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ORIGINAL ARTICLE

Mucoadhesive Gastroretentive Amoxicillin Microspheres Development using Spray Drying Technique

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ABSTRACT

Current research focused on the designing of mucoadhesive microspheres containing Amoxicillin by spray drying method using Caesalpinia pulcherrima galactomannan (CPG) and sodium alginate for H. pylori treatment. Spray dried mucoadhesive microspheres were developed using 2^3 factorial designs with amoxicillin concentration, polymer mixture containing sodium alginate with CPG and Flow rate as variables. Various parameters were applied for the evaluation of developed formulation like morphology-size, DSC, in vitro mucoadhesion, FTIR, XRD, degree of swelling, entrapment efficiency, release, in vitro H. pylori growth inhibition and in vitro drug studies. With rise in polymer content, mucoadhesion increases for the developed formulation. Percentage yield of developed formulations was detected in between 15% to 50.32%, 53.07% to 89.43% of entrapment efficiency, degree of swelling in range of 0.638 to 1.363. Developed mucoadhesive amoxicillin microspheres prolonged gastric retention with drug release and recorded better H. pylori eradication as compared to plain drug which escalate bioavailability.

Keywords: *Caesalpinia pulcherrima galactomannan, Amoxicillin trihydrate and sodium alginate, H. pylori, mucoadhesive microspheres, spray drying*

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INTORDUCTION

Amoxicillin is orally absorbed semi-synthetic antibiotic commonly used conventional treatment for stomach and duodenal ulcers brought on by H. pylori infection in combination of proton pump inhibitors and second antibiotics as triple therapies which are active clinically. Whereas thorough inhibition of *H. pylori* cannot takes place with such triple therapies as its diminutive retention of dosage form at low pH, less penetration of antimicrobials in mucosal lining at *H. pylori* location, Amoxicillin deteoriation at stomach pH. In order to treat *H. pylori*, amoxicillin serves as a model medicine. Due to its acid solubility, it is challenging to regulate its medication release for prolonged duration at gastric pH[1-3].

Treatment of *H .pylori* is not completely clearance. One of the most cause of *H. pylori's* partial eradication is minimum duration for residence of formulation at gastric pH. Hence required concentration of antimicrobial agent con not reached gastric mucous layer where *H. pylori* exits. Another reason the

amoxicillin is degradation in the gastric acid at stomach pH. Delivering drug for longer duration of time at infection location is one of most approaches for improvement in efficacy of antibiotic therapy[4].
Mucoadhesive drug delivery systems have wide acceptance due to its site-specificity for systemic and local drug delivery, this approaches is beneficial for eradication of *H.pylori* infection. Polymer and gastric mucosa exhibits strong interaction due to mucoadhesion leads to enhancement of contact time, local effectiveness, prolongs drug absorption. Effective absorption and better drug bioavailability are the prime advantages of mucoadhesive microspheres increases the contact with tissue and dosage forms. The study of spray dried natural polysaccharides microspheres development is right by required outcome obtained in the dosage forms for clearance of *H. pylori*[5-7].
H. pylori can be treated by using amoxicillin as a model drug and its gastric solubility made difficult to regulate drug release for longer period of time at gastric pH[8]. In the current research, *Caesalpinia pulcherrima* galactomannan (CPG)and sodium alginate were applied to develop spray dried microspheres. Finally, to achieve drug release in controlled manner, the developed formulation was characterized by using evaluation factors such as yield, micromeritic properties, *in vitro* drug release, scanning electron microscopy (SEM), entrapment efficiency, thermal properties, swelling behavior, *in vitro H. Pylori* growth inhibition study and *in vitro* mucoadhesive properties of particles.

MATERAL AND METHODS

Materials

Cachet Pharmaceutical Pvt. Ltd., Rajasthan, India provided gift sample of amoxicillin trihydrate.CPG wasisolated in MET's Institute of Pharmacy, Bhujbal Knowledge City,Nashik. All other chemicals and reagents are of analytical grade.

Methodology for formulation development

Using spray-drying technology, microspheres were fabricated using combination of sodium alginate with CPG and drug for $2^3$for factorial design[9]. Accurately weighed sodiumalginate were dissolved in distilled water and heated at 40˚C. Polymer were allowed to swell for 30minute and homogeneous polymer solution was formed using mechanical stirrer. Drug (Amoxicillin) was mixed into the polymeric solution and volume was made up 200 mL. The resulting mixture was spray dried for microsphere development using a LU-222 ADVANCED lab spray drier (Labultima, India) with the process criterias viz. outlet temperature 120°C, inlet temperature160°C, spray pressure 2kg/cm, pump set 2 and 4 mL/min [2].

Design of Experiment (DOE)

Factorial design experiments

Current research applied 2^3 randomized reduced factorial design where three components were investigated at 2 levels to find out how the selected variables interacted. [10-11]. Variables chosen as amoxicillin concentration (X1), proportion of polymers such as sodium alginate with CPG(X2) and feed rate as (X_3) at two diverse levels. Actual values are mentioned in Table 1 and the experimental design was coded.

Table 1: Experimental variables of factorial design with their coded levels and actual values

Batch	drug (X1)	Polymer		Feed rate (X3)	Yield (%)	Entrapment Efficiency (%)	Particle Size(µm)	Muco adhesion (%)	Degree of Swelling (a)
		SA	CP (X2)						
B1	700	500	250	2	30.00	75.28 ±03.24	3.8	73.16±1.67	0.966±0.016
B2	900	500	250	2	36.20	53.07 ±1.29	4.2	75.28±0.67	0.735± 0.32
B3	700	500	500	2	16.72	53.91 ±0.99	9.5	68.86±2.56	0.638 ±0.14
B4	900	500	500	2	35.28	87.18 ±03.5	8.7	71.32±3.46	0.872 ±0.042
B5	700	500	250	4	25.12	66.13 ±1.23	6.4	81.85±1.78	1.237±0.063
B6	900	500	250	4	50.32	54.12 ±0.46	5.6	90.69±0.23	1.146± 0.038
B7	700	500	500	4	28.50	89.43 ±1.78	3.4	91.14±0.56	1.363±0.42
B8	900	500	500	4	15.00	67.35±02.84	7.5	89.25±1.43	1.273±0.042

Characterization of microspheres

Morphological examination

Microspheres surface characteristics were examined using SEM (Scanning electron microscopy). Little amount of powder sample was mounted on aluminum foil and inserted in the SEM chamber after gold sputtering (TEOL Model JSM-6390LV)[12]. SEM images were captured at electron beam acceleration voltages of 20 kV.

Yield and entrapment efficiency

For determination of entrapment efficiency, hundred milligram microspheres precisely weighed and crushed in a glass mortar, followed by subjecting powder in 0.1 N HCl (10 mL of pH 1.2). Drug content

was tested after 24 hours, from filtrate UV spectrophotometerically at the maximum wavelength of 228 nm (Shimadzu, Japan). As per the findings, microspheres containing desired dose were selected for dissolution study [13-14].

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100 \quad \text{---(1)}$$

Differential scanning calorimetry (DSC) analysis

DSC study of API, sodium alginate, CPG along with the two polymers was carried out separately using Pyris Diamond TG/DTA Thermogravimetric /Differential Analyzer (PLACE) to evaluate any possible drugpolymer interaction at the level. The proportion of drug and polymer chosen was same as that in the final formulation. Sealed aluminium pans containing powder was subjected for heating in at a rate of 10 ºC/min from 60 ºC to 400 ºC under nitrogen stream of 20 mL/min. DSC curves modifications were evaluated both with the positions of maxima and minima[15-16].

Powder X-ray Diffraction

The X-ray powder diffraction pattern was obtained with a Bruker AXS Advance Diffractometer in between 2 and 90 ° the 2-range utilising Cu Ka radiation with a Ni filter; 1.5406 Aº (40 kV; 30 mA and 1° min_1 scanning rate). Ni-filtered radiation OF Cu Ka was used to measure data of single crystal on a Nonius CAD4 automatic four-circle diffractometer (λ=1.5406 Aº). A least-squares fit of 50 centred reflections (2 θ range: 22-32°) produced the unit cell dimensions. PXRD was studied at SAIF Department, Cochin[17-18].

Surface characterization of microspheres by scanning electron microscopy (SEM)

Formulation batch which exhibited suitable steadiness between the buoyancy and the entrapment efficiency were subjected for surface morphology using scanning electron microscope. SEM study was studiedat SAIF Department, Cochin (TEOL Model JSM-6390LV). SEM micrographs were collected while the microspheres were held on the sample holder. [19].

***In vitro* mucoadhesiveness of microspheres**

Developed formulations were characterized for mucoadhesion properties by *in vitro* wash-off test. Freshly sliced goat stomach (2 cm^2) were rinsed with physiological saline solution and was fixed on glass support with thread. One hundred mg microspheres of were spread over rinsed wet tissue specimen was then positioned at room temperature environment-controlled compartment at 90% RH. After 20 minutes, tissues were removed and positioned at a 45-degree angle. 0.1 N HCL (pH 1.2) solution was used for rinsing of stomach with 22 mL/minutes flow rate. After 5 min. administration of formulations, spectrophotometrically active pharmaceutical ingredient concentration from collected aliquot was studied [20]. Amount of formulation equal to API concentration in perfusate was computed. The proportion of microspheres that are attached was determined by following equation:

$$\% \text{ Mucoadhesion} = \frac{\text{Amount of drug in washout liquid}}{\text{Actual drug concentration in applied microsphere}} \times 100 \quad \text{---(2)}$$

Swelling study

Microspheres (50 mg) was kept at 37±0.5°C in with intermittent shaking in 10 mL of distilled water in a glass vial. Microspheres were withdrawn at regular intervals after 3 hrs and calculated the weighed microspheres[21]. Swelling ratio was computed using formula as follows:

$$\% \text{ Swelling ratio} = \frac{We - Wo}{Wo} \quad \text{---(3)}$$

Where, Wo= dried microspheres initial weight

We = swollen microspheres weight

***In vitro* dissolution study**

USP Type II dissolution test apparatus (Electrolab, TDP-06PMumbai, India) was studied for detection of drug release in 900 mL medium. Microcapsules containing 100 mg of drug were precisely weighed and wrapped in muslin cloth before tying to paddle. 0.1N concentrated Hydrochloric acid (pH1.2) was used as dissolution media at 100 rpm maintaining temperature 37.7 ± 0.5 ºC. During dissolution study, sink conditions occurred. Different time intervals with altered pH of dissolution medium was done for simulation of GI transit condition. For 8 hours, pH of 1.2 was maintained for dissolution media using 0.1 N Hydrochloric acid. During the dissolution investigation, 10 mL aliquot was removed at 1-hour intervals to 8 h and substituted same amount of fresh medium in its place equivalently. Filtrate was studied for absorbance at 228 nm [5-7].

Fourier transform infrared analysis (FTIR)

API, sodium alginate, CPG, physical mixture, formulation batch were measured FTIR on simardzu IR affinity-1. The sample were put defuse reflectance spectroscopy. Wave number range 4000-400 cm^{-1} at room temperature was applied for scanning of spectra.

***In vitro* growth inhibition studies**

H. pylori utilized in the study was separated from a 57-year-old human patient with a stomach ulcer at Wockhardt Hospital in Nashik, Maharashtra, India. Spray dried microspheres were tested for growth inhibition *in vitro* using *H. pylori* broth culture. Brain-heart infusion was incubated for *H. pylori* broth culture alongwith Skirrow supplement. In a microaerophilic environment, at 37°C Brucella broth was cultured with *H. pylori* strain for 7 days. Colony counter and urease activity test was applied for detection of growth of bacteria. The colonies had counted in culture plate and calculated 100 colonies in 1mL. To determine effectiveness of dosage form on *H. pylori* growth eradication, nutrient broth (10 mL) was inoculated loopful with stock culture of *H.pylori* to create a bacterial flora[22].

Urease test

Urease, the enzyme produced by the bacteria and fungi, hydrolyses urea and release ammonia and carbon dioxide in which on reaction of ammonia, formation of ammonium carbonate develops increase in pH of the medium. After addition of phenol red in the medium changes color to red from yellow which indicates existence of urease activity. *H. pylori* colonies were pickedup from the culture on nutrient agar and agar slop was inoculated with the colonies of *H. pylori* which was incubated at 37ºC for 18 hrs. Any change of colour in the inoculated medium was observed[22-24].

RESULTS AND DISCUSSION

Currently mentioned spray drying methodology emerged as appropriate for development of CPG and sodium alginate microspheres loaded with amoxicillin trihydrate. Spray drying technology is uni-step process, simple, uncomplicated, fast feed drying and ideal for drug entrapment.

Statistical analysis

Statistical analysis applied to study response variables and for selection of ideal statistical tests, most suitable model can be obtained by fitting outcome of evaluation parameters to detect values of independent formulation variables for production of optimum response. The current research studied factorial design for the optimization of formulation and for detection of interactions between selected factors if any. 2^3 full factorial design utilised with independent variables like drug concentration, polysaccharide concentration and feed rate at 2 levels were studied for its impact on % entrapment efficiency, % mucoadhesion and % drug release. Outcome of responses are mentioned in Table 1. Outcome of studied responses as per fitted trial formulations in the 2^3factorial matrix applied to obtain suitable response models. Statistically one-way ANOVA ($p < 0.05$) was applied as models for evaluation. The % drug release of spray dried microspheres (Table 1) was detected as65.22 to 97.00 % with regression analysis of (r^2= 0.9997) which depicted that variable of the study significantly affected drug release from the formulation. As per the response surface curve obtained, (Figure 1) drug release rises with increment in viscosity of applied polymer concentration. Drug entrapment increases with increases in polymer concentration. For % drug release, obtained regression equation was:

% Drug Release=+80.73 – 0.86* A + 6.49 * B + 1.99 * C + 2.35*A*B- 8.13 * A*C-2.31*B* (r^2= 0.9997, F value= 508.93 , p< 0.05 i.e. significant) ---------- (4)

Table 1 indicated entrapment efficiency in the range of53.07 ±1.29 to 89.43 ±1.78 %. Rise in entrapment efficiency detected with rise in polymer concentration.

Morphological examination

All batches of manufactured spray-dried microspheres were smooth surfaced spherical shape (Figure1). Microspheres surface were free from cracks, ruptures and findings of morphology would detected as distinct and circular in nature with exterior smooth surface which indicates good mucoadhesion property of pattern in stomach mucosa.

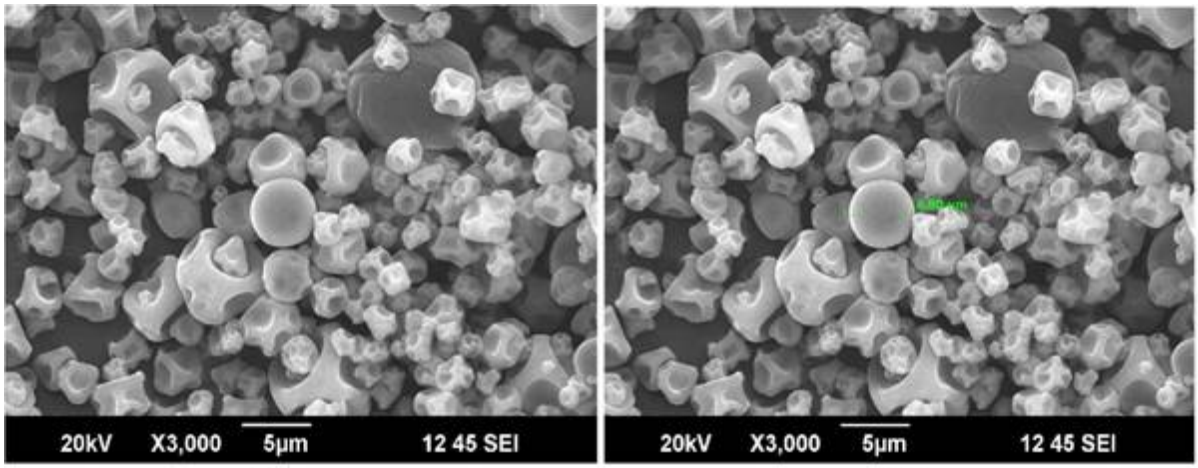

Figure 1:(a) and (b) SEM micrograph of Optimized batch

Yield and entrapment efficiency

The range of % yields for various formulations was found to be between 15.32% and 50.32%. The range of drug entrapment efficiency for several microspheres batches was discovered to be between 53.071.29% and 89.431.78%. Rise in entrapment efficiency detected with rise in polymer concentration.

Particle size analysis

Due to variations in formulation composition, formulated microspheres particle sizes ranged 12.5 to 19.5 µm.

Thermal analysis

The DSC graph of pure amoxicillin trihydrate, sodium alginate with CPGand drug loaded microspheres were studied and presented in Figure 2. At 120°C, pure amoxicillin displayed an exothermic peak which disappeared in the microspheres formulation of CPG polymer as drug dispersion has caused a polymer to form at the molecular level. Polymers showed peak at higher temperature at 340°C.

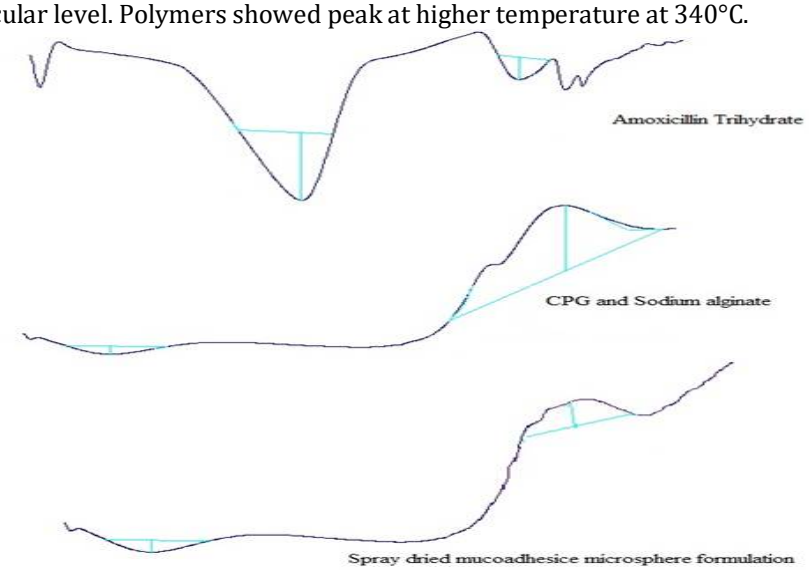

Figure 2: DSC spectra of Amoxicillin trihydrate, CPG and sodium alginate, spray dried mucoadhesive microsphere formulation.

XRD analysis

Figure 3 reported the XRD studies estimated for a pure amoxicillin, CPG polymer blank and developed microspheres formulation. These experiments are beneficial for examining the drug's crystallinity in polymeric microspheres. The figure shows that the amoxicillin trihydrate intensity peak is slightly reduced which indicatesslow drug crystallinity reduction in formulation.

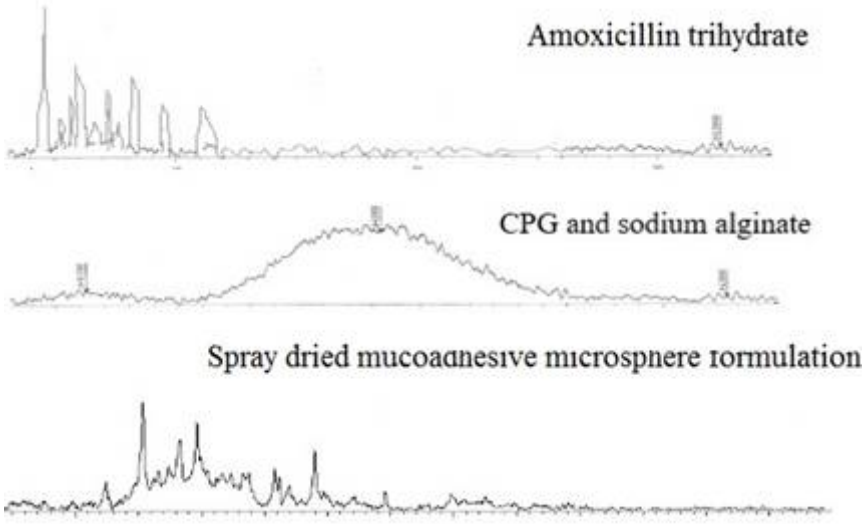

Figure 3: X- ray diffractogram of Amoxicillin trihydrate , *Caesalpinia pulcherrima* galactomannan (CPG) and sodium alginate, Spray dried mucoadhesive microsphere formulation.

***In vitro* mucoadhesion**

The findings of the present investigation contribute to the long-term adherence of the prepared microspheres to the site specific absorption for stomach mucosa. % Mucoadhesion was more at feed rate of 4 mL/min during the spray drying operation.

***In vitro* swelling studies**

The degree of swelling determined from microsphere swelling investigations were expressed using equation 3. Microspheres were evaluated for swelling properties by polymers present in the formulation. Microspheres with greater concentrations of CPG and sodium alginate were used to measure maximal swelling (degree of swelling)(Table 1).

***In vitro* drug release study**

Figure 4 displays drug release profile from various microsphere formulation batches. Drug release profile up to 8 hrs shows the highest concentration of CPG polymer The results indicated that the CPG polymer formulation was released and extended period of time.

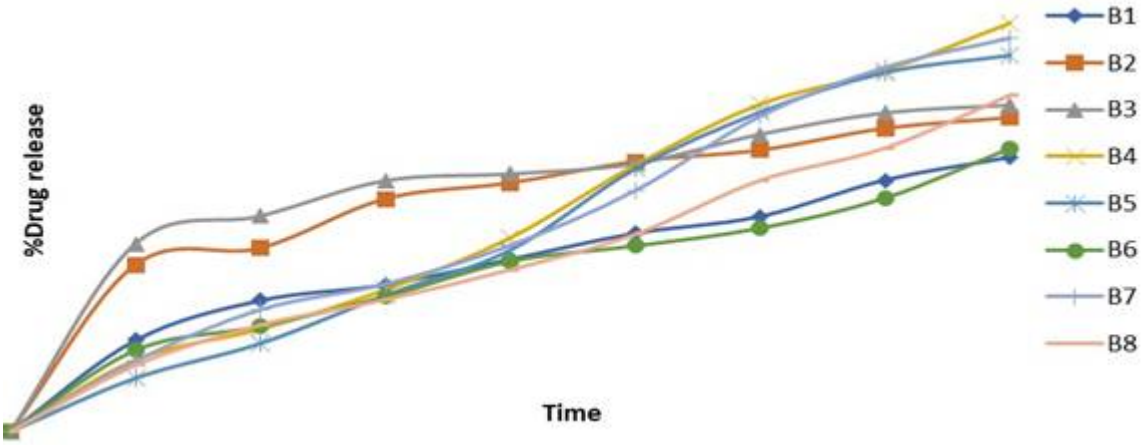

Figure 4: *In vitro* drug release of spray dried microspheres formulation

FTIR studies

FTIR was utilised, and the results are shown in Figure 5, to identify interactions and structural alterations between the excipient and amoxicillin. Plain amoxicillin FTIR spectra displayed pronounced peaks at 1620-3292 cm^{-1} and a few noticeable peaks at 846 - 600 cm^{-1}. The drug characteristic peak was also presented. Sodium alginate and CPG polymer formulations was detected with broaden and reduced intensity, indicated free from chemical interactions amongst drug , polymer, and during the production of microspheres.

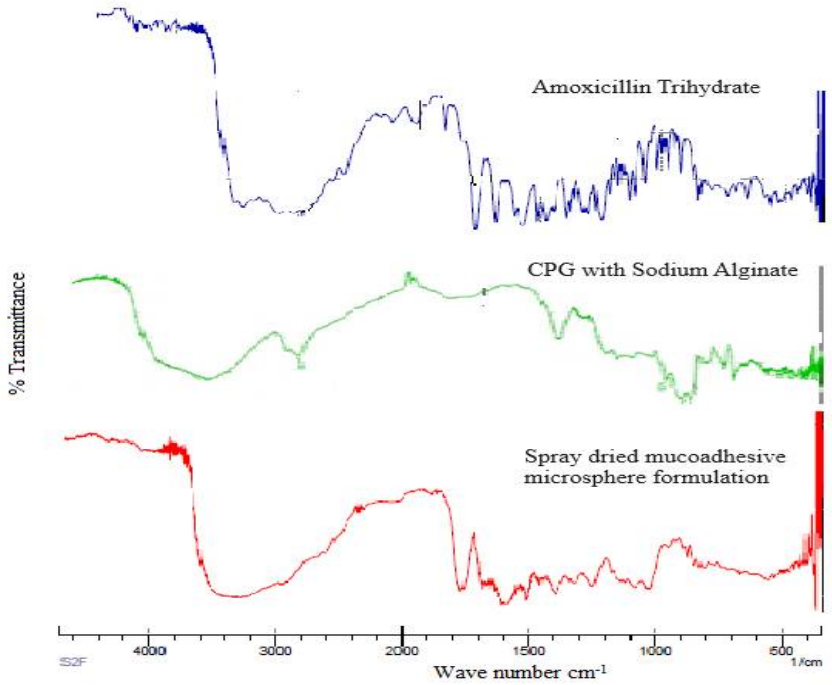

Figure 5: FTIR spectra of Amoxicillin trihydrate , polymer combination of sodium alginate with CPG, Spray dried mucoadhesive microsphere formulation.

***In vitro* growth inhibition studies**

H. pylori in vitro eradication was assessed for spray dried drug-loaded microspheres as mentioned in Figure 6. The microspheres and amoxicillin antimicrobial action were examined as growth inhibition(%) and measured from colony counts present in a specific tube containing only *H. pylori*. The proportion of growth inhibition reduced with increased incubation time. After 2 and 7 hours, microspheres showed a growth inhibition of around 51% and 75%, whereas after two and seven hours, pure API inhibited growth by around 58% and 100%, respectively. However, the stomach have a brief 2-hour residence time for API. After 12 hours of incubation, *H. pylori* completely inhibited from all batches. Overall, as per outcome of study spray dried amoxicillin microspheres prolonged gastric retention and drug release and recorded improved *H. pylori* eradication than pure amoxicillin.

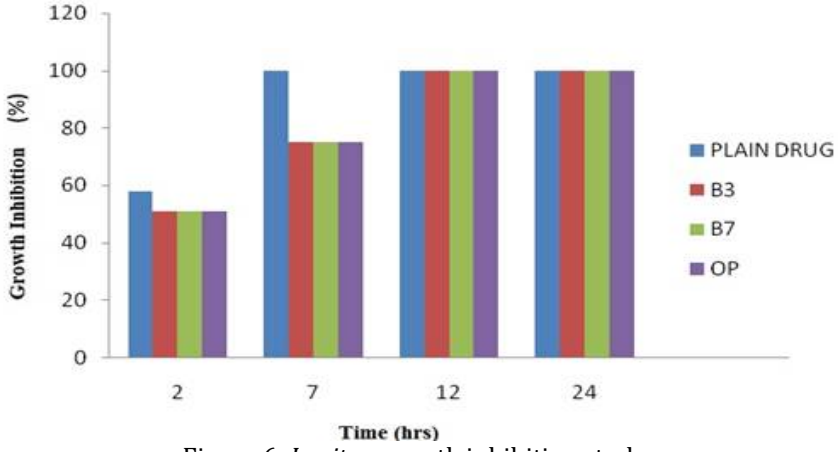

Figure 6: *In vitro* growth inhibition study

CONCLUSION

Thus the findings of the current study indicates that the developed spray dried mucoadhesive microsphere formulation using combination of sodium alginate with CPG acts as *H. pylori* growth suppression with controlled release medication. The applied polymer combination showed improvement in mucoadhesion of spray dried formulation. Thus, amoxicillin loaded spray dried microspheres using combination of sodium alginate with CPG might get rid of infection from *H. pylori*.

DECLARATION OF INTEREST

No conflicts of interest were reported by the authors.

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