RESEARCH ARTICLE

Design and evaluation of controlled release mucoadhesive microspheres of amoxicillin for anti *Helicobacter pylori* therapy

N Venkateswaramurthy, R Sambathkumar, P Perumal[1]

Departments of Pharmaceutics and [1]Pharmaceutical Chemistry, J.K.K. Nataraja College of Pharmacy, Komarapalayam, Namakkal District, Tamilnadu, India

The aim of this study was to develop controlled release mucoadhesive microspheres of amoxicillin trihydrate for the treatment of peptic ulcer disease caused by *Helicobacter pylori (H. pylori)*. Microspheres were prepared by solvent evaporation technique using carbopol 974P, hydroxypropyl methyl cellulose K4M (HPMC K4M) and Eudragit RS 100. The prepared microspheres were subjected to evaluation for particle size, incorporation efficiency, *in vitro* mucoadhesion and *in vitro* drug release characteristics. Absence of drug-polymer interaction was confirmed using differential scanning calorimetry analysis and fourier transform infrared spectrophotometry. The prepared microspheres showed a strong mucoadhesive property. The polymer concentration influenced the *in vitro* drug release significantly in 0.1N HCl. The particle sizes of systems ranged between 123±8.35 μm and 524±11.54 μm. Percent drug entrapment and release profiles of amoxicillin trihydrate in 0.1 N HCl were determined using high-performance liquid chromatography. The percentage drug entrapment and percentage yield of formulations were about 56.71±1.66% to 88.32±0.65% and 39.20±1.62% to 92.40±1.32%, respectively. The stability of the drugs was assessed in 0.1 N HCl. The results further substantiated that mucoadhesive microspheres improved the gastric stability of amoxicillin trihydrate (due to entrapment within the microsphere). From the above results, it was concluded that the mucoadhesive microspheres of amoxicillin trihydrate has feasibility for eradicating *H. pylori* from the stomach more effectively because of the prolonged gastrointestinal residence time and controlled release of drug from the formulation.

Key words: *Amoxicillin, carbopol 974P, controlled release, H. pylori, hydroxypropyl methyl cellulose K4M, microspheres, mucoadhesive*

INTRODUCTION

Helicobacter pylori (*H. pylori),* a Gram-negative human gastric bacterium, infects approximately 30-50% of adults in the developed world and over 90% of inhabitants in the developing world.[1] *H. pylori* normally causes a lifelong chronic gastritis and peptic ulcer disease. The infection plays an important role in peptic ulcer disease and gastric B-cell MALT (mucosa-associated lymphoid tissue) lymphoma and is associated with gastric adenocarcinoma[2-4] and it predicted that by 2020 to enter the top ten of leading causes of death worldwide.[5] The International Agency for Research and Cancer (IARC, USA) classified *H. pylori* as a group I carcinogen, a definite cause of human gastric cancers.[6] Amoxicillin (α-amino-hydroxybenzylpenicillin) is a semi-synthetic antibiotic, belonging to the b-Lactam family, which is effective for bacterial infection treatment, especially for *H. pylori* infection.[7] However, therapies using conventional oral amoxicillin capsules cannot completely eradicate *H. pylori* infections, allowing recolonization.[8,9] The incomplete eradication of *H. pylori* is mainly due to the short residence time of antimicrobial agents in the stomach so that effective antimicrobial concentration cannot be achieved in the gastric mucous layer or epithelial cell surfaces where *H. pylori* exists.[10,11] The minimum inhibitory concentration of less than or equal to 0.01-0.1 mg/L determined *in vitro* implies that if successful local delivery were achieved, lower doses of antibiotic may be effective.[12] It has therefore been proposed that local delivery could increase drug levels in the gastric mucus and mucosa

Address for correspondence:
Mr. N. Venkateswaramurthy,
Department of Pharmaceutics, J.K.K Nataraja College of Pharmacy, Komarapalayam- 638 183, Tamil Nadu, India.
E-mail: murthyvenki@rediffmail.com

Access this article online

Quick Response Code:

Website: www.asiapharmaceutics.info

DOI: 10.4103/0973-8398.97125

to effective bactericidal levels and extend the contact time of drugs with the organism.[13]

Mucoadhesive drug carriers may prolong the residence time in the GI tract because they can adhere to the mucus surface, resulting in an effective localized drug concentration.[14] Among the mucoadhesive drug carriers mucoadhesive microspheres have some advantages, e.g. a much more intimate contact with the mucus layer, lightweight and a smaller dose variation due to the large number of microspheres administered.[15]

The aim of this work was to prepare amoxicillin trihydrate-loaded mucoadhesive microspheres using the mucoadhesive polymers for *H. pylori* eradication therapy. To achieve therapeutic needs of the drug, the mucoadhesive drug delivery system should have mucoadhesive and controlled release property. In this study, carbopol 974P and HPMC K4M were used to achieve the mucoadhesive and controlled release property. Eudragit RS 100 is used as matrix polymer to disperse mucoadhesive polymers and also it has mucoadhesive property.[16] Carbopol 974P has good mucoadhesive ability but there are few reports on the application of carbopol 974P for the extended release dosage forms. The gelling nature of carbopol 974P is important for controlled release of drugs, but the gelling nature depends upon the pH of the medium.[17] The maximum gelling of carbopol 974P was achieved at pH 7-7.5 due to the ionization of carboxyl groups.[18,19] *H. pylori* colonizes in the stomach where the pH is acidic in nature. But in acidic pH, carbopol 974P does not dissociate completely and it forms less viscous gel, which significantly affects its release property.[20] To optimize the controlled release property and mucoadhesiveness a combination of carbopol 974P and HPMC K4M were used in this study. The gelling nature of HPMC K4M was not affected by pH of the environment because of its non-ionic nature.[21]

MATERIALS AND METHODS

Materials

Amoxicillin trihydrate was obtained as gift sample from Sandoz Pharma Ltd. Mumbai, India, Eudragit RS 100 was a gift sample from Microlabs, Bangalore, India. Hydroxypropyl methyl cellulose K4M (HPMC K4M) was obtained as gift from Colorcon Asia Pvt. Ltd., Mumbai, India and Carbopol 974P was a gift from BF Goodrich Co., Germany. All other reagents and chemicals used were of analytical grade.

Preparation of microspheres

Amoxicillin trihydrate loaded microspheres were prepared by solvent evaporation method. Acetone/liquid paraffin solvent system was used. Agglomeration of microspheres was prevented by using 0.75% w/v Span 80. Eudragit RS 100 was dissolved in 10 ml acetone and carbopol 974P, HPMC K4M and amoxicillin trihydrate were dispersed in it [Table 1]. This homogeneous final dispersion was cooled to 5°C and poured slowly with stirring (700 rpm) into 80 ml of liquid paraffin containing 0.75% w/v span 80, which was previously cooled to 5°C. The obtained emulsion was stirred at 40°C for 40 min. The suspension of microspheres in liquid paraffin was filtered and microspheres were washed by n-hexane and dried in vacuum at room temperature overnight.

Stability study of amoxicillin trihydrate 0.1 N HCL

It was reported that amoxicillin trihydrate was unstable in acidic (0.1 N HCl) solutions.[22] Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from microspheres. Hence, in order to calculate correct amount of the drug release the degradation rate constant and half life were determined by following method.

Amoxicillin trihydrate (50 mg; powder) was dissolved in 250 ml of 0.1 N HCL (pH 1.2) and vibrated in a water bath maintained at 37°C. After complete dissolvation of amoxicillin trihydrate 3 ml of samples were collected at 0, 1, 2, 4, and 8 h and mixed with 1 ml of 0.3M NaOH to prevent further degradation. The samples were then filtered through a 0.45 µm nylon membrane filter and concentration was determined by HPLC as per the method reported earlier.[23] The HPLC (Shimadzu scientific instruments, MD, USA) comprises a UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A). The wavelength of the UV detector was 230 nm and a reversed-phase column (Luna 5m C8, Phenomenex, USA) was used. The column temperature was maintained

Table 1: Formulation composition of the mucoadhesive microspheres of amoxicillin trihydrate

Formulation code	Eudragit Rs 100 (% w/v)	Carbopol 974P (% w/v)	HPMC K4M (% w/v)	Amoxicillin trihydrate (% w/v)
FA1	2	1	1	5
FA2	4	1	1	5
FA3	6	1	1	5
FA4	8	1	1	5
FA5	6	0.5	1	5
FA6	6	1.5	1	5
FA7	6	2	1	5
FA8	6	1	0.5	5
FA9	6	1	1.5	5
FA10	6	1	2	5

at 30°C, the flow rate was 1 ml/min, and the mobile phase consisted of an aqueous 0.05 M phosphate buffer containing 0.1% v/v triethylamine (pH 3.0)–methanol (90/10 % v/v).

The main peak area of amoxicillin trihydrate was measured at 0, 1, 2, 4, and 6 h. The degradation of amoxicillin trihydrate was assumed to follow first order kinetics. Degradation rate constant and degradation half life were calculated using following first order equation.[24]

$$C = C_0 e^{-kt}$$

In which C is the concentration of drug remaining at time t, C_0 is the initial concentration of drug, k is the degradation constant. The half life ($t_{1/2}$) was determined from the degradation constant.

Morphological analysis

Surface and cross-sectional morphologies of the beads were observed with a Scanning Electron Microscope (SEM) (JSM-6360A, Jeol Ltd, Tokyo, Japan) at an accelerating voltage of 20 kV. Prior to examination, samples were prepared on aluminum stubs and coated with gold under argon atmosphere by means of a sputter coater.

Particle size analysis

Particle size and size distribution of the raw material were measured by a laser-based particle size analyzer (Mastersizer, Malvern Instruments, and U.K). The particles were dispersed in n-Hexane prior to analyzing and the particles were suspended mechanically by magnetic stirring during the measurement.

Determination of drug content and encapsulation efficiency

The drug content in microspheres was measured after extraction from the microspheres. 5 mg of microspheres were weighed accurately and crushed in to powder using glass mortar and pestle. The powdered microspheres were suspended in 15 ml of NaOH solution (pH 10). The mixture was vortexed at 2500 rpm for 1 min and then for a further 2 h at 1000 rpm. The solution was then filtered through a 0.45 µm syringe filter to determine the amount of drug loaded in the microspheres and the filtrate was analyzed by HPLC.

$$\text{Drug content in microspheres} = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \times 100 \quad (1)$$

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug encapsulated}}{\text{Theoretical drug encapsulated}} \times 100 \quad (2)$$

Determination of yield of microspheres

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was then calculated using formula given below.

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \times 100 \quad (3)$$

Fourier transform infrared spectrophotometry

Infrared red spectra for pure amoxicillin trihydrate, blank microspheres, amoxicillin-loaded microspheres were obtained on a fourier transform infrared spectrophotometry (FTIR)-Shimadzu (84005) using the KBr disk method (2mg sample in 200mg KBr). The scanning range was 450–4000 cm^{-1}.

Differential scanning calorimetry

The thermal analysis of pure drug, formulations and blank microspheres was carried out using Universal V4.2E TA Instruments to evaluate possible drug-polymer interaction. Approximately 3mg of sample was accurately weighed into a 40-µl aluminum pan and sealed with a punched lid. A temperature range of 10–300°C was scanned using a heating rate of 10°C min^{-1}. A nitrogen purge of 50 ml min^{-1} was used in the oven.

X-Ray powder diffractometry

X-ray powder diffractometry (XRD) was carried out to investigate the effect of the microencapsulation process on the crystallinity of the drug. Powder XRD patterns were recorded on a Bruker AXS D8 Advance diffractometer using Ni-filtered, Cu Kα radiation with 2θ interval defined from 20 to 95° with a step size of 0.05°. The XRD patterns of pure drug, formulations and blank microspheres were recorded.

In vitro evaluation of mucoadhesiveness

The mucoadhesive properties of the mucoadhesive microspheres were evaluated by *in vitro* wash-off test as reported by Lehr *et al*.[25]

A 1x1 cm piece of stomach mucosa was mounted on to a glass slide with cyanoacrylate glue and rinsed with 0.1 N HCL. Microspheres were spread (~50) on a wet rinsed tissue specimen and the prepared slide was hung on to one of the grooves of a USP tablet disintegrating test apparatus which contained 900 ml of 0.1 N HCL at 37±0.5°C. When the disintegrating apparatus operated, the tissue specimen was given slow regular up and down movement in the test fluid.

At the end of 30 min, 1 h and at the hourly intervals up to 6 h, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate.

$$\text{Percentage of Mucoadhesiveness} = \frac{\text{Number of microspheres adhered at the end of 6 hour}}{\text{Number of microspheres spread}} \times 100 \quad (4)$$

Stability of amoxicillin trihydrate in microspheres in pH 1.2

Stability of amoxicillin trihydrate present within the microspheres was analyzed at pH 1.2 by the following method. Fifty milligrams of mucoadhesive microspheres were suspended in 30 ml of 0.1 N HCL in four graduated centrifuge tube with lid. The tube was then placed in a thermostatic vibrator and vibrated at a speed of 100 rpm at 37±1°C for 1, 2, 4, and 6 h, respectively. Whole samples were withdrawn at different time interval and neutralized with NaOH solution (0.05 M) to adjust the pH of sample to approximately 5.0 in order to prevent further degradation of drug. The samples were taken out at different time intervals and microspheres were collected separately by filtration. The drug content of the filtrate (amount of drug released from microspheres) and microspheres (amount drug entrapped in microspheres) were determined separately by HPLC method as described earlier.

In vitro dissolution studies

Drug release from mucoadhesive microspheres of amoxicillin trihydrate was determined by using USP dissolution test apparatus with stirrer at 100 rpm (Disso 2000, Labindia)). 900 ml of 0.1 N HCl (pH 1.2) was used as the dissolution medium and the temperature was maintained at 37°C±0.2C. A sample of microspheres equivalent to 500 mg of drug was used in each test. Samples were taken at appropriate time intervals and replaced with an equal volume of fresh dissolution medium. The withdrawn samples were filtered through 0.45 µm syringe filter and neutralized with NaOH solution (0.014 M) to adjust the pH of sample to approximately 5.0 in order to prevent the further degradation of drug. The samples were analyzed by RP-HPLC as described above. These experiments were conducted in triplicate.

Amoxicillin trihydrate was reported to be unstable in mediums with low pH.[22] Therefore, the results obtained from the dissolution study will underestimate the amount of the drug released from microspheres. Correct amount of the drug released were calculated using degradation rate constant. The following equation was used to correct dissolution data of amoxicillin trihydrate.[23]

$$dc/dt = dQ/Vdt - kC \quad (5)$$

Where C is the concentration of the drug at time t, Q the total amount of the drug released at time t, V the volume of the release medium, and k the first order degradation constant.

Kinetics of drug release

In order to understand the mechanism and kinetic of drug release, the drug release data of the *in vitro* dissolution study were analyzed with various kinetic model like zero order (fraction drug release *vs* time),[24] first order (log percentage drug remaining *vs* time),[24] Higuchi model (fraction drug release *vs* square root of time),[26] and Peppas model equation, %R = Ktn, where %R is the percentage drug release, K is the kinetic constant, and n is the release exponent and is a measure of release mechanism were applied to interpret mechanism and kinetics of drug release.[27] R^2 values were calculated for the linear curves obtained by regression analysis of the above plots. The n-values could be obtained from the slope of the above equation. If the value of n is 0.43 or less, the release mechanism follows Fickian diffusion, while the higher values ($0.43 < n < 0.85$) indicates a non-Fickian model (anomalous transport). The non-Fickian model corresponds to coupled diffusion/polymer relaxation. If the n-value is 0.85, the drug release follows zero order and case II transport.

RESULTS AND DISCUSSION

One of the important factors related to microspheres as reported by Lee *et al.*,[28] is the viscosity of the polymer solution. Polymer concentrations of 2%, 4%, 6% and 8% w/v were selected for studies to determine optimum concentration of matrix polymer. Flake formation was observed when Eudragit RS 100 concentration was used at a level 2%, 4% w/v, whereas maximum sphericity was observed at the 6% w/v level. Non-spherical microspheres were found when polymer concentration was used at the 8% w/v level. Therefore, 6% w/v of Eudragit RS 100 in acetone was found to be the optimum concentration for the polymer solution.

The half–life ($t_{1/2}$) of amoxicillin trihydrate was determined from the pseudo–first order degradation rate constant. Degradation rate constant used to correct the drug release data obtained in acidic media. The degradation rate constant and the degradation half-life of the amoxicillin trihydrate in pH 1.2 were found to be 0.0972 $hr^{-1}$and 7.128 hrs.

The morphology of the microspheres was examined by scanning electron microscopy [Figure 1]. The view of the microspheres showed a spherical shape with a smooth surface morphology. The mean particle size increased with increasing polymer concentration. Due to the higher concentration of the polymer in the internal phase large emulsion droplets

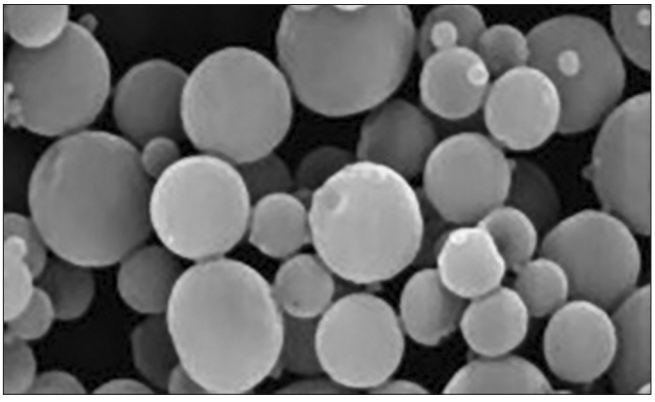

Figure 1: SEM photograph of prepared mucoadhesive microspheres of amoxicillin trihydrate (FA6)

Table 2: Physico-chemical characteristics of the mucoadhesive microspheres of amoxicillin trihydrate

Formulation code	Percentage yield	Encapsulation efficiency (%)	Particle size in µm (Mean±SD)	Percentage of Mucoadhesiveness
FA1	39.20±1.62	56.71±1.66	123±8.35	63.00±0.33
FA2	66.57±1.75	62.59±1.09	202±9.24	65.00±1.53
FA3	85.26±1.12	71.42±1.85	271±8.11	66.66±0.67
FA4	87.00±1.53	88.32±0.65	524±11.54	70.67±0.33
FA5	83.69±1.28	67.36±1.03	216±9.33	55.67±0.67
FA6	87.81±1.45	74.88±0.99	345±8.29	77.33±0.67
FA7	90.68±1.15	76.55±1.75	397±10.27	88.67±0.67
FA8	84.47±1.18	69.67±1.16	221±9.53	61.67±0.33
FA9	89.25±1.26	75.31±1.54	353±10.31	71.00±0.58
FA10	92.40±1.32	77.00±1.88	402±9.38	74.00±0.58

were formed which would not undergo size reduction at the shear force energy supplied to the system and eventually get precipitated leading to an increase in the mean particle size.[29]

The encapsulation efficiency of the prepared microspheres was shown in Table 2. The encapsulation efficiency of the prepared microspheres varied from 56.71±1.66% to 88.32±0.65%. The encapsulation efficiency was increased progressively with increasing the Eudragit RS 100, HPMC K4M, and carbopol 974P concentrations. The contribution of a high polymer concentration to the encapsulation efficiency can be interpreted in two ways. First, when highly concentrated, the polymer solidifies rapidly on the surface of the dispersed phase and prevents drug diffusion across the phase boundary.[30] Secondly, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets.[31] The production yield was very high for all the formulations ranging from 39.20±1.62% to 92.40 ± 1.32%. The production yield was increased with increased in the polymer concentrations.

Figure 2 demonstrates the FTIR spectra of amoxicillin trihydrate showing characteristic peaks[32] at 1581.63 cm^{-1} (COO^- Asymmetric Stretching), 1688.79 cm^{-1} (Amide I C = O Stretching), 1775.51 cm^{-1} (b– Lactum C = O Stretching). Characteristic peaks of amoxicillin trihydrate were also present in FTIR spectrum of amoxicillin trihydrate-loaded microspheres with slight broadening and reduction in intensity [1581.63cm^{-1} (COO^- Asymmetric Stretching), 1687.71cm^{-1} (Amide I C = O Stretching), 1775.57 cm^{-1} (β–Lactum C = O Stretching), indicating the absence of chemical interaction between amoxicillin trihydrate and polymers.

Figure 3 demonstrates the DSC thermogrames of pure amoxicillin trihydrate, blank microspheres, and amoxicillin trihydrate-loaded microspheres. The endothermic peak of amoxicillin trihydrate is at about 132.22°C. However, blank microsphere did not show any endothermic peak because of their amorphous nature of excipients, Eudragit RS 100, HPMC K4M, and carbopol 974P. Endothermic peak corresponding to the amoxicillin trihydrate were observed in amoxicillin trihydrate-loaded microspheres at 131.53°C. However, there

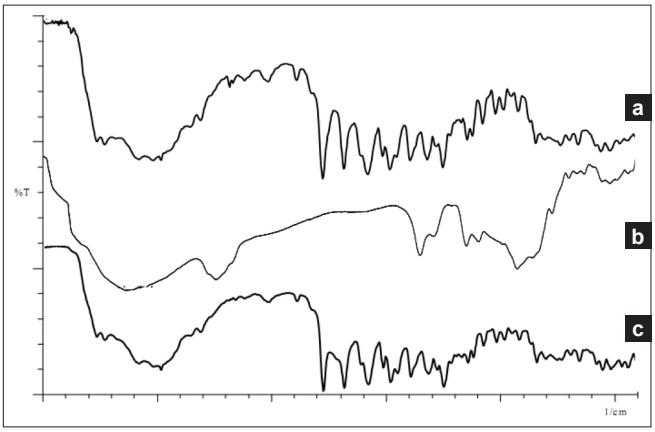

Figure 2: FTIR spectrum of pure amoxicillin trihydrate (a), blank microsphere (b), and amoxicillin trihydrate-loaded microsphere (c)

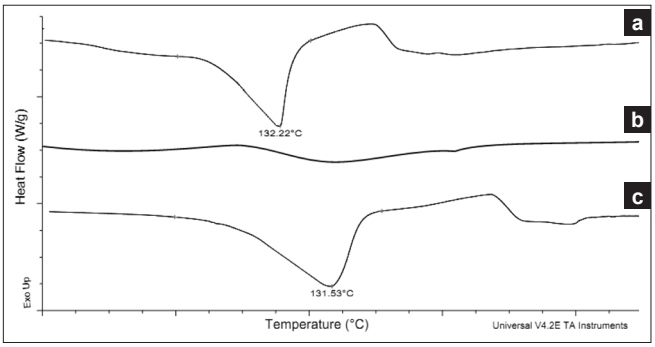

Figure 3: DSC thermogram of pure amoxicillin trihydrate (a), blank microsphere (b), and amoxicillin trihydrate-loaded microsphere (c)

was a slight decrease in the melting point of drug when prepared in the form of microspheres. It was also observed that there was a noticeable reduction in the enthalpy of the crystals in comparison with pure amoxicillin trihydrate. The evaluation of the thermograms obtained from DSC revealed no interaction between the polymer and the drug in the microspheres.

In order to investigate the physical nature of the encapsulated drug, the powder X-ray diffraction technique was used. Diffraction patterns amoxicillin trihydrate, physical mixture and drug loaded microsphere formulation were studied [Figure 4]. The powder XRD patterns of pure amoxicillin

trihydrate 2θ values appeared at 12.20°/15.15°/16.26°/18.1°/19.39°/26.6°/28.76°. All these peaks of amoxicillin trihydrate were detectable in both physical mixtures and microparticles, indicating the crystalline state of amoxicillin trihydrate within the samples. Thus, X-ray results were confirmed by the DSC data.

The study of *in vitro* mucoadhesive study revealed that all the batches of prepared microspheres had good mucoadhesive property ranging from 55.67±0.67% to 88.67±0.67%. As shown in Table 2 the remaining percentage was slightly increased with increasing the Eudragit RS 100 concentrations. It may due to the mucoadhesive nature of Eudragit RS 100.[16] By increasing the concentration carbopol 974P in the microspheres, the better retention effect was observed. Similarly, increasing the HPMC concentration retention effect was increased. The change in retention effect was less than carbopol with respect to concentration it may be due to strong mucoadhesive nature of carbopol than HPMC K4M. The maximum retention effect was observed in the formulation FA 7. These studies suggested that the spherical matrix of microspheres can interact with mucosubstrate on the surface of the stomach, and adhere to mucosa more strongly and could stay in stomach for prolong period for more effective *H. pylori* clearance.

Figures 5-7 showed the drug release profiles from various formulations of microspheres. An initial burst effect was observed in all the batches of microsphere formulations. The initial burst release may be due to the crystals of amoxicillin trihydrate adsorbed on the surface of microspheres. Decrease in the rate and extent of drug release was observed with the increase in polymer concentration microspheres and is attributed to an increase in the density of the polymer matrix and also an increase in the diffusional path length, which the drug molecules have to traverse. The release of drug from these gels was characterized by an initial phase of high release (burst effect). However, as gelation proceeds, the remaining drug was released at a slower rate followed by a second phase of moderate release. This bi-phasic pattern of release is a characteristic feature of matrix diffusion kinetics.[33] The initial burst effect was considerably reduced with increase in polymer concentration.

The effect of concentration of carbopol 974P and HPMC K4M on *in vitro* drug release is shown in Figures 6 and 7, respectively. Due to the chemical nature of carbopol 974P, peak swelling of the polymer was reported at pH 7-7.5.[18,19] So pH 1.2 is not the most favorable condition for the swelling of carbopol 974P to form a gel. It has been reported that under pH 1.2, the extent of carbopol 974P swelling is less than that

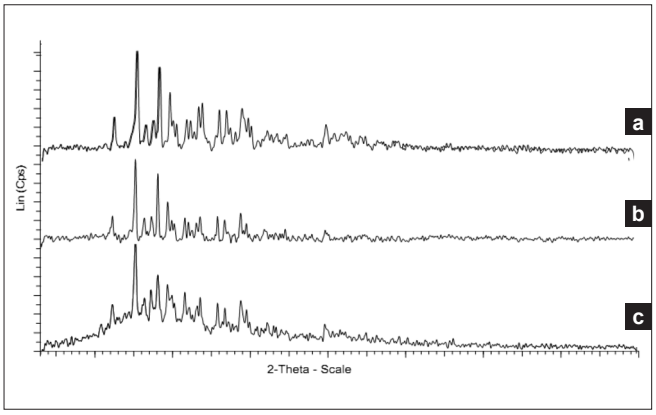

Figure 4: XRD pattern of pure amoxicillin trihydrate (a), physical mixture (b), and amoxicillin trihydrate-loaded microsphere (c)

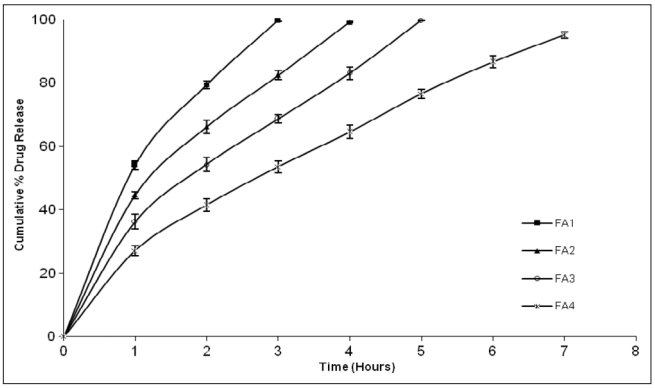

Figure 5: Effect of Eudragit RS 100 on the *in vitro* drug release characteristics of mucoadhesive microspheres of amoxicillin trihydrate in pH 1.2. Bars represent mean±SD (*n* = 3)

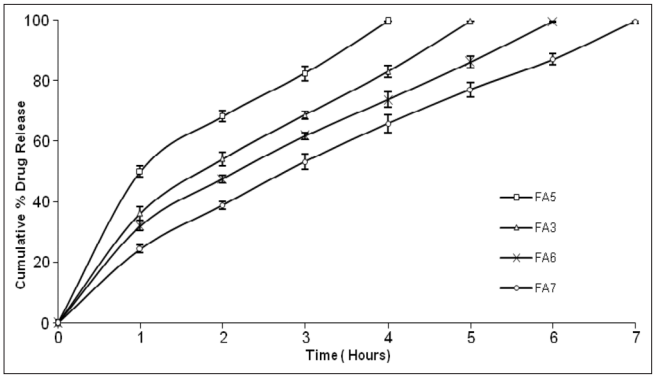

Figure 6: Effect of carbopol 974P on the *in vitro* drug release characteristics of mucoadhesive microspheres of amoxicillin trihydrate in pH 1.2. Bars represent mean±SD (*n* = 3)

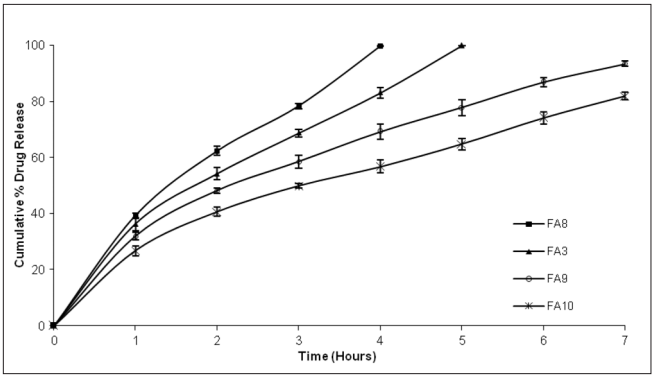

Figure 7: Effect of HPMC K4M on the *in vitro* drug release characteristics of mucoadhesive microspheres of amoxicillin trihydrate in pH 1.2. Bars represent mean±SD (*n* = 3)

of HPMC K4M.[34] Carbopol 974 has a pKa of 6, so in 0.1 N HCL, would be virtually un-ionized and unable to hydrate and control the drug release.[19] Drug release retardation effect of HPMC K4M may be due to nonionic nature of the polymer. Because of its non-ionic nature, swelling is not affected by pH variation. In pH 1.2, HPMC K4M may form viscous gel around the microspheres and keep the active drug inside and limiting the release. HPMC K4M predominantly controlled the drug release since carbopol has a low solubility at pH 1.2. Drug release became sustained with increasing HPMC K4M concentration. Similar results were obtained by Prudat *et al*.[35]

When the data were plotted according to the first order equation,[24] the formulations showed a fairly good linearity, with a R^2 value of 0.6980-0.9738 whereas the same data, when plotted according to the zero order equation,[24] improved the R^2 value 0.9839–0.9984 was obtained [Table 3]. In our experiment, the *in vitro* release profiles of amoxicillin trihydrate from all the formulations could be best expressed by Higuchi's equation,[24] as the plots showed good linearity with R^2 value (0.9900–0.999). The slope of the regression line from the Higuchi plot, which revealed the rate of drug release, thus confirmed that the mode of release was diffusion. For further confirmation of the diffusion mechanism, the data were fit into Korsmeyer *et al.*,[27] equation, which showed good linearity with comparatively high slope (n) value (0.5426–0.6499).

The slope of the regression line from the Higuchi plot[26] confirmed the diffusion mechanism, while to further confirm the diffusion mechanism, the data were fit into the Korsmeyer *et al,*[25] equation which showed high linearity with a comparatively high slope (n) value (0.5426–0.6255). This n-value, however, appears to indicate a coupling of diffusion and erosion mechanism, called anomalous diffusion. This indicated that drug release from the microspheres follows a non-Fickian trend as reported earlier.[36,37]

There are several reports regarding amoxicillin trihydrate stability in acidic solutions.[14] Amoxicillin trihydrate in acidic pH degraded in to amoxicillin penamaldic acid and amoxicillin penicilloic acid.[38] In the present study, no drug degradation product peaks were detectable by HPLC from samples taken from inside the microspheres until the 6 h of the release studies. And also, the cumulative amount of amoxicillin trihydrate released from microspheres was determined from the concentration in the release medium and from the amount of drug remaining in the microspheres as a function of time. These two methods resulted in nearly same release profiles [Figure 8]. This study confirmed that amoxicillin trihydrate was stable inside the microspheres.

CONCLUSION

Mucoadhesive microspheres of amoxicillin trihydrate were prepared to increase the local concentration of the antibiotic in the stomach to eradicate *H. pylori* infection. The main goal of this study was to optimize mucoadhesiveness and controlled release property. The results shown in the study clearly demonstrate that amoxicillin trihydrate can remain stable in the acidic environment of the stomach. Mucoadhesive microspheres can effectively control the release of amoxicillin trihydrate and also provide longer residence time in the fasted stomach. It is concluded that designed targeted delivery system could possibly treat the colonization of *H. pylori* in an effective manner.

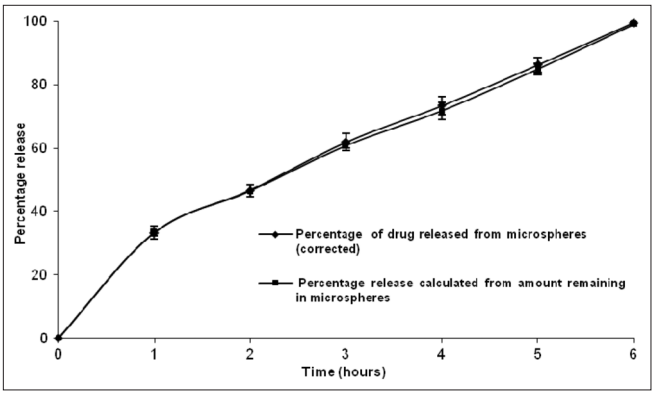

Figure 8: Stability of amoxicillin trihydrate in mucoadhesive microspheres in pH 1.2 Bars represent mean±SD (*n* = 3)

Table 3: *In vitro* drug release kinetic of mucoadhesive microspheres of amoxicillin trihydrate

F code	Zero order plot		First order plot		Higuchi plot	Korsemeyer peppa's plot	
	K_0	R^2	K_1	R^2	R^2	*n*	R^2
FA1	–*	–*	–*	–*	–*	–**	–**
FA2	17.9870	0.9956	0.5726	0.8239	0.9981	–**	–**
FA3	15.5920	0.9984	0.5396	0.7024	0.9900	–**	–**
FA4	11.3290	0.9950	0.1817	0.9144	0.9953	0.6255	0.9999
FA5	16.3030	0.9979	0.6964	0.7673	0.9918	–**	–**
FA6	13.3125	0.9980	0.3813	0.7050	0.9924	0.5950	0.9990
FA7	12.3403	0.9961	0.3044	0.6980	0.9940	0.6499	0.9987
FA8	19.7430	0.9955	0.7116	0.7607	0.9911	–**	–**
FA9	10.1307	0.9839	0.1604	0.9557	0.9990	0.5531	0.9982
FA10	08.8160	0.9906	0.0958	0.9738	0.9941	0.5426	0.9971

*Insufficient data points to apply kinetics due to rapid release profiles **Insufficient data points to apply Korsmeyer-Peppas equation up to 70%

REFERENCES

1. Kleanthous H, lee CK, Monath TP. Vaccine development against infection with *Helicobacter pylori*. Br Med Bull 1998;54:229-41.
2. Marshall BJ, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet 1984;1:1311-5.
3. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, *et al*. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ 1991;302:1302-5.
4. Wotherspoon AC, Ortiz-hidalgo C, Falzon MR, Isaacso PG. *Helicobacter pylori* associated gastritis a primary B-cell gastric lymphoma. Lancet 1991;338:1175-6.
5. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997;349:1498-504.
6. Schistosomes, liver flukes and *Helicobacter pylori*. IARC working group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. IARC Monogr Eval Carcinog Risks Hum 1994;61:1-241.
7. Risbud MV, Hardilar AA, Bhat SV, Bhonde KR. pH sensitive freeze-dried chitosan-polyvinyl pyrolidone hydrogels as controlled release system for antibiotic delivery. J Control Release 2000;68:23-30.
8. Kawabami E, Ogata SK, Portorreal AC, Magni AM, Pardo ML, Patrício FR. Triple therapy with clarithromycin, amoxicillin and omeprazole for *Helicobacter pylori* eradication in children and adolescents. Arq Gastroenterol 2001;38:203-6.
9. Lin CK, Hsu PI, Lai KH, Lo GH, Tseng HH, Lo CC, *et al*. One-week quadruple therapy is an effective salvage regimen for *Helicobacter pylori* infection in patients after failure of standard triple therapy. J Clin Gastroenterol 2002;34:547-51.
10. Cooreman MP, Krausgrill P, Hengels KJ. Local gastric and serum amoxycillin concentrations after different oral application forms. Antimicrob Agents Chemother 1993;37:1506-9.
11. Atherton JC, Cockayne A, Balsitis M, Kirk GE, Hawley CJ, Spiller RC. Detection of the intragastric sites at which *Helicobacter pylori* evades treatment with amoxicillin and cimetidine. Gut 1995;36:670-4.
12. Hirschl AM, Rotter ML. Amoxicillin for the treatment of *Helicobacter pylori* infection. J Gastroenterol 1996;31 Suppl 9:44-7.
13. Conway BR. Drug Delivery Strategies for the treatment of *Helicobacter pylori* Infections. Curr Pharm Des 2005;11:775-90.
14. Nagahara N, Akiyama Y, Nakao M, Tada M, Kitano M, Ogawa Y. Mucoadhesive microspheres containing amoxicillin for clearance of *Helicobacter pylori*. Antimicrob Agents Chemother 1998;42:2492-4.
15. Vasir JK, Tambwekar K, Garg S. Bioadhesive microspheres as a controlled drug delivery system. Int J Pharm 2003;255:13-32.
16. Lopedota A, Trapani A, Cutrignelli A, Chiarantini L, Pantucci E, Curci R, *et al.* The use of Eudragit RS 100/cyclodextrin nanoparticles for the transmucosal administration of glutathione. Eur J Pharm Biopharm 2009;72:509-20.
17. Singla AK, Chawla A, Singh A. Potential applications of carbomer in oral mucoadhesive controlled drug delivery system: a review. Drug Dev Ind Pharm 2000;26:913-24.
18. Lubrizol. Formulating controlled release tablets and capsules with carbopol polymers. Pharm Bull 2011;31:1-22.
19. Perez-Marcos B, Ford JL, Armstrong DJ, Elliott PN, Rostron C, Hogan JE. Influence of pH on the release of propranolol hydrochloride from matrices containing hydroxypropyl methylcellulose K4M and carbopol 974. J Pharm Sci 1996;85:330-4.
20. Bonacucina G, Martelli S, Palmieri GF. Rheological, mucoadhesive and release properties of Carbopol gels in hydrophilic cosolvents. Int J Pharm 2004;282:115-30.
21. Bravo SA, Lamas MC, Salomon CJ. Swellable Matrices for the Controlled-Release of Diclofenac Sodium: Formulation and *in vitro* Studies. Pharm Dev Technol 2004;9:75-83.
22. Erah PO, Goddard AF, Barrett DA, Shaw PN, Spiller RC. The stability of amoxicillin, clarithromycin and metronidazole in gastric juice: Relevance to the treatment of *Helicobacter pylori* infection. J Antimicrob Chemother 1997;39:5-12.
23. Chun MK, Sah H, Choi HK. Preparation of mucoadhesive microspheres containing antimicrobial agents for eradication of *H*. pylori. Int J Pharm 2005;297:172-9.
24. Bourne DW. Pharmacokinetics. In: Banker GS, Rhodes CT, editors. Modern Pharmaceutics. 4th ed. New York: Marcel Dekker Inc; 2002. p. 67-92.
25. Lehr CM, Bowstra JA, Tukker JJ, Junginger HE. Intestinal transit of bioadhesive microspheres in an *in situ* loop in the rat. J Control Release 1990;13:51-62.
26. Higuchi, T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci 1963;52:1145-9.
27. Korsemeyer RW, Gurny R, Docler E, Bur P, Peppas NA. Mechanism of solute release from porous hydrophilic polymer. Int J Pharm 1983;15:25-35.
28. Lee JH, Park TG, Choi HK. Development of oral drug delivery system using floating microspheres. J Microencapsul 1999;16:715-29.
29. Dubey RR, Parikh RH. Two-stage optimization process for formulation of chitosan microspheres. AAPS PharmSciTech 2004;5:E5.
30. Rafati H, Coombes AG, Adler J, Holland J, Davis SS. Protein-loaded PLGA microparticles for oral administration: Formulation, structural and release characteristics. J Control Release 1997;43:89-102.
31. Bodmeier R, McGinity JW. Solvent selection in the preparation of PLA microspheres prepared by the solvent evaporation method. Int J Pharm 1988;43:179-86.
32. Bird AE. Amoxicillin. In: Harry GB, editor. Vol 23. Analytical Profiles of Drug Substances and Excipient. San Diego: Academic Press; 1994. p. 4-51.
33. Lemoine D, Wauters F, Bouchend S, Preat V. Preparation and characterization of alginate microspheres containing a model antigen. J Pharm Sci 1998;176:9-19.
34. Li S, Lin S, Daggy BP, Mirchandani HL, Chien YW. Effect of formulation variables on the floating properties of gastric floating drug delivery system. Drug Dev Ind Pharm 2002;28:783-93.
35. Prudat-Christiaens C, Arnaud P, Allain P, Chaumeil JC. Aminophylline bioadhesive tablets attempted by wet granulation. Int J Pharm 1996;141:109-16.
36. Soppimath KS, Kulkarni AR, Aminabhavi TM. Controlled release of antihypertensive drug from the interpenetrating network poly (vinyl alcohol)-guar gum hydrogel microspheres. J Biomater Sci Polym Ed 2000;11:27-43.
37. Rokhade AP, Agnihotri SA, Patil SA, Mallikarjuna NN, Kulkarni PV, Aminabhavi TM. Semi-interpenetrating polymer network micro-spheres of gelatin and sodium carboxy-methyl cellulose for controlled release of ketorolac tromethamine. Carbohydr Polym 2006;65:243-52.
38. Blaha JM, Knevel AM, Kessler DP, Mincy JW, Hem SL. Kinetic analysis of penicillin degradation in acidic media. J Pharm Sci 1976;65:1165-70.

How to cite this article: Venkateswaramurthy N, Sambathkumar R, Perumal P. Design and evaluation of controlled release mucoadhesive microspheres of amoxicillin for anti *Helicobacter pylori* therapy. Asian J Pharm 2011;5:238-45.

Source of Support: Nil. **Conflict of Interest:** None declared.