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DESIGN, *In-vitro* AND *In-vivo* EVALUATIONS OF CLARITHROMYCIN BIOADHESIVE TABLETS FOR PEPTIC ULCER

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ABSTRACT

The present study of this research work was to formulate bioadhesive tablets of clarithromycin for the treatment of gastric ulcers caused by H. pylori bacteria. Clarithromycin is a semisynthetic macrolide antibiotic for the treatment of peptic ulcer. The tabets were formulated by synthetic polymer by carbopol- 934 for controlled drug delivery of 24 hrs. The drugs and excipients were subjected to compatibility studies using FTIR. The in process evaluation studies were performed for tablet processing. The formulated and selected tablets were performed for in- vitro dissolution studies. Adhesive properties of polymers and tablets were confirmed by ex vivo methods. The duration of binding were determined by pre-clinical studies in rabbits as in-vivo methods. The tablets were subjected to stability studies. The study finally concluded that clarithromycin bioadhesive tablets loaded by carbopol 934 polymer is a good controlled release tablets for mucoadhesion due to good release retarding mechanism and carbopol 934 is synthetic polymer for binding action.

KEYWORDS: Clarithromycin, mucoadhesion, carbopol -934, Ex-vivo methods, in- vitro studies, in -vivo studies, X - ray imaging technique.

INTRODUCTION

Macrolide antibiotics considered as the first line drugs for the treatment of peptic ulcer caused by H. pylori bacteria. Clarithromycin is effective for controlled drug delivery forms due to low half-life (3-4 hrs). The present study aims to formulation of once daily tablets. Using carbopol 934 as synthetic polymer.[1] The pre formulation studies of drug and excipients were performed, including FTIR compatibility study. The selected formulations were evaluated for drug release study by *in –vitro* dissolution test. The binding property of polymer and tablets were determined by *ex- vivo* methods using goat ilium.[2] The adhesive binding duration of tablets were confirmed by pre- clinical studies of *in- vivo* methods by X-ray imaging technique. The optimized formulation were subjected to stability studies as per ICH guidelines.[3]

MATERIALS AND METHODS

Chemicals and reagents: All chemicals, reagents and solvents used in the study were of analytical grade.

Pre formulation studies: Pre-formulation may be described as a phase of the research and development process where the formulation scientist characterizes the physical, chemical and mechanical properties of new drug substances, in order to develop stable, safe & effective dosage forms.[4]

Drug excipient compatibility studies: Drug and excipient were analyzed by IR spectral studies by using KBr pellet technique. In this method, the drug and KBr were mixed at the ratio of 1:300. Then these mixtures were pressed in to a pellet. The FTIR spectra were recorded using KBr pellet method in the region of 400-2000 cm^{-1}.[5]

Assay of clarithromycin Powder

By UV method

UV analysis: Clarithromycin Clarithromycin solution (100 µg mL-1) in 1M H_2SO_4 was scanned in UV spectrophotometer over wavelength range 200–400 nm, the wavelength of maximum absorption (λmax) was found to be at 210 nm.[6]

Formulation of Clarithromycin mucoadhesive Tablets

The component of each formulation made to the preparation of 250 tablets as shown in the tables. All the the components were sifted through mesh no (#40). Clarithromycin was mixed with polymer and followed by diluents like lactose and micro crystalline cellulose. The powder mixture were subjected to lubrication with half portions of lubricants like magnesium stearate, talc and aerosol and compressed with oblong shape punches (slugging). Which were made into size reduction (sluggs) and sieved through mesh no #16 and added remaining portion of lubricants. The powder was compressed using oblong shape punches.[7]

Table. 1: Formulation of Clarithromycin Tablets by carbopol-934 Polymer.

Dry granulation (mg/tablet)							
Ingredients	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Clarithromycin	500	500	500	500	500	500	500
Lactose	40	40	40	45	35	40	45
Carbopol 934	85	95	100	103	107	109	100
MCC	30	32	34	33	29	30	25
Magnesium stearate	10	10	10	10	10	10	10
Talc	10	10	10	10	10	10	10
Aerosol	15	15	15	15	15	15	15
Avg. wt of tablet	690	702	709	710	706	714	705

Evaluation of Mucoadhesive tablets

Tests for Content Uniformity

Physical properties of tablets the optimized formulation were selected for different physical property tests. Like thickness,hardness,friability and weight variation. Results are discussed in table no: 02.

Test for content uniformity: Determination of λ max was done by UV spectrophotometric method. The amount of drug, which is present in the tablets, were determined by Spectrophotometric method using 0.1M H_2SO_4.[8]

***In-vitro* dissolution study of clarithromycin mucoadhesive tablet**

In-vitro release study of tablets was done by using USPXXII. dissolution test apparatus for each formulations. Jar was filled with HCl buffer pH 1.2 and temperature was maintained at 37±0.5 0C. Paddle was revolved at 100 rpm speed. Five ml of sample was withdrawn after interval of 2 hour and replaced with 5 ml of fresh dissolution medium to maintain sink condition. Samples were then analyzed spectrophotometric ally for drug content at 210 nm.[9]

Determination of adhesive strength of polymer (ex- vivo)

Wihelmy's method

Take a small slide of (2×5 cm) length. Which is coated by 1% W/V solution of mucoadhesive agent. The slide were dipped in the mucin solution in beaker by maintaining the temperature 30^0C. the one end of the slide is connected to nylon thread and the other end is to keep the weights. The slides were withdrawn in different time interwels of 5,10,15,30 minutes. The experiments were performed for selected formulation.[10]

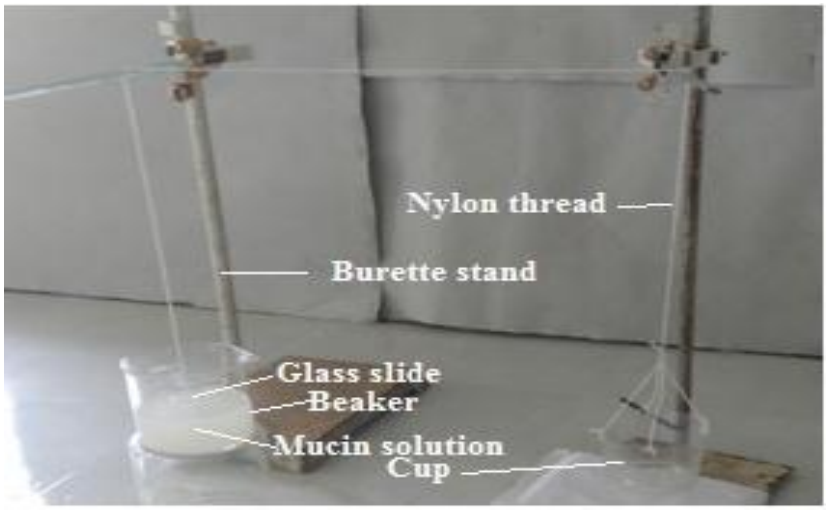

Fig. 1: Wihelmy's Method to Measure Mucoadhesive Strength.

Shear stress method: Glass plates were taken and mucoadhesive polymer were kept inside. The experiment was performed for carbopol-934 polymer. Different concentration like 1%,2% & 3% were made and arranged 3 sets of glass plates. 100gm wt rolled over the plates to improve the adhesion uniformly. The time taken to move the distance from the initial point in 15, 30, 60 minutes were determined.

Study of Mucoadhesive strength for tablets

Measurement of adhesive strength by *in –vitro* wash off test

The experiment was performed by disintegration test apparatus. The cylinder part of disintegration tester was removed. This is replaced by glass slide (10× 2 cm2). The slide was attached with stainless steel plate. The intestine part of $3\times2cm^2$ was fitted on the slide and tied with thread. The tablet was pressed with pressure and dipped the slide in 500ml of 0.1N solution and operated the machine. The time to detach the tablet from tissue surface was considered as wash of time for the tablet.[11]

Fig. 2: *in –vitro* wash off test method.

Detachment force measurement

This is the method used to measure *in -vitro* mucoadhesive capacity of different polymers.

Method: Immediately after slaughter, the intestines was removed from the goat and transported to laboratory in tyrode solution is (gm/litre); (sodium chloride 8 gm; potassium chloride 0.2 gm; calcium chloride $2H_2O$ 0.134 gm; sodium bicarbonate 1.0 gm; sodium di hydrogen phosphate 0.05 gm and glucose H_2O 1gm). During this experiment take the intestine in a specified area and place it on one glass slide and tie it. The glass slide with the intestine was affixed on one side floor below the modified physical balance. Already prepared 200 mg plain polymer tablet was pasted in another glass slide and it balanced in the assembled physical balance with a beaker in other side which is used to hold the water.[12]

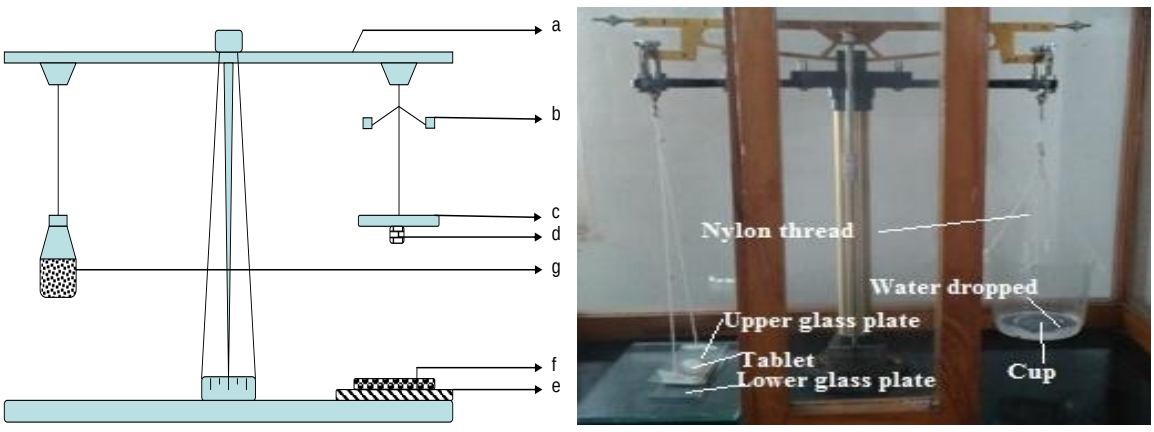

Fig. 3: Detachment force measurement method

a → Modified physical balance b → Weights c → Upper Glass Slide d → Tablet.
e → Lower glass slide f → Goat intestine g → Beaker which hold water.

***In -vivo* Bioadhesive study**

Determination of gastric retention time by X-ray imaging technique: Healthy adult Male New Zealand white strain rabbits weighing 1.5 to 2.5 kg were used for the study. The animals were housed in cages and were kept in well ventilated with 100% fresh air by air handling unit., 12 light/dark cycle were maintained. Room temperature was maintained between 22±2°C and relative humidity 50–65%. They were provided with food and water *ad libitum.*[9] All the animals were acclimatized to the laboratory for 14 days before the start of the study. The experimental protocol was approved by the Institutional Animal Ethics Committee.[13]

Drug administration: Animals were divided in to two groups. Group I administered with clarithromycin tablet coated with carbopol-934 polymer All animals were fastened for overnight with free access to water and before drug administration rabbits were feed orally with 2 ml of saline for evaluating the floating nature of the tablet within the stomach. Dose of drug is 40 mg/Kg.[14]

Radio graphical examination X-ray: After administration of standard and trial drug by intramuscular route, rabbits were anesthetized with ketamine and xylazine anesthetic agents and were exposed to X-ray to ascertain the location and nature of tablet in the stomach.[15]

Stability Studies: The purpose of stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light and to establish a re-testing for the drug substance or a self-life for the drug product and recommended storage conditions.[16] So, formulation No. F6 was subjected to determine its shelf life i.e. stability study by using accelerated stability chamber. The tablets were packed and stored in the stability chamber under desired temperature and humidity given below for six month.

RESULTS AND DISCUSSIONS

Pre formulation studies of tablets: The results of micrometric properties were performed. The results indicate that clarithromycin raw material shown passable flow property with the angle of repose of 32.92^0 and the granule ready for compression was found to be 28.76^0, it shows good flow property. The bulk density, tapped density, compressibility index and hausner ratiowere observed.it revealed that the formulated granules shown good flow characters and good compression capacity.

Drug excipient compatibility studies

FTIR analysis of Clarithromycin: An FTIR spectrum of clarithromycin was obtained in the range of 400-4000 cm^{-1} using KBr pellet technique and the peaks mentioned in standards were compared with those obtained.

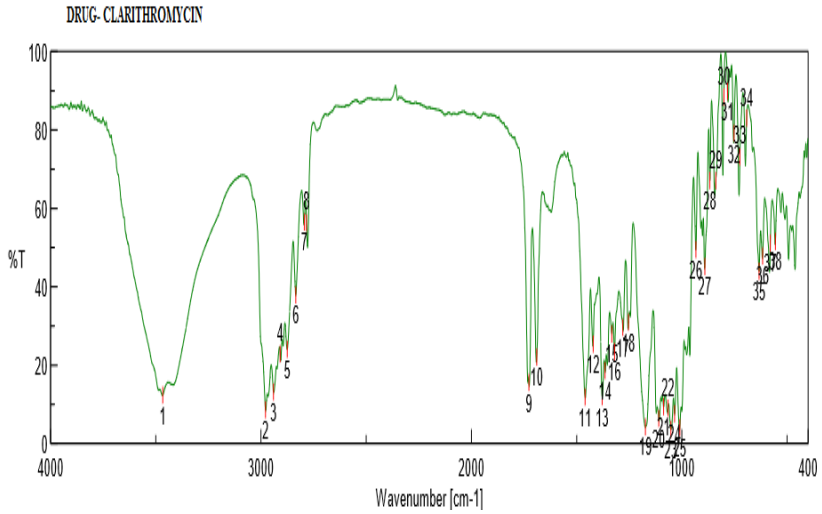

Fig. 4: FTIR spectrum of clarithromycin raw drug.

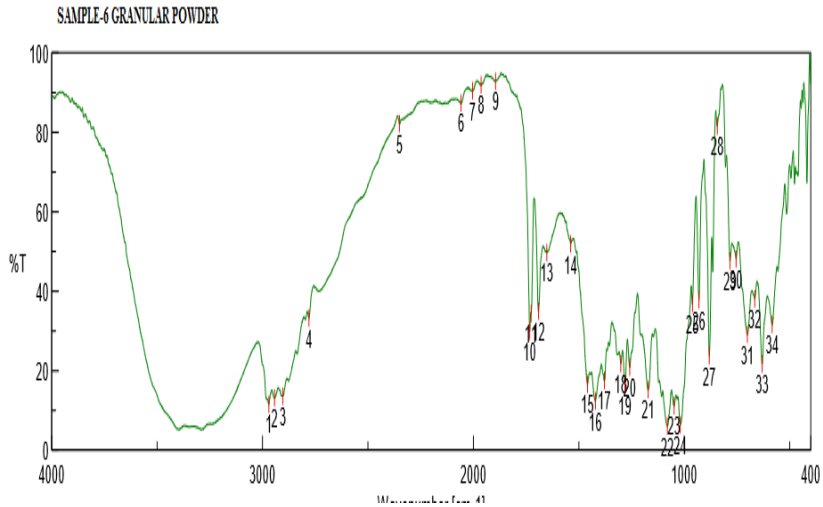

Fig. 5: FTIR spectrum of mixture of clarithromycin with polymer for compression.

FTIR spectrum of granules of the formulation

Also FTIR study of polymers, combination of polymers and whole granular powder also performed for incompatibility test. The spectrum was obtained in the range of 400-4000 cm^{-1} using KBr pellet technique and the peaks mentioned in standards were compared with those obtained. There was no evidence of any interaction between drugs and polymers.

Formulation and evaluation of clarithromycin mucoadhesive tablet

Table. 2: In-process evaluation of formulated tablets

formulations	Wt. variation mg	Thickness (mm)	Length (mm)	Breadth (mm)	Hardness (Kg/cm^2)	Friability (%)	% drug content %w/w
F1	690	5.65	10.95	9.46	6.5	0.74	93.46
F2	702	5.63	10.96	9.48	5.8	1.02	94.56
F3	709	5.65	10.96	9.46	6.2	0.85	95.62
F4	710	5.64	10.95	9.47	6.0	0.91	93.21
F5	706	5.66	10.96	9.48	5.9	0.80	92.15
F6	714	5.67	10.95	9.46	6.2	0.76	95.66
F7	705	5.64	10.95	9.47	5.9	0.86	94.23

All the tablet formulations showed acceptable pharmaco technical properties.

Table. 3: *In -Vitro* Dissolution Study Clarithromycin Mucoadhesive Tablet.

Cumulative % Drug release of different formulations							
Time (hr)	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
1	7.69	7.45	6.39	5.65	5.37	5.05	8.25
4	32.10	27.62	29.91	27.08	16.22	22.74	29.23
8	59.58	45.91	55.05	45.82	35.20	37.11	39.75
12	86.84	74.79	75.17	58.45	51.48	54.08	50.25
14	97.01	86.71	89.45	70.29	58.51	64.59	65.24
16	-	98.51	101.02	77.94	63.62	71.14	80.24
18	-	-	-	84.74	72.77	79.79	85.24
20	-	-	-	94.60	81.79	87.91	96.98
22	-	-	-	99.48	85.37	93.85	104.61
24	-	-	-	-	95.11	99.78	-

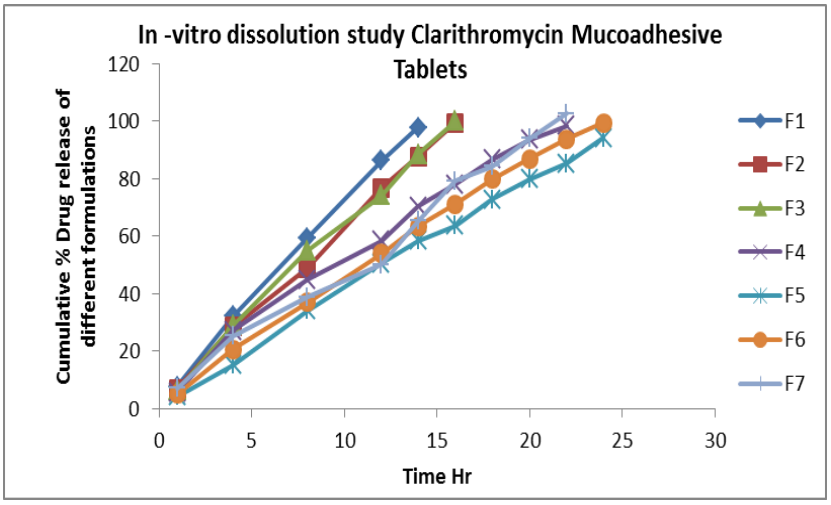

Fig. 7: *in-vitro* drug release of different formulations.

Determination of adhesive strength of polymers

Wihelmy's method

Table. 4: mucoadhesive strength of Carbopol- 934 polymer.

Time (minutes)	Mucoadhesive strength (gm).n =3
	Carbopol-934
05	0.99
10	1.39
15	1.58
30	1.61
60	2.11

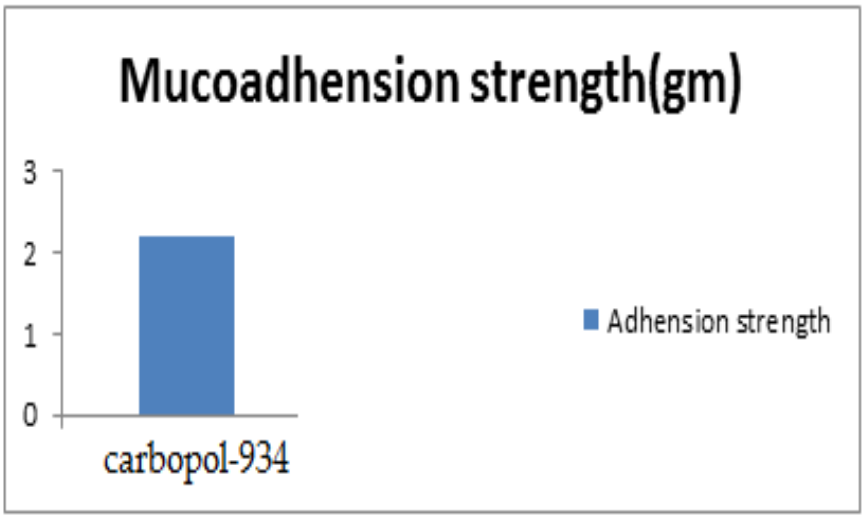

Fig. 8: Muco adhesion strength at 60th minute.

The mucoadhesive strength for carbopol-934 polymer were performed upto 60 minutes. It shows that when time continuing the adhesive strength of polymer increases. The polymer shows more adhesive strength.

Shear stress method

Table. 5: Mucoadhesive strength of different polymers.

Time (minutes)	Wt required (adhesion strength,gm)
	Carbopol-934
05	1.78
10	1.89
15	2.01
30	2.25

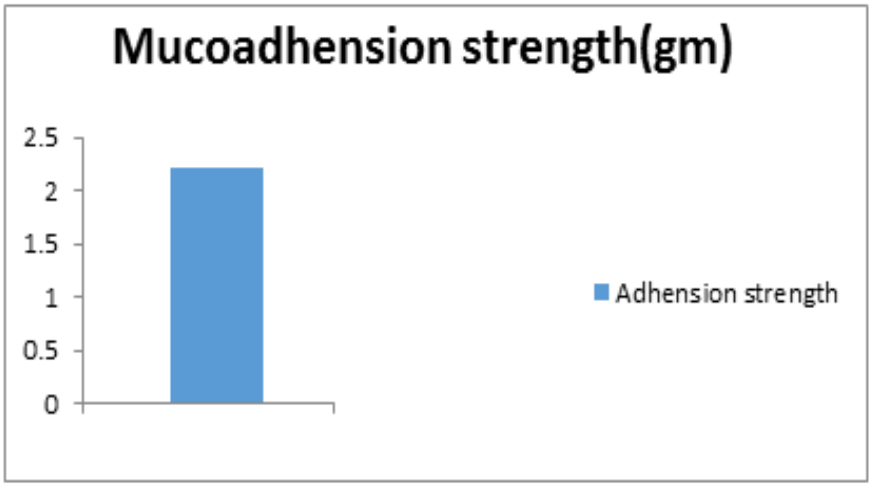

Fig. 9: Muco adhesion strength at 30th minute.

Shear stress method was performed for different polymers in different time intervals. The studies were performed for carbopol -934 polymer. The weight required to pull the glass plate in each time interval increases with increase in time. The polymer shows more adhesive strength.

Determination of adhesive strength for tablets

Detachment force method Selected formulation (F6)

Table. 6: Mucoadhesive strength by detachment force method.

TIME	5 minutes	10 minutes	15 minutes	30 minutes
Adhesion strength (gm)	26.85	50.52	74.28	99.24
Adhesive force (N)	0.265	0.4925	0.725	0.945

Adhesive force=(adhesive strength/1000)×9.81.

Detachment force method performed for to determine the adhesive strength and adhesive force. The test was carried out for different time intervals 5, 10, 15 and 30 minutes respectively. The weight required to detach the tablet from gastric mucosa is different in different time intervals. Hence, more time contact increases the adhesion strength and adhesion force.

***In-vitro* wash off test**

Table. 7: Detachment time of last four formulations.

Formulations	Time of detachments (minutes)
F4	725
F5	918
F6	1185
F7	968

In-vitro wash of test were performed by using disintegration test apparatus. Test were carried out for last four formulations like F4, F5,F6 and F7. The detachment fore varies with different time (725 to 1185 minutes). The formulation F6 tablet shows maximum time of contact (1185 minutes) with mucus layer till detachment.

Radio graphical Examination - X-Ray

After administration of standard and trial drug by intra muscular route, rabbits were anaesthetized with ketamine and xylazine anesthetic agents (2:1) and were exposed to X- ray imaging method of detection to ascertain the location and nature of tablet in the stomach. The X-ray image of group I animals at different time intervals were shown [Fig.10, 11&12]

X RAY-GROUP I

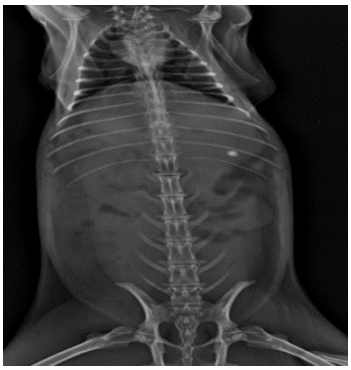
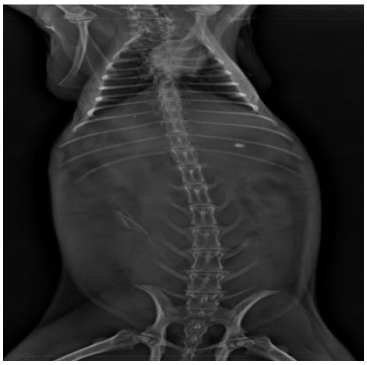
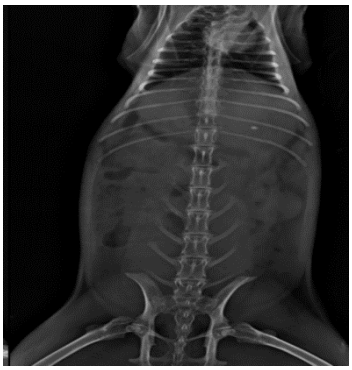

Fig. 10, 11 & 12: Clarithromycin + Chito-TSP polymer at 0th hr Clarithromycin + Chito-TSP polymer at 4th hr and Clarithromycin + Chito-TSP polymer at 8 th hour.

The gastric retention time of muoadhesive tablet were examined by using X-ray machine in rabbits. The tablets were administered by orally in the form of barium meal. The location of tablet in the rabbit stomach was identified in different time intervals of initial, 4th.8th hour respectively. The experiment were carried out. Group-I rabbits were administered by Carbopol-934. at the time of 8th hour tablets were retain in group-I rabbits. Hence, tablet with carbopol polymer shows good bio- adhesive character for long time.

Stability Studies

The stability studies were performed for selected formulation (F6) of clarithromycin mucoadhesive tablet as per the guidelines (Fig. 13). All the results evaluation studies were resembles with initial tablets. Hence, stability studies confirmed that the selected formulation (F6) has very good stable condition.

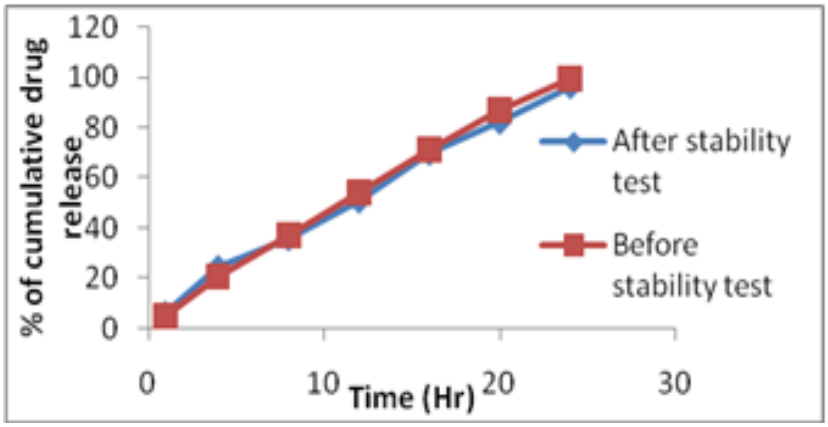

Fig. 13: *In vitro* release profiles of F6 before and after stability test.

CONCLUSION

The study was under with the aim to development of controlled release mucoadhesive tablets of clarithromycin using synthetic polymers. Here, which are drug release retarding agents. From the above results and discussion, it is concluded that the formulations of mucoadhesive tablets containing carbopol-934 and followed by excipient which are ideal or optimized formulation for controlled release mechanism for 24 hours. As it full fill all the requirement of controlled drug release and pre-clinical trials encourages the results also. The long-term stability studies prove the stable conditions of tablets.

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REFERENCES

1. Shailesh t. Prajapati, amit n. Patel, and chhagan n. Patel.formulation and evaluation of controlled-release tablet of zolpidem tartrate by melt granulation technique. Isnn pharmaceutics, 2011; 5, article id 208394, 8 page.
2. Srijan shrestha, shrawani lamichhane, aastha shrestha, junu khatri silwal, bhupendra kumar poude1 and pradeep paudel.formulation and in vitro evaluation of sustained release tablets of aceclofenac world journal of pharmaceutical sciences issn (print): 2321-3310; issn (online): 2321-3086.
3. K. J. Wadher, r. B. Kakde,[1] and m. J. Umekarformulation and evaluation of a sustained-release tablets of metformin hydrochloride using hydrophilic synthetic and hydrophobic natural polymersind An J Pharm Sci., Mar-Apr 2011; 73(2): 208-215.

4. Sudarshan singh, mohan govind and sunil b bothara. A review onIn vitro - in vivo mucoadhesive strength assessment. Phtechmed vol 1/issue 2/ Jan-Feb 2013; 221-227.
5. Rishabha malviya polimery w medycynie extraction characterization and evaluation of selected mucilage as pharmaceutical excipient, 2011; t. 41.
6. Dr. Safila Naveed1and Fatima Qamar. Simple UV spectrophotometric assay of Clarithromycin. International Journal of Pharma Sciences and Research, 09 Sep 2014; 5(5): 0975-9492, 582-585.
7. Sankar goswami& manoj sharma development of new mucoadhesive polymer from natural source dhruba pacificasian journal of pharmaceutical and clinical research, 2012; 5(3).
8. Mohan govind1 and sunil b bothara a review on in vitro - in vivo mucoadhesive strength assessment phtechmed vol 1/issue 2/jan-feb 2013; 221-225.
9. Satyabrata bhanja, c. Md zakiuddin shafeeque1, muvvala sudhakar1 mucoadhesive buccal tablets of glimeperide- formulation and evaluation international journal of pharmacy and pharmaceutical sciences, 2013; 5(4): 111-114.
10. Gavin p Andrews,Thomas, mucoadhesive polymeric platform for controlled drug delivery, europian journel of pharmaceutics and biopharmaceutics, 2009; 7: 505-518.
11. Mohammed s. Khan,rohitash k, vijaykumar m, suresh c pandey, gowda d. Vishakante1, faruqui m. Ahmed, aquil r sidiqui development and evaluation of nasal mucoadhesive nanoparticles of an analgesic drug. Scholars research library der pharmacia lettre, 2012; 4(6): 1846-1854.
12. Formulation and in vitro evaluation of combined floating mucoadhesive tablet of clarithromycin by using natural polymers international journal of research in pharmaceutical and biomedical Sciences, 2014; 5(3): 214-216.
13. Sudarshan singh, mohan govind and sunil b bothara. A review onIn vitro - in vivo mucoadhesive strength assessment. Phtechmed, Jan-Feb 2013; 1(2): 221-227.
14. Satyabrata bhanja, c. Md zakiuddin shafeeque1, muvvala sudhakar1 mucoadhesive buccal tablets of glimeperide- formulation and evaluation international journal of pharmacy and pharmaceutical sciences, 2013; 5(4): 111-114.
15. Singh sudarshan, bothara sunil bin vivo mucoadhesive strength appraisal of gum manilkara zapotabrazilian journal of pharmaceutical sciences, Jul.,/Sep., 2015; 51(3).
16. Paulo renato oliveira, cassiana mendes, lilian klein, maximiliano da silva sangoi, larissa sakis bernardi, [1]and marcos antônio segatto silva formulation development and stability studies of norfloxacin extended-release matrix tabletsbiomed res int., 2013; 716736.