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Controlled release mucoadhesive microspheres of clarithromycin for the treatment of *Helicobacter Pylori* infection

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ABSTRACT

The aim of this study was to develop controlled release mucoadhesive microspheres of clarithromycin for the treatment of peptic ulcer disease caused by Helicobacter pylori (H. pylori). Clarithromycin mucoadhesive microspheres were prepared by using carbopol 974P and Hydroxypropyl methyl cellulose K4M (HPMC K4M) and Eudragit RS 100. The prepared microspheres were subjected to evaluation for percentage yield, particle size, incorporation efficiency, in vitro mucoadhesion and in vitro drug release characteristics. Absence of drug-polymer interaction was confirmed by using differential scanning calorimetry analysis and fourier transform infrared spectrophotometry. X- Ray diffraction study was conducted to analyze physical nature of entrapped drug. The stability of the clarithromycin in 0.1N HCl was determined to correct the dissolution data and also the stability of entrapped clarithromycin was analyzed in 0.1N HCl. 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 microspheres ranged between 118.5±6.51 µm to 493.23±11.23 µm. The percentage drug entrapment and percentage yield of formulations were about 52.62±0.72 to 87.97±0.83% and 37.53±1.43 to 89.33±1.46% respectively. In vitro release was conducted in 0.1N HCl using high-performance liquid chromatography. The results further substantiated that mucoadhesive microspheres of clarithromycin improved the gastric stability of clarithromycin (due to entrapment within the microsphere). The formulation FC6 selected as best formulation based on release profile, mucoadhesiveness and mucous turnover rate. From the above results, it was concluded that the mucoadhesive microspheres of clarithromycin 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 of the formulation.

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

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INTRODUCTION

Helicobacter pylori (H. Pylori) infects approximately 50% of the adult population and is associated with a wide range of upper gastrointestinal diseases including gastritis, peptic ulcer disease and gastric cancer [1]. The most widely recommended treatment in international guidelines for the eradication of *H. pylori* is combination of two

antibiotics (clarithromycin plus amoxicillin or metronidazole) with a proton pump inhibitor (PPI) for at least 14 days [2–6]. However, since the microorganism was discovered, the eradication rate has fallen considerably with this regimen [7]. There could be several reasons for the failure antibiotic therapy against *H. pylori*. One the reason is that conventional tablets or capsules do not remain in the stomach long. Therefore, antibiotics might not have enough time to diffuse in to the mucosa layer where *H. Pylori* resides, and the antibiotic concentrations in the gastric mucus cannot reach minimum inhibitory concentrations [8, 9]. Stomach specific drug delivery systems can prolong the residence time of antibiotics in the stomach and continuously release the antibiotics in the infected area.

The objective of this work was to prepare microspheres of clarithromycin by using the mucoadhesive polymers for *H. pylori* eradication therapy. To achieve therapeutic needs dosage form should have mucoadhesive and controlled release property. In this study carbopol 974P and HPMC K4M were used achieve the mucoadhesive and controlled release property. Eudragit RS 100 used as matrix polymer to disperse mucoadhesive polymer and also it has mucoadhesive property [10]. Carbopol 974P is a weak acidic polymer, it form hydrogen bonding with mucin glycoprotein and this interaction is pH sensitive. Although carbopol has many advantages e.g Good gel forming ability and mucoadhesive ability, there are few reports on the application of carpopol to the extended release dosage forms. This is due to ionic nature and high sensitivity of carbopol to the pH of the medium [11]. Gelling nature of carbopol is important for drug release property. The maximum swelling of the polymer is at pH 7-7.5. *H. pylori* colonize in stomach where the pH is acidic. But in acidic pH carbopol does not dissociate completely resulting in a less viscous gel, it significantly affect its release property [12]. To optimize the mucoadhesive and controlled release property combination of carbopol and HPMC K4M was used in this study. Because HPMC K4M is a non ionic polymer its release property is not affected by pH of the environment [13].

MATERIALS AND METHODS

Materials

Clarithromycin, Eudragit RS 100 and Hydroxypropyl methyl cellulose K4M (HPMC K4M) were collected as gift samples from Macleods Pharmaceuticals limited, Mumbai. All other reagents and chemicals used were of analytical grade.

Preparation of Microspheres

Clarithromycin loaded microspheres were prepared by a solvent evaporation method by using acetone/liquid paraffin solvent system. Agglomeration of microspheres was prevented by using 0.75% w/v Span80. Eudragit RS 100 was used to form a matrix of microspheres and mucoadhesive polymer were chosen to produce mucoadhesion is carbopol 974P and HPMC K4M. Eudragit RS 100 and clarithromycin were dissolved in acetone; carbopol 974P and HPMC K4M were dispersed in it (Table 1). The total volume of acetone was 30 ml. This homogeneous final dispersion was cooled to 5°C and poured slowly with stirring (700 rpm) into 300 ml of liquid paraffin containing 0.75% w/v span 80, which was previously also 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.

Table 1: Formulation composition of the mucoadhesive microspheres of clarithromycin

Formulation code	Eudragit RS 100(% w/v)	Carbopol 974P(% w/v)	HPMC K4M(% w/v)	Clarithromycin (% w/v)
FC1	2	1	1	5
FC2	4	1	1	5
FC3	6	1	1	5
FC4	8	1	1	5
FC5	6	0.5	1	5
FC6	6	1.5	1	5
FC7	6	2	1	5
FC8	6	1	0.5	5
FC9	6	1	1.5	5
FC10	6	1	2	5

Stability study of clarithromycin 0.1 N HCL

It was reported that clarithromycin unstable in acidic (0.1N HCl) solutions [14]. Therefore the results obtained from the dissolution study will underestimate the amount of the drug released from microspheres. In order to calculate correct amount of the drug release the degradation rate constant and half life were determined by following method.

Clarithromycin (50 mg; powder) dissolved in 250 mL of 0.1 N HCL (pH 1.2) and vibrated in a water bath maintained at 37°C. After clarithromycin was completely dissolved 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 0.45µm nylon membrane filter and concentration determined by HPLC as per the method reported earlier [15]. The HPLC (Shimadzu Scientific Instruments, MD, USA), consisting of a UV detector (SPD-10A), a pump (LC-10AD), and an automatic injector (SIL-10A. The wavelength of the UV detector was 210 nm and a reversed-phase column (Luna 5µm C8, Phenomenex, USA) was used. The mobile phase flow rate was 1ml/min, and the mobile phase consisted of aqueous 0.07M potassium dihydrogen phosphate-acetonitrile (65/35).

The main peak area of clarithromycin at 0, 1, 2, 4, and 6 h was measured. The degradation of clarithromycin was assumed to follow first order kinetics. Degradation rate constant and degradation half life were calculated by using following first order equation [15].

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

In which C is the concentration of drug remaining at time t, C_0 is the initial concentration of drug 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 (HITACHI-SEM MODEL S – 450) 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 (780 AccuSizer, Particle sizing systems, Inc, Santa Barbara, Calif., USA). 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. Accurately weighed 5 mg microspheres, 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 and room temperature. In order to determine the amount of drug loaded in the microspheres, the solution was filtered through a 0.45µm syringe filter and the filtrate was analyzed by HPLC.

$$\text{Drug content in microspheres} = \frac{\text{weight of drug in microspheres}}{\text{weight of microspheres}} \text{x} 100$$

$$\text{Encapsulation efficiency} = \frac{\text{Actual drug encapsulated}}{\text{Theoretical drug encapsulated}} \text{x} 100$$

Determination of yield of microspheres

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

$$\% \text{ yield} = \frac{\text{Mass of microspheres obtained}}{\text{Total weight of drug and polymer}} \text{x} 100$$

Fourier Transform Infrared Spectrophotometry (FTIR)

Infrared red spectra for pure clarithromycin, blank microspheres, clarithromycin loaded microspheres were obtained on a FTIR-Shimadzu (84005) using the KBr disk method. 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^{o}C was scanned using a heating rate of 10^{o}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 K[alpha] radiation with 2[theta] interval defined from 20 to 95[degrees] with a step size of 0.05[degrees]. 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 [16]. A 1x1 cm piece of stomach mucosa was mounted on to a glass slide with cyanoacrylate glue and rinsed with 0.1N 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 the 900 ml of 0.1N HCL at 37± 0.5^{o}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.

$$\text{Percentage of Mucoadhesiveness} = \frac{\text{Number of microspheres adhered at the end of 6 hour}}{\text{Number of microspheres spread}} \text{ x } 100$$

***In vitro* dissolution studies**

Drug release from mucoadhesive microspheres of clarithromycin was determined using USP dissolution test apparatus 2 (Paddle) with stirrer at 100 rpm (Disso 2000, Labindia). 900 ml of 0.1N HCl (pH 1.2) was used as the dissolution medium and the temperature was maintained at 37ºC±0.2°C. 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 0.45µm syringe filter and neutralized with NaOH solution (0.03 M) to adjust the pH of sample to approximately 5.0 in order to prevent the further degradation of drug before analyzed by RP-HPLC as described above. These experiments were conducted in triplicate.

Clarithromycin was reported to be unstable in mediums with low pH [15]. 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 by using degradation rate constant. The following equation was used to correct dissolution data of clarithromycin [15].

$$dc/dt = dQ/Vdt - kC$$

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), first order (log percentage drug remaining *vs* time), Higuchi model (fraction drug release *vs* square root of time), and Peppas model equation, %R = Kt n, 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 [17]. 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.

Stability of clarithromycin in microspheres in pH 1.2

Stability clarithromycin present within the microspheres were analyzed at pH 1.2 by following method. Microspheres containing equivalent to 50 mg of drug were suspended in 30 ml of 0.1 N HCL in six 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, 5 and 6 h, respectively. The whole samples were withdrawn at different time interval and neutralized with NaOH solution (0.03 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 [15].

Accelerated stability studies

The optimized formulation were stored in a stability chamber (Remi CHM- 10 S®, India) at 40 ± 2°C and humidity of 75 ± 5% RH for 6 months and observed for the drug content, mucoadhesiveness and *in vitro* drug release 0, 30, 90, and 180 days. The zero time samples were used as controls.

RESULTS AND DISCUSSION

One of the important factors related to microspheres as reported by Lee *et al.,* [18] 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 clarithromycin 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 clarithromycin in pH 1.2 were found to be 4.052 h^{-1} and 0.171h respectively.

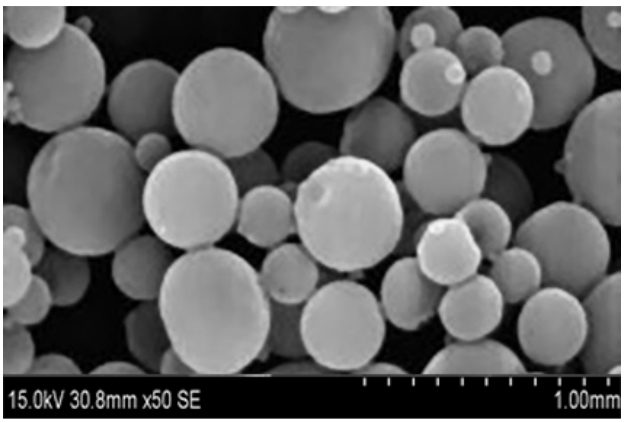

Figure 1: SEM photograph of formulation FC6

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. The higher the concentration of the polymer in the internal phase, the more polymers would be present in each droplet. Therefore, large emulsion droplets 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 [19]. The mean diameter clarithromycin loaded microspheres was found to be in the range of 118.5±6.51 µm to 493.23±11.23 µm.

The incorporation efficiency of the prepared microspheres is shown in Table 2. The incorporation efficiency of the prepared microspheres varied from 52.62±0.72% to 87.97±0.83%. The encapsulation efficiency increased progressively with increasing the Eudragit RS 100, HPMC K4M and carbopol 974P concentrations ($P < 0.05$). The contribution of a high polymer concentration to the encapsulation efficiency can be interpreted in two ways. First, the highly concentrated polymer precipitates faster on the surface of the dispersed phase and prevents drug diffusion across the phase boundary [20]. Secondly, the high concentration increases viscosity of the solution and delays the drug diffusion within the polymer droplets [21]. The production yield was very high for all the formulations ranging from 37.53±1.43% to 89.75±1.43%. The production yield was increased with increased in the polymer concentrations ($P < 0.05$).

Table 2. Physico-chemical characteristics of the mucoadhesive microspheres of clarithromycin

Formulation code	Percentage yield (Mean ± SD)	Encapsulation efficiency (%) (Mean ± SD)	Particle size in µm (Mean ± SD)	Percentage of Mucoadhesiveness (Mean ± SE)
FC1	37.53±1.43	52.62±0.72	118.21±6.51	58.67±0.67
FC 2	65.08±1.54	59.80±2.07	187.08±11.51	60.00±0.58
FC 3	83.97±0.88	69.89±2.13	257.78±07.35	61.67±0.88
FC 4	85.77±1.48	87.97±0.83	493.23±11.23	65.67±0.67
FC 5	81.54±1.67	64.77±0.66	192.54±8.46	50.33±0.88
FC 6	86.76±1.49	71.66±1.72	310.47±10.69	72.67±0.88
FC 7	89.33±1.46	74.87±1.52	370.92±12.73	84.33±0.33
FC 8	83.44±1.13	67.71±1.44	197.65±12.69	56.33±0.67
FC 9	86.77±1.24	75.79±1.71	322.74±10.24	65.67±0.33
FC10	89.75±1.43	73.53±1.19	379.35±05.98	70.00±1.16

Figure 2 demonstrate the FTIR spectra of clarithromycin characteristic peaks at 1729.22 cm^{-1} (Lactone carbonyl), 1690.50 cm^{-1} (Ketone carbonyl), 3450.15 cm^{-1} (Hydrogen bonding between OH Groups), and 1375.25 cm^{-1} (CH_2). Characteristic peaks of clarithromycin were also present in FTIR spectrum of clarithromycin loaded microspheres with slight broadening and reduction in intensity. Peaks at 1733.29 cm^{-1} (Lactone carbonyl), 1699.43 cm^{-1} (Ketone carbonyl), 3457.58 cm^{-1} (Hydrogen bonding between OH Groups), and 1373.32 cm^{-1} (CH_2), indicating the absence of chemical interaction between clarithromycin and polymers. In blank microspheres Eudragit characteristic peaks observed at 1728.58 cm^{-1} due to the C=O stretching vibration. In addition, HPMC K4M showed a characteristic band at 3455.67 cm^{-1} due to O–H stretching vibration. Carbopol showed a characteristic band at 1728.58 cm^{-1} due to C=O stretching.

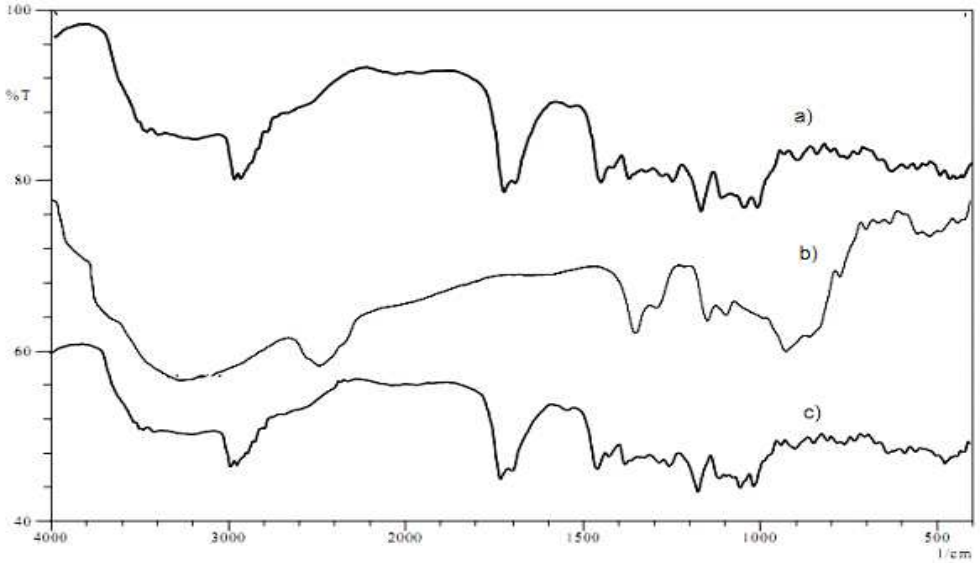

Figure 2: FTIR spectrum of pure clarithromycin (a), blank microsphere (b), and clarithromycin -loaded microsphere (c)

Figure 3 demonstrate the DSC thermogrames of pure clarithromycin, blank microspheres, and clarithromycin loaded microspheres. The endothermic peak of clarithromycin observed at about 221.19°C. However in blank microspheres the excipients Eudragit RS 100, HPMC K4M and carbopol 974P did not show any endothermic peak because of their amorphous nature [23-25]. The DSC thermogram of clarithromycin raw material corresponds to the thermogram of the polymorph ''Form II" that can be justified by the presence of an endothermic peak at 225.99°C attributed to the melting process [26]. No endothermic peak corresponding to the clarithromycin were observed in clarithromycin loaded microspheres. The absence of detectable crystalline domains in the microspheres clearly indicates that drug was molecularly dispersed in the microspheres.

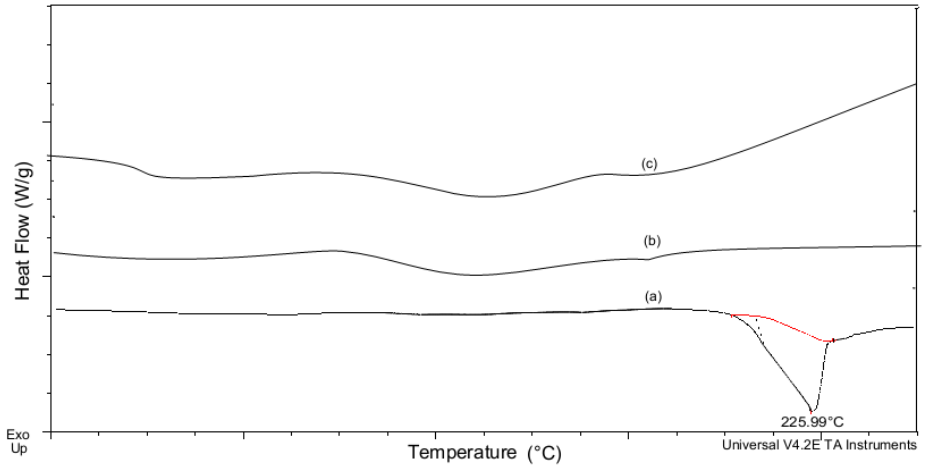

Figure 3: DSC thermogram of pure clarithromycin (a), blank microsphere (b), and clarithromycin loaded microsphere (c)

In order to investigate the physical nature of the encapsulated drug, the powder X-ray diffraction technique was used. Diffraction patterns clarithromycin, physical mixture and drug loaded microsphere formulation were studied (Figure 3). The powder XRD patterns of pure clarithromycin 2θ values appeared at 8.66° / 9.60° / 10.92° / 11.59° / 13.89° / 15.28° / 17.42° / 18.30° / 19.17° / 20.58° [22]. The powder XRD patterns of the clarithromycin loaded microspheres were completely different from those of pure drugs and showed no characteristic peaks. This demonstrates that the drugs were in an amorphous state in the solid dispersions (Figure 4).

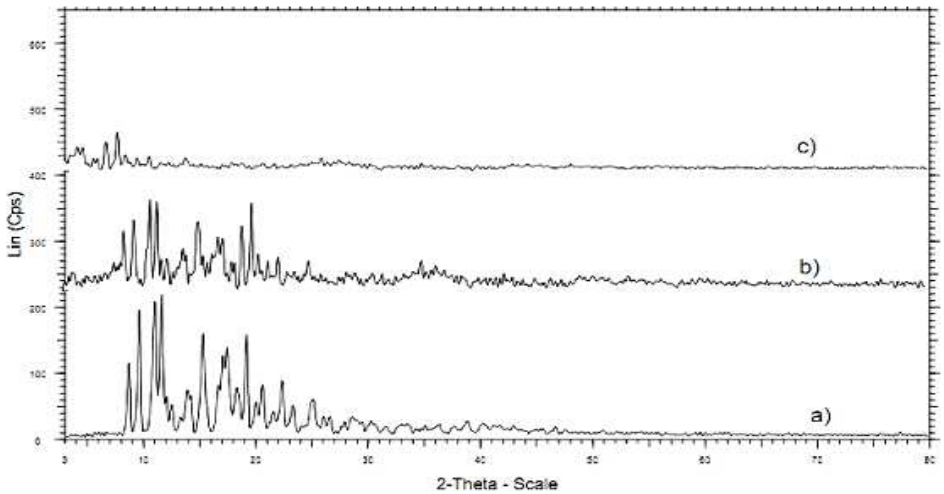

Figure 4: XRD pattern of pure clarithromycin (a), physical mixture (b), and clarithromycin loaded microsphere (c).

The study of *in vitro* mucoadhesion revealed that all the batches of prepared microspheres had good bioadhesive property ranging from 58.67±0.67 to 84.33±0.33%. As shown in Table 2 the remaining percentage was slightly increased with increasing the Eudragit RS 100 it is due mucoadhesive nature of Eudragit RS 100 polymer ($P < 0.05$)

[27]. By increasing the Concentration carbopol in the microspheres, the better retention effect was observed ($P < 0.05$). Similarly increasing the HPMC concentration retention effect was increased ($P < 0.05$). 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 K4 M. The maximum retention effect was observed in the formulation FC 7. These studies suggest 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. Figure 5-7 shows the drug release profiles from various formulations of microspheres. An initial burst effect was observed in all the batches of microsphere formulations. To provide an effective local action for eradication of *H. pylori* ideally it would be required to release drug rapidly in the initial stages to obtain the desired concentration following by slow release of the drug in order to replace drug lost, for example by gastric emptying.

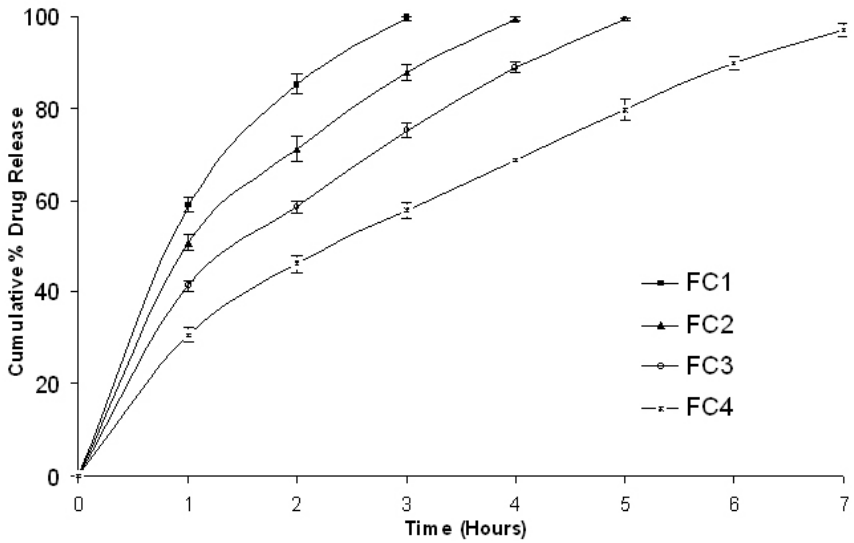

Figure 5 : Effect of Eudragit RS 100 on the *in vitro* drug release characteristics of mucoadhesive microspheres of clarithromycin in pH 1.2. Bars represent mean±SD (n = 3)

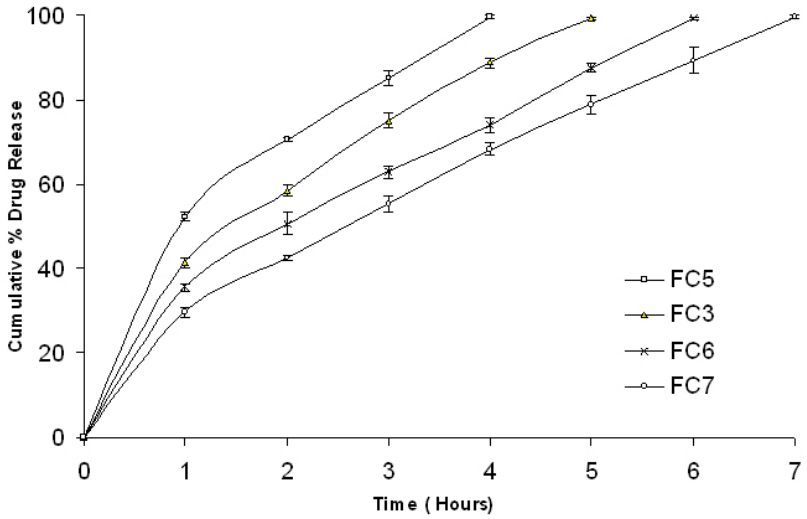

Figure 6 : Effect of carbopol 974P on the *in vitro* drug release characteristics of mucoadhesive microspheres of clarithromycin in pH 1.2. Bars represent mean±SD (n = 3)

Decrease in the rate and extent of drug release was observed with the increase in polymer concentration microspheres and is attributed to 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 [28]. The initial burst effect was considerably reduced with increase in polymer concentration.

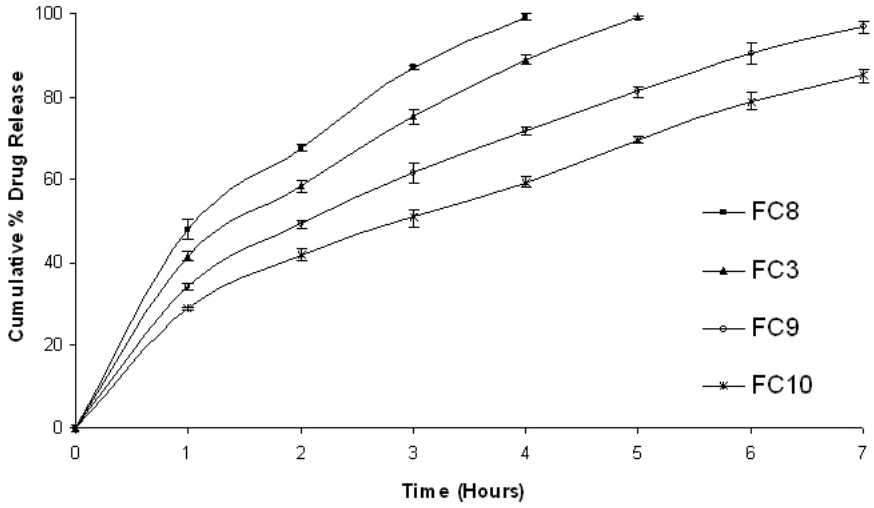

Figure 7: Effect of HPMC K4M on the *in vitro* drug release characteristics of mucoadhesive microspheres of clarithromycin in pH 1.2. Bars represent mean±SD (n = 3)

The effect of concentration of carbopol on *in vitro* drug release from mucoadhesive microspheres is shown in Figure 6. Increasing the proportion of carbopol 974P sustained the drug release ($P < 0.05$). Similarly the effect of concentration of HPMC K4M on *in vitro* drug release from mucoadhesive microspheres is shown in Figure 7. Drug release retards while increasing the Increasing the proportion of HPMC K4M ($P < 0.05$). HPMC K4M is significantly influenced the drug release properties than carbopol 974P ($P < 0.05$). Carbopol 974P peak swelling is reported at pH 7-7.5 [29]. So, pH 1.2 is not a most favorable condition for the swelling of carbopol to form a gel. It has been reported that under pH1.2, the extent of carbopol 974P swelling is less than that of HPMC *in vitro* [30]. Carbopol 974 unable to hydrate in 0.1 N HCL because of its pKa (6) and un-ionized nature [29]. Drug release retardation effect of HPMC may be due to nonionic nature of the polymer. Because of its nonionic nature, swelling is not affected by pH variation. In pH 1.2 HPMC may form viscous gel around the microspheres, keeping the active drug inside and limiting the release. HPMC K4M predominantly controlled release since carbopol has a low solubility at pH 1.2. Increasing the proportion of HPMC, sustained the drug release effect increases. Similar results were obtained by Prudat *et al*.,[31].

When the data were plotted according to the first order equation [32], the formulations showed a fairly good linearity, with a R^2 value of 0.7478–0.9856 whereas the same data, when plotted according to the zero order equation [32], improved the R^2 value 0.9852–0.9983 (Table 3). In our experiment, the *in vitro* release profiles of clarithromycin from all the formulations could be best expressed by Higuchi's equation [33], as the plots showed good linearity with R^2 value (0.99–0.9995). The slope of the regression line from the Higuchi plot, which reveals 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.,* [17] equation. Which showed high linearity with a comparatively high slope (n) value (0.5804–0.5973) (Table 3).

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

F code	Zero order plot		First order plot		Higuchi plot	Korsemeyer peppa's plot	
	K	R^2	K	R^2	R^2	n	R^2
FC1	---*	---*	---*	---*	---*	---**	---**
FC2	16.2790	0.9859	-0.6227	0.8662	0.9991	---**	---**
FC3	14.6220	0.9909	-0.4467	0.8182	0.9979	---**	---**
FC4	10.9857	0.9905	-0.2075	0.902	0.9978	0.5804	0.9998
FC5	15.6280	0.9963	-0.6398	0.8025	0.9979	---**	---**
FC6	12.6088	0.9983	-0.3402	0.7478	0.9900	0.5180	0.9998
FC7	11.6789	0.9970	-0.0144	0.9856	0.9927	0.5973	0.9937
FC8	17.2840	0.9882	-0.5807	0.8662	0.9969	---**	---**
FC9	10.3389	0.9852	-0.2033	0.9169	0.9995	0.5352	0.9999
FC10	9.3489	0.9938	-0.1115	0.9761	0.9943	0.5378	0.9977

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

The good linearity observed with the zero order equation. The slope of the regression line from the Higuchi plot indicates the rate of drug release and thus confirmed that the mode of release was diffusion, while to further confirm the diffusion mechanism, the data were fit into the Korsmeyer *et al.*, equation which showed high linearity with a comparatively high slope (n) value (0.5804–0.5973). This n-value, however, appears to indicate a coupling of diffusion and erosion mechanism, called anomalous diffusion. This indicates that drug release from the microspheres follows a non-Fickian trend as reported earlier [34, 35].

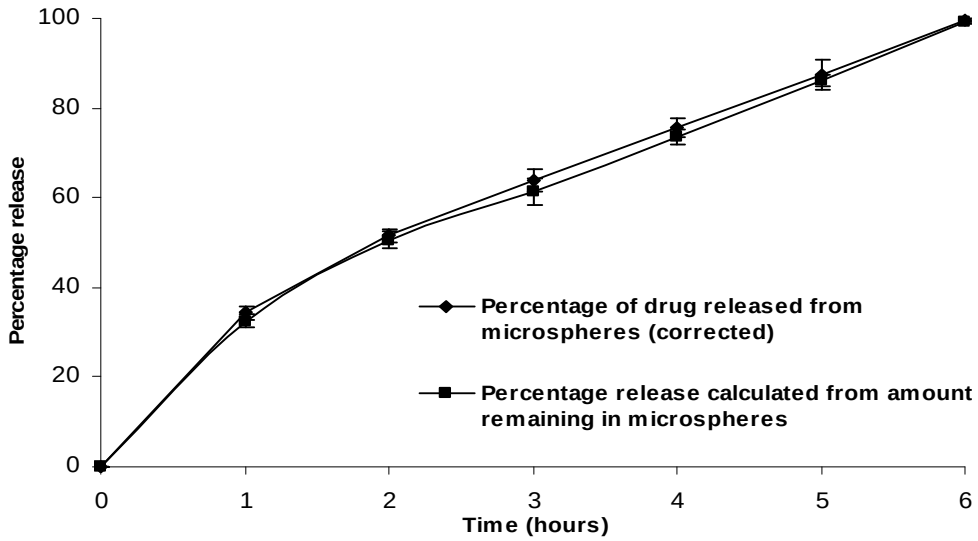

Figure 8: Stability of clarithromycin in mucoadhesive microspheres in pH 1.2 Bars represent mean±SD (n = 3)

There are several reports regarding clarithromycin stability in acidic solutions[14]. Clarithromycin degrades in acidic media into 5-O-desosaminyl-6-O-methyl-erythronolide A, with loss of the cladinose sugar [37]. In the resent 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 cumulative amount of clarithromycin 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 confirms that the drug remaining inside the microspheres were stable.

The optimized formulation (FC6) were stored in a stability chamber (Remi CHM- 10 S®, India) at 40 ± 2°C and humidity of 75 ± 5% RH for 6 months and observed for the drug content, mucoadhesiveness and *in vitro* drug

release 0, 30, 90, and 180 days. The zero time samples were used as controls. No remarkable changes were observed in drug content, mucoadhesiveness and *in vitro* drug release in stability studies.

CONCLUSION

Mucoadhesive microspheres of clarithromycin 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 clarithromycin can remain stable in the acidic environment of the stomach. Mucoadhesive microspheres can be effectively controls the release of clarithromycin 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.

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Declaration of interest
The authors report no conflict of interest. The authors alone are responsible for the content and writing of the article.

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