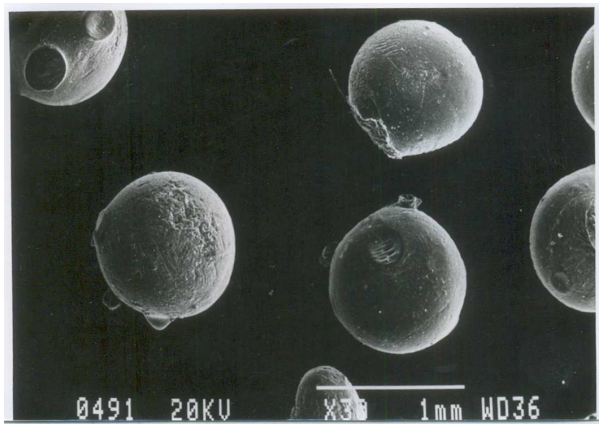

Figure 1. SEM micrograph of alginate-carbopol 934P microbeads

Table 1 Formula for preparation of microbeads

Sr. No.	Formulation Code	Sodium Alginate : Carbopol-934P ratio	Amount of drug	% Yield of microbeads	% Entrapment of drug in microbeads	Average size of microbeads (µm
1.	SACM-1	7:1	200 mg	94.5±2.6	68.2±2.5	980±3.2
3.	SACM-2	5:1	200 mg	93.1±2.5	69.5 ±2.7	953±2.2
5.	SACM-3	3:1	200 mg	93.7±3.0	72.3±1.6	925±1.7
7.	SACM-4	1:1	200 mg	96.7±1.7	78.0±2.1	890±2.8

SACM-1= microbeads containing Sodium alginate: Carbopol-934 (7:1 w/w, ratio)
SACM-2= microbeads containing Sodium alginate: Carbopol-934 (5:1 w/w, ratio)
SACM-3= microbeads containing Sodium alginate: Carbopol-934 (3:1 w/w, ratio)
SACM-4 =microbeads containing Sodium alginate: Carbopol-934 (1:1 w/w, ratio)

The increase in size of microbeads observed with decrease of alginate ratio or increase in the ratio of carbopol in polymer mixture for preparation of microbeads. On increasing the ratio of carbopol, the viscosity of mixture increases. Increase in viscosity will be responsible for formation of the big droplet of the beads, higher viscous droplet take more time to fall down from orifice and this will also contain more mass then less viscous gel mixture. So this is responsible for increase in size of microbeads. A less increase in yield of microbeads was observed with increase of the carbopol ratio with respect to alginate in different formulations [Table 1].

In vitro drug releases profile was observed for all four formulations in PBS [pH-7.4] and SGF [pH-1.2] medium. In SGF [pH-1.2] medium formulation SACM1 showed 56.3% release in 5 hours, where the formulation SACM4 showed 59.4 % of release of clarithromycin. After 10 hours the release was found to be maximum 95.7% and minimum 70.8 % for SACM4 and SACM2 formulation, respectively. After 24 hours almost all the drug content had been released [Fig 2].

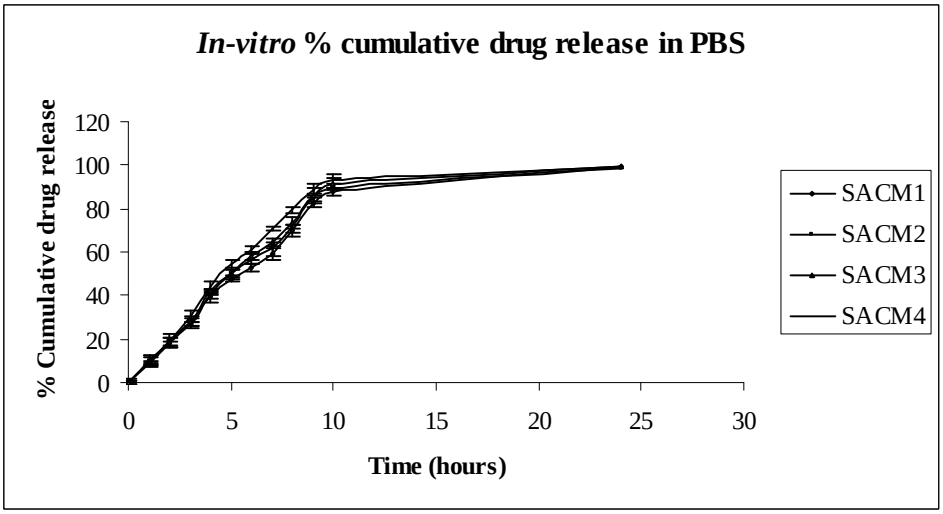

Figure 2. *In vitro* cumulative %drug release in PBS (pH-7.4)
SACM-1= microbeads containing Sodium alginate: Carbopol-934 (7:1 w/w, ratio)
SACM-2= microbeads containing Sodium alginate: Carbopol-934 (5:1 w/w, ratio)
SACM-3= microbeads containing Sodium alginate: Carbopol-934 (3:1 w/w, ratio)
SACM-4 =microbeads containing Sodium alginate: Carbopol-934 (1:1 w/w, ratio)

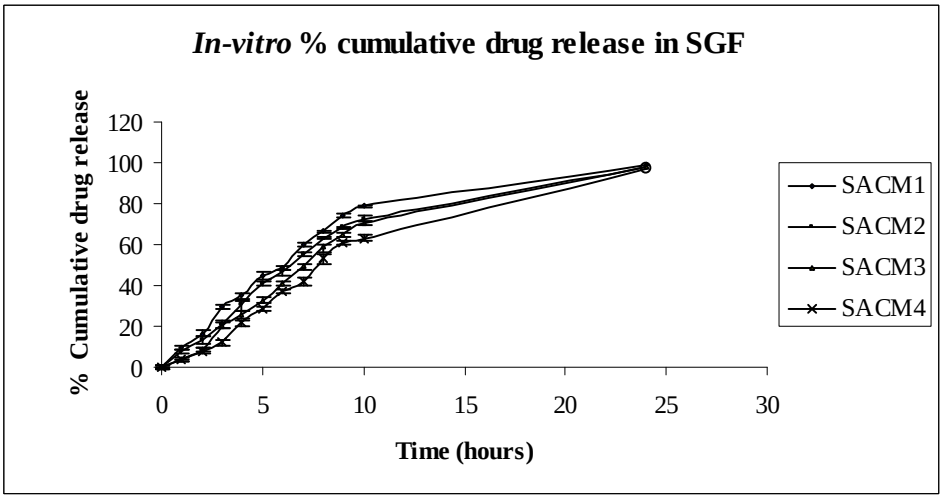

Figure 3. *In vitro* cumulative %drug release in SGF (pH-1.2)
SACM-1= microbeads containing Sodium alginate: Carbopol-934 (7:1 w/w, ratio)
SACM-2= microbeads containing Sodium alginate: Carbopol-934 (5:1 w/w, ratio)
SACM-3= microbeads containing Sodium alginate: Carbopol-934 (3:1 w/w, ratio)
SACM-4 =microbeads containing Sodium alginate: Carbopol-934 (1:1 w/w, ratio)

Similarly in case of PBS [pH-7.4] medium, the drug release was 47.8, 49.8, 49.8 and 54.3% after 5 hours for SACM1, SACM2, SACM3 and SACM4 formulation, respectively. After 10 hours drug release was 87.5, 89.8, 91.5 and 93.4% for formulations SACM1, SACM2, SACM3 and SACM4, respectively. After 24 hours almost drug was found to be released [Fig. 3].

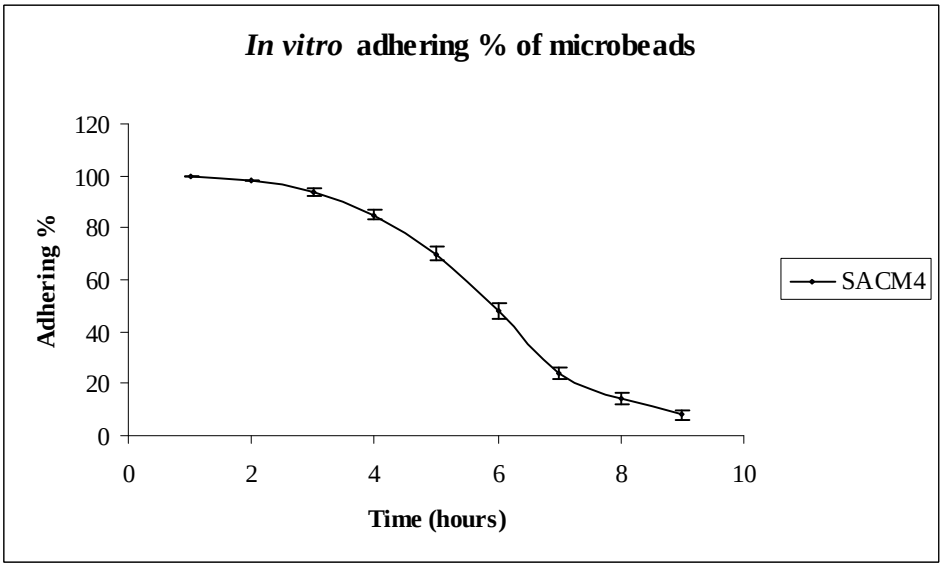

Figure 4. *In vitro* study of mucoadhesion
SACM-4 = microbeads containing Sodium alginate: Carbopol-934 (1:1 w/w, ratio)

Initially more release rate was also observed in SGF [pH-1.2] medium than PBS [pH-7.4], because of more solubility of drug in acidic medium than in basic medium. The drug, which is present in outer region of the matrix, get dissolved fast and released immediately. After 4-5 hours it was found that the release rate of drug was decreased from all formulations, this may be due to swelling of outer part of the matrix which made outer layer of matrix compact/tight and inhibits the rate of diffusion of drug out side from matrix. During this period, in SGF, the decrease in release rate was less then that of PBS, because of less swelling of alginate matrix in acidic medium then basic except SACM2 and SACM3 in SGF medium. After 7-8 hours, again increase in cumulative% drug release was observed. This may be due to more swelling and bursting/breakdown of the spherical matrix containing drug and release of the remaining amount of the drug from matrix. [Fig 2, 3]. *In vitro* mucoadhesive property of microbeads was studied and observed that 50% of microbeads were remain adhered with mucous membrane for about 5 hours and after 9 hours about 8% [Fig.4].

This showed that formulation met our requirement for releasing the drug in stomach for extended period of time and may give the positive effect in eradication of *H. pylori* bacteria. *In vivo* mucoadhesive property of microbeads was studied and observed about 72% were remained adhered with mucus membrane of the stomach even after 5 hours, but after 7 hours it was observed only 37% as shown in [Fig.5]. *In vivo* study showed that the microbeads containing drug might provide it locally for extended period of time to eradicate the *H. pylori* microorganism from stomach.

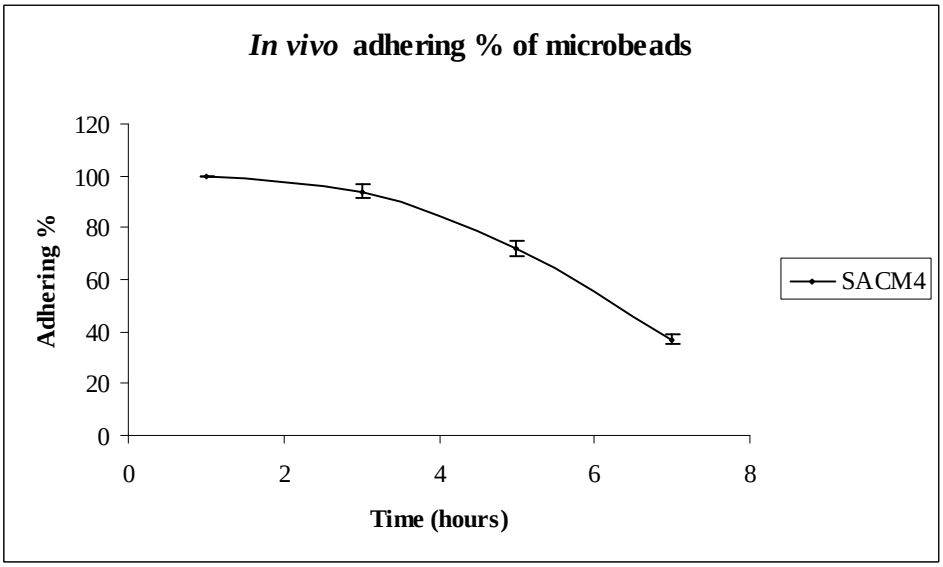

Figure 5. *In vivo* study for mucoadhesion
SACM-4 = microbeads containing Sodium alginate: Carbopol-934 (1:1 w/w, ratio)

In vitro antibacterial activity was performed on isolated culture of *H. pylori*. From this study % growth inhibition was calculated and it was observed that the pure drug shows more effectiveness then the formulation up to 3-4 hours only. After that the effectiveness of the drug entrapped formulation increases than pure drug for remaining time period against bacteria, which is attributed to the sustained release property of formulation, which makes enable it to deliver the antibiotic to deliver at infected site for longer period of time. The local availability of antibiotic at infected area could eradicate the more number of bacteria from gut and improves the patient's compliance as compare to the marketed formulations [Fig.6].

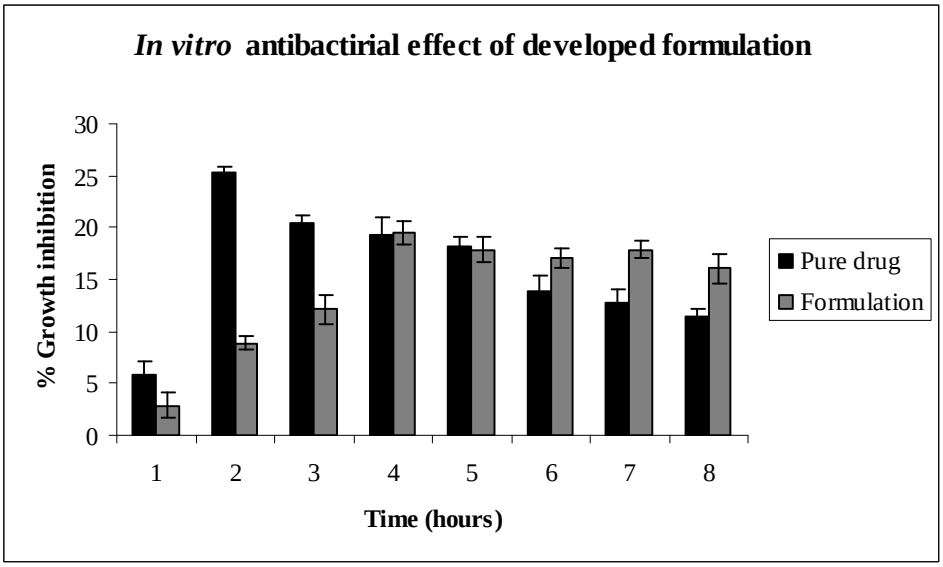

Figure 6. *In vitro* % growth inhibition effect of developed formulation on bacteria

In vitro stability study of the formulation was performed by placing the optimized formulation at different temperature conditions and observation was made at different time intervals. It has been found that formulation placed at 4°-8^{0}C shows no degradation after even after 30 days when compared with standard. At room temperatures after 30 days only 2.7% degradation was observed while formulation placed at 60^{0}C after 20 and 30 days showing 4.7, 6.4 and 6.9, 17.7% decrease in drug concentration. More stability of the formulation was observed at the lower temperature [Table 2].

CONCLUSION

In the present study, we investigated the potential of alginate-carbopol microbeads for the specific delivery of clarithromycin to the infection site. The developed system could be able to deliver the drug for longer period of time at stomach which will eradicate the organism from infected site. Consequently system showed great promise in eradication of *H. pylori* infection *in vitro* antibacterial activity of formulation .

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