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PHARMACEUTICAL CHARACTERIZATION OF AMOXICILLIN TRIHYDRATE AS MUCOADHESIVE MICROSPHERES IN MANAGEMENT OF H. PYLORI

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ABSTRACT: *Helicobacter pylori* (*H. pylori*) infect more than half of the world population, making it one of the most prevalent infections. *H. pylori* is now accepted as the most common cause of histologic gastritis and is responsible for the majority of cases of peptic ulcer disease and gastric cancer. Approximately 1 in 6 (17%) persons with *H. pylori* infection will develop peptic ulcer disease, and each year 1% to 2% of these will experience a major or life-threatening complication, and this basically occurs due to short gastric residence time of antimicrobial agents, keeping that in mind mucoadhesive microspheres were prepared to increase gastric residence time using solvent evaporation method. The (mucoadhesive) sustained release of amoxicillin trihydrate is desired because of its short biological half-life. Predominantly to treat *H. pylori* infections, the mucoadhesive is desired to be confined to the upper gastrointestinal tract. Amoxicillin trihydrate mucoadhesive microspheres were prepared using Eudragit RS100 as matrix and HPMC K4M as mucoadhesive polymer for the potential use of treating gastric and duodenal ulcers, which were associated with *H.pylori.* The morphological characteristics of the mucoadhesive microspheres were studied under scanning electron microscope. The percentage yield of microspheres of all formulation was in the range of 78.90% to 90.95%. The drug content determination showed that even if the polymer composition was changed the solvent evaporation process was highly efficient to give microspheres having maximum drug loading. In termination, the prolonged gastrointestinal residence time and enhanced amoxicillin trihydrate stability resulting from the mucoadhesive microspheres of amoxicillin trihydrate might make contribution to *H. pylori* clearance.
Keywords: Mucoadhesive microsphere, Gastric residence time, Amoxicillin Trihydrate.

INTRODUCTION

Amoxicillin trihydrate (a-amino-hydroxybenzyl - penicillin) is a semi synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in a standard eradication treatment of gastric *H. pylori* infection combined with a second antibiotic and an acid-suppressing agent[1–3]. These triple therapies are proved to be effective in clinical application. However, some other reports and clinical trials indicate that the therapies cannot bring out complete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation[4-5]. One reason for the incomplete eradication of *H. pylori* is probably 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[6-7]. The other may be the degradation of amoxicillin trihydrate in gastric acid[8-9]. Therefore, some researchers had prepared and reported new amoxicillin trihydrate formulations, such as float tablet, mucoadhesive tablet and pH-sensitive excipient composition mucoadhesive microspheres etc., which were able to reside in the gastrointestinal tract for an extended period of time for a more effective *H. pylori* eradication[10–15]. Among these formulations, mucoadhesive microspheres have gained considerable attention due to their ability to adhere to the mucus layer, as well as to release the drug in a sustained manner. The rationale of this cram was to design and characterizes amoxicillin trihydrate mucoadhesive microspheres for *H. pylori* eradication therapy.

MATERIALS AND METHODS

Materials

Amoxicillin trihydrate (powder) and Eudragit RS100 was obtained as gift sample from Ranbaxy Pvt. Ltd Gurgaon**,** Hydroxypropylmethyl cellulose (K4M) was obtained as gift sample from Orchid Lab, Chennai, Span 80 was purchased from Loba Chemicals. Pvt. Ltd. Mumbai., Light Liquid Paraffin, Acetone AR, Concentrated HCl LR, Potassium dihydrogen phosphate AR, Sodium hydroxide LR were purchased from Nice Chemicals Pvt. Ltd. Cochin.

Methods

Preparation of Mucoadhesive Microspheres

Amoxicillin trihydrate microspheres were formulated using solvent evaporation technique, Using Eudragit RS100 as matrix polymer. Eudragit was dissolved in required quantity of acetone, the HPMC K4M and drug was dispersed with the polymer solution. The dispersed content was placed drop wise in light liquid paraffin containing span80 maintained at 40^{o}C while stirring at 750±50 rpm. The solvent, acetone was then removed by continuous stirring at room temperature for three hours to produce spherical microspheres. The microsphere were than separated from liquid paraffin by filtration through whatmann filter paper, the microspheres were collected and washed three times with n-hexane and dried using vacuum filtration. The product was then air-dried to obtain microspheres[16].

Physico-chemical and Morphological characterization of microspheres

The shape and surface characterization of microspheres were observed under a Scanning Electron Microscope (ZEOL JSM–5610). The microspheres were mounted directly on the SEM sample stub, using double-sided sticking tape, and coated with gold film (thickness 200 nm) under reduced pressure (0.001 torr) and photographed. The spectral characterizations for microsphere were done by using FTIR and XRD, Change in crystallinity were determined by DSC.

Determination of Percentage yield of microspheres

Thoroughly dried microspheres were collected and weighed accurately. The percentage yield was than calculated using formula given[17].

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

Swelling studies

A known weight of microspheres was placed in a glass vial containing 10ml of distilled water at 37-±0.5^{0}C in incubator with occasional shaking. The microspheres were periodically removed, blotted with filter paper and their changes in weights were measured during the swelling until equilibrium was attained. Finally, the weight of the swollen microspheres was recorded after a period of 3 hours, and the swelling ratio (SR) was then calculated from the formula. The studies were carried out in triplicate[18].

$$\text{Swelling Ratio (SR)} = \frac{We - W_O}{W_O}$$

Where,

Wo = Initial weight of the dry microspheres,

We = weight of the swollen microspheres at equilibrium swelling in the media.

In vitro wash-off test

The mucoadhesive property of microspheres was evaluated by an *In vitro* adhesion testing method known as wash-off method. Freshly excised piece of intestinal mucosa (2 x 2 cm) from goat were mounted on to glass slides (3 x 1 inch) with cyanoacrylateglue. Two glass slides were connected with a suitable support, about 100 microspheres were spread on to each wet rinsed tissue specimen and immediately thereafter the support was hung on to the arm of a USP tablets disintegrating test machine. When the disintegrating test machine was operated, the tissue specimen was given slow, regular up-and-down moment in the test fluid (900ml of 0.1N HCl) at 37± 0.5^{0}C. At the end of 30 min, at the end of one hour, and at the hourly intervals up to 5 hours, the machine was stopped and number of microspheres still adhering to tissue was calculated. The studies were carried out in triplicate[19].

Determination of drug content

Accurately weighed 100mg microspheres, were crushed in glass mortar and pestle and powder microspheres were suspended in 100ml of 0.1N HCl. After 12 hours the solution was filtered and the filtrate was analyzed for the drug content using UV-Visible spectrophotometer.

Encapsulation efficiency

Encapsulation efficiency was calculated using the following formula

$$\text{Encapsulation efficiency} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100$$

***In vitro* dissolution studies**

Dissolution studies were carried out for all the formulation, employing USP XXIII apparatus (Basket method) at 37± 0.5^{0}C rotated at constant speed of 50 rpm using 0.1N HCl as the dissolution medium. A sample of microspheres equivalent to 100mg of amoxicillin trihydrate was used in each test. An aliquot of the sample was periodically with drawn at suitable time interval and the volumes were replaced with fresh dissolution medium in order to maintain the sink condition. The sample was analyzed spectrophotometrically at 272 nm[20].

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, first order, Higuchi's, Peppas and Coefficient of correlation (r) values were calculated for the liner curves by regression analysis of the above plots[21].

RESULTS AND DISCUSSION

The microspheres were prepared by a solvent evaporation method, which was developed earlier by Bogataj. The different polymers, of which the microspheres were composed, were chosen with regard to their structure and mucoadhesive properties. Since most of mucoadhesive polymers are very hydrophilic and are not soluble in acetone (CMCNa, Carbopol), another acetone soluble polymer was used to form the matrix of microspheres and connected all other components. For that reason Eudragit RS100 as matrix polymer and HPMC K4M as potential mucoadhesives or as reference polymers for the preparation of microspheres. The spectral analysis shows that there was no appearance or disappearance of any characteristic peaks of pure drug amoxicillin trihydrate in the physical mixture of drug and polymer, which confirms the absence of chemical interaction between drug and polymers and method of preparation (figure 2 & 3). The DSC spectral analysis also reveals the same (figure 4 & 5). The XRD analysis of final formulation (figure 6, 7 & 8) shows that the peak intensity of Amoxicillin trihydrate slightly reduced it indicates that the crystallinity of amoxicillin trihydrate slowly reduced in formulation.

Morphological characterization of microspheres

The microspheres of all batches were found to be spherical and free flowing (Figure 1). The size range of different batches of microspheres was in the range of 533–588.02mm (Table 1). The drug entrapment efficiency analysis showed that the entrapment of drug within each batch of microspheres ranges from 80.22 to 81.46%w/w. The percentage yield of microspheres of all formulation was in the range of 78.90% to 90.95%. The microsphere prepared by this method was found to be discreet, spherical, and it was observed by scanning electron microscopy (SEM) (Figure 1) & (Table 1). The packing properties of the drug and the formulation widely depend upon bulk density. It has been stated that, bulk density values less than 1.2gm/cm3 indicate good flow and values greater than 1.5gm/cm3 indicate poor flow characteristic. It is seen from (Table 1) that the bulk density values are less than 1.2gm/cm3 indicating good flow characteristics of the microspheres. Angle of repose less than or equal to 40° indicates free flowing properties of the microcapsules. The angle of repose for all the formulations (F1-F8) is seen to be between 21°03' and 23°30' indicating good flow property.

Drug content

The drug content determination shows that even if the polymer composition was changed the process was highly efficient to give microspheres having maximum drug loading (Table 1). The entrapment efficiency was in the range of 72.03% to 82.15% (Table 1). Microspheres of amoxicillin trihydrate exhibited good mucoadhesive properties in the *in vitro* wash off test. The result of *in vitro* wash off test was shown in (Table 3).Swelling ratio was in the range of 0.2904 to 0.733 for all formulations shown in (Table 4) which indicates those polymers used in concentration are having better capability of swelling.

Drug release behavior

In-vitro drug release studies were carried out with formulations F1–F8 in 0.1N Hydrochloric acid for 12 hours in USP XXIII basket type dissolution tester. At the lowest concentration of the polymer the drug release from F1-F4 was within seven hours but as the concentration of the polymer increased up to maximum extent the drug release from F5-F8 was delayed up to 12 hours and further mucoadhesive strength confirmed the sustained release drug profile. The results of in vitro release study were summarized in (Table 2) (Figure 9).

Drug release kinetics

The *in-vitro* release data have been plotted according to the following models of data treatment, cumulative percent drug release versus time, log of cumulative drug retained versus time, and erosion plot of $(1\text{-}t/m)^{1/3}$

versus time (Table 4). Kinetics studies were done which shows that all the formulations exhibited anomalous (non-fickian transport) diffusion mechanism and follow zero order kinetic.

Accelerated stability studies

Stability studies were carried out with the optimized formulation F7 for 3 month in two condition i.e. 25ºC/60%RH and 40ºC/75%RH. As per ICH guidelines, the formulations were subjected to drug assay and *in vitro* dissolution studies. The statistical analysis of the parameters dissolution data, after storage for three month showed no significant change indicating that the two dissolution profiles were similar.

CONCLUSION

Amoxicillin trihydrate mucoadhesive microspheres were prepared successfully by using the solvent evaporation method. Polymer-drug ratio influences the particle size as well as drug release pattern of microsphere. The obtained microspheres are fine and free flowing, the method followed is economical to get reproducible microspheres, and the drug:polymer ratio has an impact on the drug encapsulation efficiency and *in vitro*. The release from adhered microspheres is influenced by at least four parameters: type of mucosa, mucoadhesion strength and swelling of polymers, and retardation properties of microspheres. The yield was high and encapsulation efficiency was good for all the preparation, but was highest for F3 formulation. The assessment of release kinetic showed that drug release from amoxicillin trihydrate mucoadhesive microsphere followed the matrix-Higuchi model (Diffusion-controlled drug release mechanism). From the study, it was concluded that the amoxicillin trihydrate mucoadhesive microsphere prepared with HPMC K4 and Eudragit RS100 shows delayed release rate of drug as the concentration of both the polymer were simultaneously increased. The formulation F7 (Amoxicillin trihydrate 250mg equivalent to 235 mg of Amoxicillin, HPMC K4M 480mg, Eudragit RS100 1000mg) was selected as optimized formulation for further factorial design; with 96.15% of drug release at 12^{th} hour. The prepared microspheres proved to be good candidate for site-specific drug release.

Table 1: Technological characterization of formulated Amoxicillin Trihydrate powder blend and microsphere formulation*

Parameters	Amoxicillin trihydrate	F1	F2	F3	F4	F5	F6	F7	F8
Angle of repose	40.28 ± 0.31	24.15 ± 0.55	25 39 ± 0.51	25.23± 0.90	26.51 ± 0.92	22. 56 ± 0.19	22.24 ± 1.05	22.27 ± 0.93	25.10 ± 0.15
Bulk density* (gm/cm3)	-	0.592 ± 0.10	0.624 ± 0.18	0.624 ± 0.12	0.596 ± 0.10	0.622 ± 0.18	0.610 ± 0.12	0.598 ± 0.15	0.622 ± 0.12
Arithmetic mean diameter (mm)	-	533	572.53	577.57	589.71	560.27	567.96	578.1	588.02
Entrapment Efficiency in %	-	80.22	75.04	79.03	78.56	80.10	82.15	72.03	81.46
Percentage drug content	-	80.22 ± 0.99	75.04 ± 1.08	79.03 ± 1.09	78.56 ± 0.47	80.10 ± 1.00	82.15 ± 0.61	72.03 ± 1.55	81.46 ± 1.11
Percentage Yield	-	82.64	78.90	90.95	86.53	80.50	83.16	80.93	82.64

***All values are mean ± S.D. for n=3**

Table 2: *In vitro* drug release profile of various formulations *

Time in hours	F1	F2	F3	F4	F5	F6	F7	F8
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	24.61 ± 1.1	27.17 ± 0.78	21.30 ± 1.64	22.56 ± 0.43	13.84 ± 0.74	14.36 ± 0.74	10.77 ± 0.33	12.31 ± 0.35
2	33.97 ± 1.26	39.11 ± 0.57	29.34 ± 1.2	31.40 ± 0.45	24.17 ± 0.5	25.20 ± 0.29	16.98 ± 0.14	19.04 ± 0.07
3	52.10 ± 1.78	60.34 ± 0.95	47.44 ± 0.71	49.51 ± 0.7	41.73 ± 1.54	44.30 ± 0.86	28.86 ± 1.69	30.42 ± 0.81
4	64.17 ± 1.01	86.81 ± 0.89	58.98 ± 1.37	61.06 ± 0.15	54.78 ± 0.70	56.34 ± 1.15	40.81 ± 0.19	41.86 ± 1.8
5	84.52 ± 1.1	93.96 ± 1.67	71.09 ± 1.07	76.77 ± 0.35	62.77 ± 1.54	64.34 ± 0.76	50.77 ± 0.9	52.86 ± 0.81
6	95.74 ± 0.73	99.60 ± 0.75	82.25 ± 0.9	95.64 ± 0.46	72.85 ± 0.93	76.99 ± 0.11	60.28 ± 1.31	61.35 ± 0.49
7	99.86 ± 0.23	-	92.95 ± 1.08	99.76 ± 0.34	78.89 ± 0.97	82.54 ± 1.35	70.86 ± 1.07	73.47 ± 0.65
8	-	-	99.09 ± 1.53	-	84.44 ± 0.34	91.70 ± 0.73	78.94 ± 1.79	80.54 ± 0.75
9	-	-	-	-	96.69 ± 0.41	95.79 ± 0.8	81.42 ± 1.68	84.57 ± 0.59
10	-	-	-	-	99.26 ± 0.31	99.38 ± 0.30	84.42 ± 1.06	90.15 ± 0.45
11	-	-	-	-	-	-	94.10 ± 1.66	94.22 ± 0.25
12	-	-	-	-	-	-	96.15 ± 0.88	96.78 ± 0.32

***All values are mean ± S.D. for n=3**

Table 3: Technological characterization for mucoadhesion *in vitro* wash-off test

Mean Percentage of microspheres adhering to tissue *(n = 3)*						
0.1 N HCl						
Formulation Code	**0.5 hr**	**1 hr**	**2 hr**	**3 hr**	**4 hr**	**5 hr**
F1	74 (5.4)	64.66 (4.96)	65.33 (5.36)	63.66 (5.94)	58.33 (2.6)	61.33 (4.08)
F2	85 (3.52)	86.33 (4.37)	76 (5.73)	78.66 (1.94)	76 (5.26)	77.66 (4.14)
F3	76.66 (4.27)	74 (3.57)	68.33 (3.04)	66.66 (3.77)	64.66 (6.24)	64.66 (4.97)
F4	93.66 (4.31)	86.66 (2.40)	82.66 (5.71)	83.66 (3.00)	78 (2.56)	76.66 (3.28)
F5	72.66 (7.56)	65 (5.54)	66 (3.03)	62.66 (4.87)	63.33 (7.77)	65.66 (5.34)
F6	83 (6.26)	80 (2.5)	77.66 (1.96)	77 (2.59)	75 (1.33)	73.66 (6.83)
F7	69 (1.44)	70 (2.85)	66 (3.03)	65.66 (4.65)	64.66 (3.21)	60.33 (2.53)
F8	88.33 (2.35)	85.33 (3.58)	86 (2.32)	84 (2.38)	83.33 (2.42)	81 (1.23)

Number in parenthesis indicates the coefficient of variance (CV) or percentage relative standard deviation (% RSD).CV = (Standard deviation / Mean)* 100

Table 4: Technological characterization for Swelling Ratio Entrapment Efficiency of various formulations

Formulation Code	0.5 hr	1 hr	2 hr	3 hr
F1	0.3477 ± 0.0061	0.4401 ± 0.0006	0.5068 ± 0.0009	0.609 ± 0.013
F2	0.436 ± 0.0006	0.5246 ± 0.0008	0.6318 ± 0.0012	0.696 ± 0.0252
F3	0.4208 ± 0.0006	0.4718 ± 0.0013	0.6114 ± 0.0013	0.677 ± 0.0096
F4	0.46 ± 0.0006	0.5403 ± 0.0004	0.6598 ± 0.0004	0.723 ± 0.004
F5	0.2904 ± 0.0004	0.4276 ± 0.0003	0.4818 ± 0.0004	0.588 ± 0.004
F6	0.3861 ± 0.0005	0.4599 ± 0.0006	0.5345 ± 0.0007	0.634 ± 0.0055
F7	0.3914 ± 0.0013	0.4667 ± 0.0005	0.5766 ± 0.0007	0.663 ± 0.0066
F8	0.4464 ± 0.0005	0.5369 ± 0.0004	0.6839 ± 0.0005	0.733 ± 0.009

***All values are mean ± S.D. for n=3**

Table 5: *In vitro* release kinetic data for various formulations

Formula code	Zero order		First order		Higuchi's	Korsmeyer-Peppas	
	Rate constant K_O	Correlation coefficient R	Rate constant K_1	Correlation coefficient r	Correlation coefficient R	n	Correlation coefficient r
F1	14.5047	0.9909	-0.7719	-0.8637	0.9733	0.7766	0.9902
F2	17.1453	0.9823	-0.8234	-0.9138	0.9724	0.7883	0.9861
F3	12.3462	0.9932	-0.4901	-0.8986	0.9778	0.7861	0.9932
F4	14.4215	0.9947	-0.7147	-0.8596	0.9667	0.8143	0.9904
F5	10.0082	0.9872	-0.4081	-0.8991	0.9803	0.8723	0.9931
F6	10.1750	0.9825	-0.4276	-0.9213	0.9813	0.8644	0.9914
F7	8.3368	0.9877	-0.2552	-0.9608	0.9744	0.9352	0.9932
F8	8.4348	0.9863	-0.2744	-0.9705	0.9765	0.8901	0.9941

Figure 1: Scanning electron micrograph of amoxicillin trihydrate microspheres

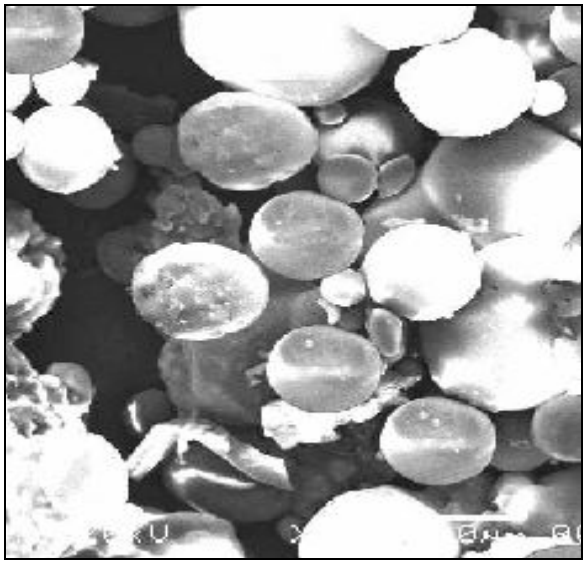


Figure 2: FTIR Spectra of Amoxicillin trihydrate

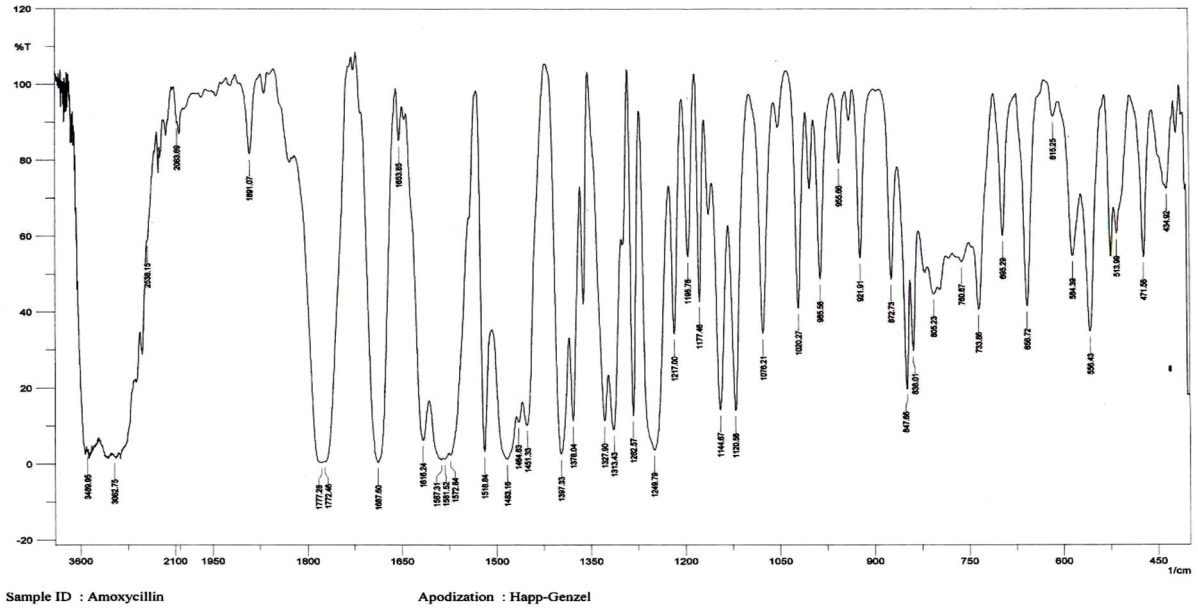


Figure 3: FTIR Spectra of Physical Mixture of Amoxicillin trihydrate, HPMC K4 and Eudragit RS100

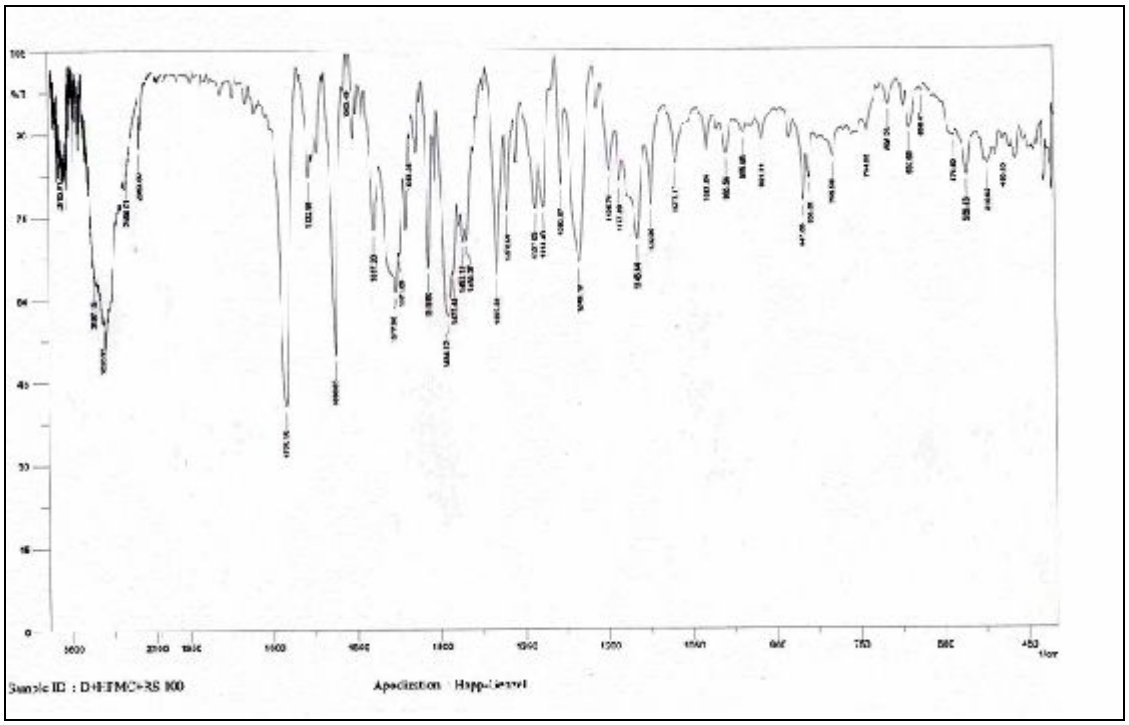


Figure 4: DSC Spectra of Amoxicillin trihydrate

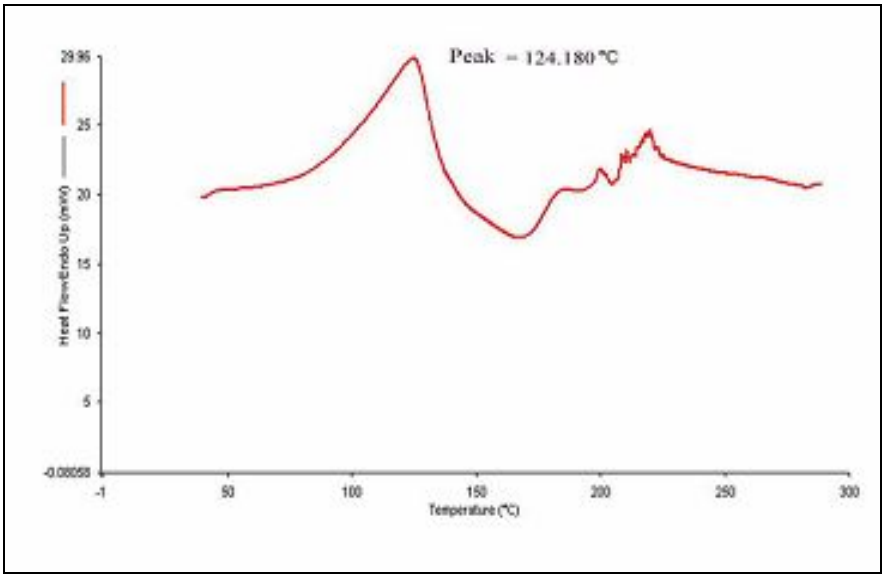


Figure 5: DSC Spectra of Physical mixture of Amoxicillin trihydrate HPMC K_4M and Eudragit RS100

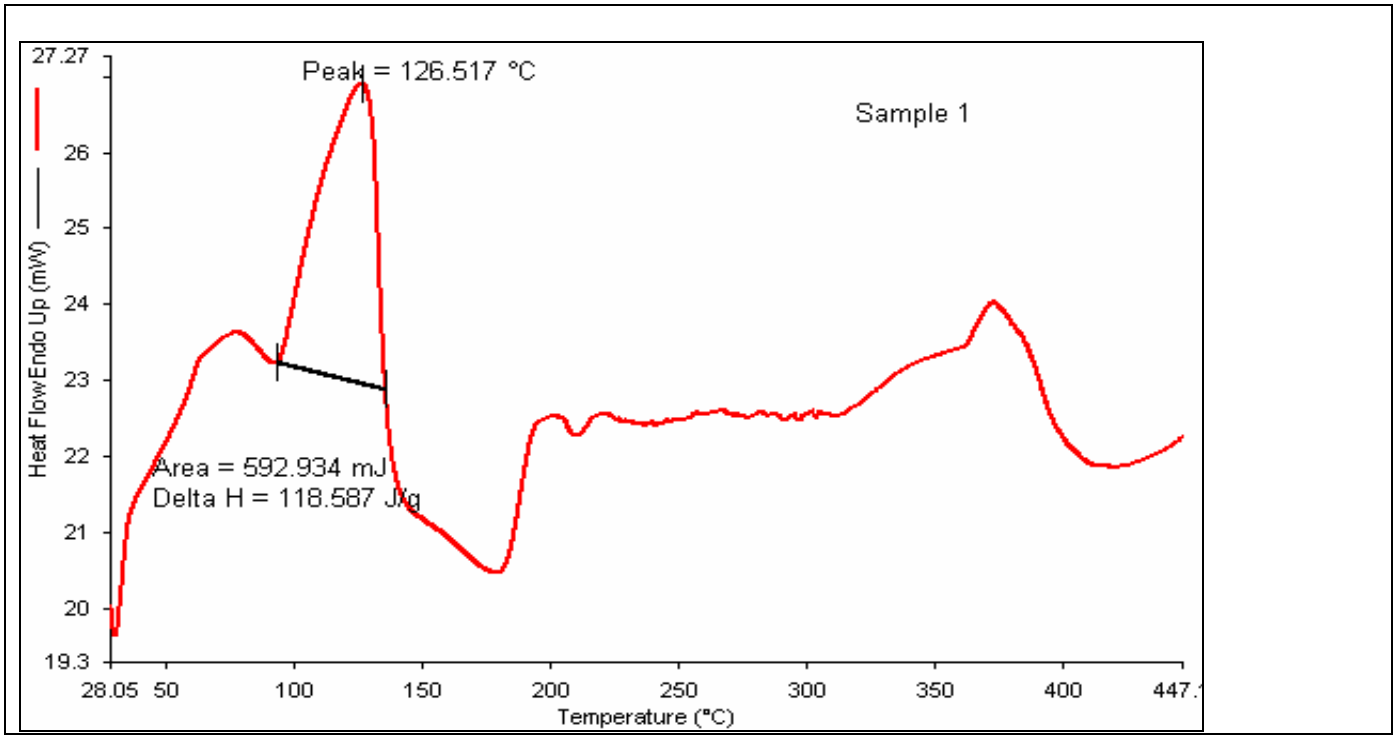


Figure 6: XRD Pattern of Amoxicillin Trihydrate

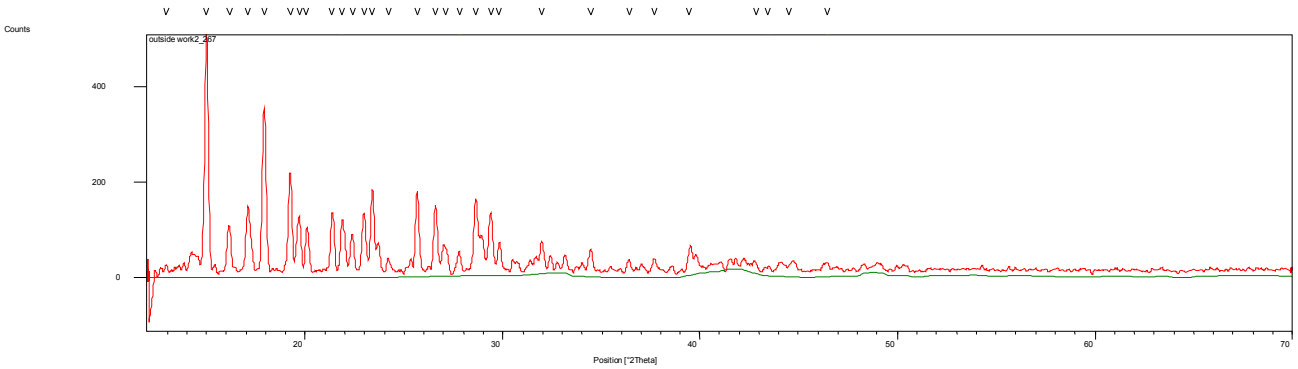


Figure 7: XRD Pattern of Physical mixture of Amoxicillin trihydrate HPMC K_4M and Eudragit RS100

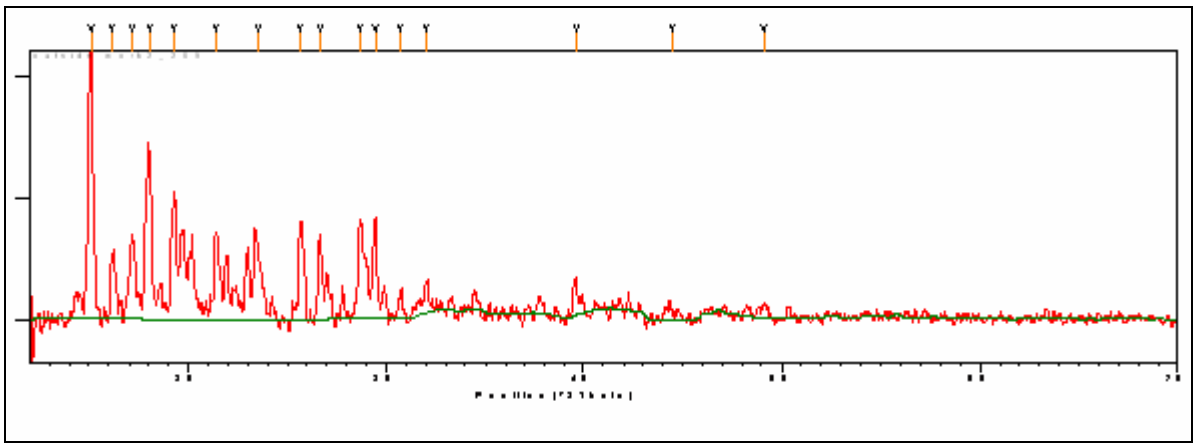


Figure 8: XRD Pattern of Formulation

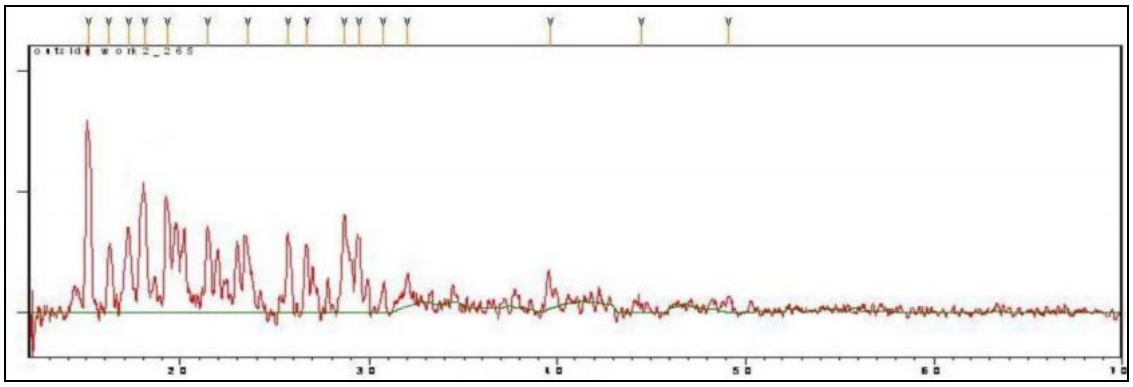
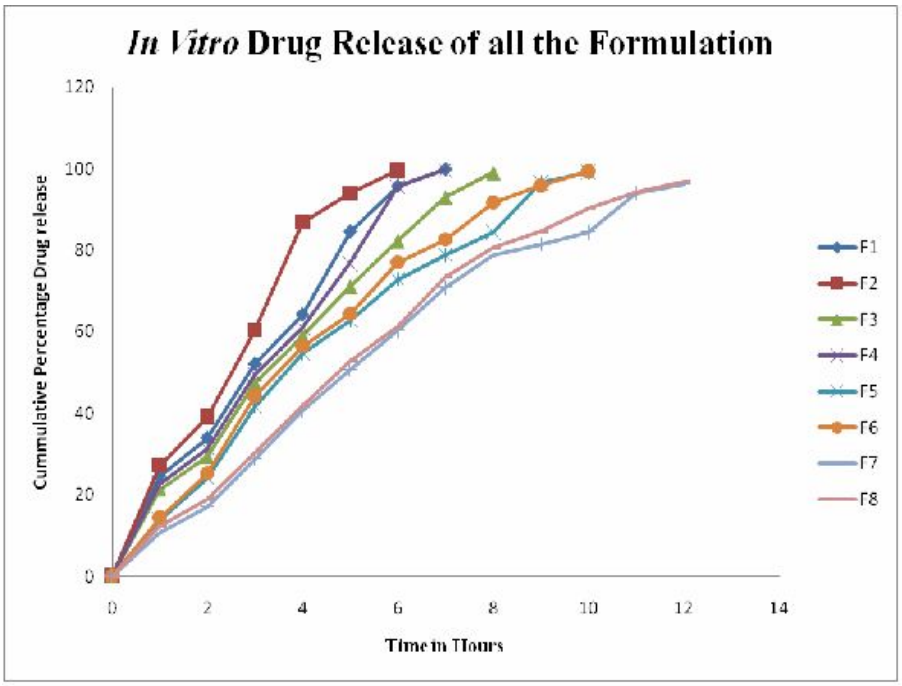


Figure 9: In vitro drug release F1-F8 Formulation



REFERENCES

1. Suleymanlar I., *et.al.* Response to triple treatment with omeprazole, amoxicillin trihydrate and clarithromycin for Helicobacter pylori infections in continuous ambulatory peritoneal dialysis patients, Adv. Perit. Dial, 1999, 15, 79-81.
2. Vakil N. and Cutler A., Ten-day triple therapy with ranitidine, bismuthcitrate, amoxicillin trihydrate and clarithromycin in eradicating Helicobacter pylori, Am. J. Gastroenterol, 1999, 94 (5), 1197-1199.
3. Buzas G.M. and Szekely E., Eradication of Helicobacter pylori in peptic ulcer patients, Orv. Hetil 1999, 140 (3), 121-124.
4. Lin C.K., Hsu P.I. and Lai K.H., One-week quadruple therapy is an effective salvage regimen for Helicobactere pylori infection in patients after failure of standard triple therapy, J. Clin. Gastroenterol, 2002, 34 (5), 547-551.
5. Kawabami E., Ogata S.K. and Portorreal A.C., Triple therapy with clarithromycin, amoxicillin trihydrate and omeprazole for Helicobacter pylori eradication in children and adolescents. Arq. Gastroenterol 2001, 38 (3), 203-206.
6. Cooreman M.P, Krausgrill V. and Hengels K.J., Local gastric and serum amoxycillin concentrations after different oral application forms, Antimicrobial Agents Chemotherapy, 1993, 37, 1506-1509.
7. Atherton J.C, Cockayne V., Balsitis M., Kirk G.E., Hawley C.J. and Spiller R.C., Detection of the intragastric sites at which Helicobacter pylori evades treatment with amoxicillin trihydrate and cimetidine, Gut, 1995, 36, 670-674.
8. Axon A.T., The role of acid inhibition in the treatment of *H. pylori.* Infection. Scand., J. Gastroenterol, 1994, 29, 16-23.
9. Giacomo F., Mariano L., Silvana M., Domenico S., and Gaetano G., Amoxicillin trihydrate-loaded polyethylcyanoacrylate nanoparticles: influence PEG coating on the particle size, drug release rate and phagocytic uptake, Biomaterials, 2001, 22, 2857-2865.
10. Hilton A.K. and Deasy P.B., *In vitro* and *in vivo* evaluation of an oral sustained-release floating dosage form of amoxicillin trihydrate, Int. J. Pharm, 1992, 86, 79-88.
11. Chen C.J, Jacob J., Klein A.B, Chayapruks T. and Mathiowitz E., Bioadhesive polymers for stomach targeted drug delivery, Proceedings 24th International Symposium on Controlled Release of Bioactive Materials. Stockholm 1997, 259-260.
12. Nagahara N., Akiyama Y., Nakao M., Tada M., Kitano M. and Ogawa Y. Mucoadhesive microspheres containing amoxicillin trihydrate for clearance of *H. pylori.* Antimicrob. Agents Chemother 1998, 42 (10), 2492-2494.
13. Wang J, Tauchi Y, Deguchi Y, Morimoto K, Tabata Y and Ikada Y. Positively charged gelatin microspheres as gastric mucoadhesive drug delivery system for eradication of *H. pylori*. Drug Deliv 2000, 7 (4), 237-243.
14. Clausen AE and Bernkop-Schnurch A. Direct compressible polymethacrylic acid–starch

compositions for site-specific drug delivery, J. Control. Release, 2001, 75 (1–2), 93-102.
15. Cuna M., Alonso M.J. and Torres D., Preparation and *in vivo* evaluation of mucoadhesive microparticles containing amoxicillin–resin complexes for drug delivery to the gastric mucosa, Eur. J. Pharm. Biopharm, 2001, 51, 199-205.
16. Bogataj M., Mrhar A., Kristl A. and Kozjek F., Eudragit E microspheres containing bacampicillin: preparation by solvent removal methods, J. Microenacapsul, 1991, 8, 401–406.
17. Arul B., Kothai R. and Sangameswaran B. *et.al.,* Formulation and evaluation of micro spheres containing isoniazid, Indian Journal of Pharmaceutical Science ,2003, 65, 640-642.
18. Raghavendra C. Mundargi *et.al.,* Formulation and *in-vitro* evaluation of noval starch-based tableted microsphere for controlled release of amplicillin, Carbohydrate polymer, 2008, 71, 42-53.
19. Lehr CM, Bowstra JA, Tukker JJ and Junginer HE. Intestinal transit of bioadhesive microspheres in an in situ loop in the rat. Journal of Controlled Release 1990, 13, 51-62.
20. Zhepeng Liua, Weiyue Lua, Lisheng and Qianb *et.al., In vitro* and *in vivo* studies on mucoadhesive microspheres of amoxicillin, Journal of Controlled Release, 2005, 2, 135-144.
21. Paulo Costa and Jose Manuel Sousa Lobo. Modeling and comparison of dissolution profiles, European Journal of Pharmaceutical Sciences, 2001, 13, 123-133.

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