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CLARITHROMYCIN AND RABEPRAZOLE MUCOADHESIVE MICROSPHERES FOR ANTI *HELICOBACTER PYLORI* THERAPY: FORMULATION AND *INVITRO* EVALUATION

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ABSTRACT

The aim of the present investigation was to formulate and evaluate the mucoadhesive microsphere of clarithromycin and rabeprazole using hydroxyl propyl methyl cellulose K4M, hydroxyl propyl methyl cellulose K100M and carbopol 971p. Microspheres were prepared by non-aqueous emulsification solvent evaporation method using liquid paraffin as a cross linking agent. The prepared microspheres were characterized for micromeritic properties such as particle size determination, tapped density, angle of repose and Carr's index. Batch CF5 and OF5 shows the highest adhesion property i.e. 7.9 hrs and 7.57 hrs respectively. Likewise batch CF5 and OF5 also shows highest % drug entrapment i.e. 83.0% and 95.22% respectively and declared as an optimized batches. Finally these batches were filled in capsules and evaluated for general appearance, weight variation, content uniformity and cumulative drug release .

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Key Words

Mucoadhesive, Microspheres,
Gastro retentive, *Helicobacter pylori*, Oral drug delivery system,
HPMC K4M, HPMC K100M

INTRODUCTION

Mucoadhesion is topic of current interest in the design of drug delivery system. The term 'mucoadhesive' is commonly used for materials that bind to the mucin layer of a biological membrane. Mucoadhesive drug delivery system utilizes the property of bioadhesion of certain polymers which become adhesive on hydration and can be used for targeting a drug to a particular region of the body for extended periods of time^[1,2,3]. Mucoadhesive drug delivery systems promises several advantages that arise from localization at a given target site, prolonged residence time at the site of drug absorption and an intensified contact with the mucosa increasing the drug concentration gradient^[4,5]. Hence, uptake and consequently bioavailability of the drug is increased and frequency of dosing reduced with the result that patient compliance is improved^[6-7]. Rabepazole Sodium belongs to the class of anti-secretory compounds that do not exhibit anticholinergic or histamine H₂-receptor antagonist properties, but suppress gastric acid secretion by inhibiting the gastric H⁺/K⁺-ATPase at the secretory surface of the gastric parental cell. *Helicobacter pylori* (*H.pylori*) are a gram-negative bacillus responsible for one of the most common infections found in humans worldwide^[8,9,10]. *H.pylori* causes gastric diseases, such as peptic ulcer, gastric mucosa associated lymphoma. 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^[11,12]. The main objective of this work was to formulate and evaluate the mucoadhesive microspheres^[13,14,15] containing clarithromycin and rabepazole^[16,17,18] as an anti-*H. pylori* agents.

MATERIAL AND METHODS

Clarithromycin and rabepazole was a kind gift from Ajanta Pharmaceutical Ltd., Mumbai, India. Carbopol 971p, HPMCK 4M, HPMC K100M were supplied by Lubrizol Pvt. Ltd., Mumbai. All other chemicals were of analytical grade and were purchased from Concept Pharma, Aurangabad, Maharashtra, India.

Formulation of microspheres

The microspheres were prepared by non-aqueous emulsification solvent evaporation method. Briefly, clarithromycin and polymers were mixed in ethanol and dichloromethane. The slurry was introduced in to 200 ml of liquid paraffin while being stirred at given rpm by mechanical stirrer for given time to allow the solvent to evaporate completely and the microspheres were collected by filtration. The microspheres were washed repeatedly with petroleum ether (40 –50 °C) until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in desiccators over fused calcium. The same procedure was followed for preparation of rabepazole microsphere.

Evaluation of microspheres

1) Particle size determination

Particle size was determined by using an optical microscope under regular polarized light, and the mean particle size was calculated by measuring 50-100 particles with the help of a calibrated ocular micrometer^[1,2].

2) Tapped Density

The sample of about 10 g of powder is carefully introduced into a 25 ml graduated cylinder. The cylinder was dropped at 2 second intervals onto a hard wood surface 100 times from a height of 1 inch. It was calculated by using equation given below,

$$D_o = M / V_p$$

Where,

D_o = bulk density

M = weight of samples in grams

V_p = final tapped volumes of granules in cm³

3) Angle of repose

The Angle of repose (θ) i.e. flow property of the microspheres which measures the resistance to particle flow was calculated as,

$$\tan \theta = 2H / D$$

Where,

$2H / D$ is the surface area of the free standing height of the microspheres heap that is formed after making the microspheres flow from the glass funnel^[19,20].

4) Carr's Index

The percentage compressibility of microspheres was calculated according to equation^[21] given below,

$$\% \text{ Compressibility} = \frac{D_o - D_f}{D_o} \times 100$$

Where,

D_f = bulk density

D_o = Tapped density

5) Adhesion property

A freshly cut of 5 cm long piece of pig intestine obtained from a local abattoir within 1 hr of killing the animal was cleaned by washing with isotonic saline solution. An accurate weight of microspheres was placed on mucosal surface which was attached over a polyethylene plate that fixed in an angle of 40° relative to the horizontal plane, and 0.1N HCl warmed at 37°C was peristaltically pumped at a rate of 5 ml/min over the tissue. The duration for completely washing of microspheres from pig intestine was recorded and averaged from five determinations^[22,23].

6) Drug entrapment efficiency

Microspheres equivalent to 20 mg of the drug clarithromycin were taken for evaluation. The amount of drug entrapped was estimated by crushing the microspheres and extracting with aliquots of 0.1N HCl repeatedly. The extract was transferred to a 100 ml volumetric flask and the volume was made up using 0.1N HCl. The solution was filtered and the absorbance was measured after suitable dilution spectrophotometrically at 282 nm against 0.1N HCl as a blank. The amount of drug entrapped in the microspheres was calculated by the following formula,

Calculated drug concentration

$$\% \text{ Drug entrapment} = \frac{\text{-----}}{\text{-----}} \times 100$$

Theoretical drug concentration

Same procedure was repeated for rabeprazole microspheres and absorbance was measured at 284 nm.

7) Formulation of capsule single unit dosage form of optimized batch

Capsules size was selected according to the volume of microsphere needed to deliver the drug dose. The contents were filled in the tarred capsule, which were then reweighed. Care was taken to fill the capsule completely in order to maintain a uniformity of weight. The optimized batch containing clarithromycin and rabeprazole was evaluated for general appearance, weight variation, content uniformity and *in-vitro* drug release studies.

8) Evaluation of capsule

a) General appearance

The capsules were visually evaluated for texture, pinholes, deformity, and appearance.

a) Weight variation

This test was conducted on 20 capsules. Each capsule was individually weighed to find out weight variation. Average weight was calculated.

b) Content uniformity test

10 capsules were accurately weighed, remove the hard gelatin shells and powder equivalent to 20 mg of drug clarithromycin and dissolved it in 1N NaOH. Drug content was calculated by measuring absorbance at wavelength 282 nm. Same procedure is followed for rabeprazole at wavelength 284 nm.

c) *In-vitro* drug release

The release rate of drug clarithromycin and rabeprazole from capsule was determined using USP dissolution testing apparatus I (Basket type). The dissolution test was performed using 900 ml of 0.1 N HCl, at $37 \pm 0.5^\circ\text{C}$ and 100 rpm. A sample (5 ml) of the solution was withdrawn from the dissolution apparatus at various sampling time 0 min, 1hr, 2hr, 3hr, 4hr up to 08hrs and the samples were replaced with fresh dissolution medium to avoid sink condition. The samples

were filtered through Whatman filter paper no. 41. Absorbance of these solutions was measured at 282 nm for clarithromycin and 284 nm for rabeprazole [24,25].

RESULT AND DISCUSSION

The present study was carried out to develop mucoadhesive drug delivery system in the form of microsphere dosage form in combination of clarithromycin and rabeprazole. The prepared microspheres of clarithromycin and rabeprazole gave good micromeritic results. CF5 and OF5 batches show

Table No.01: Formulation of microspheres

Batches	CF1	CF2	CF3	CF4	CF5	CF6	OF1	OF2	OF3	OF4	OF5	OF6
Clarithromycin (mg)	300	300	300	300	300	300	---	---	---	---	---	---
Rabeprazole (mg)	---	---	---	---	---	---	300	300	300	300	300	300
Carbopol 971p (mg)	300	300	150	150	150	150	300	300	150	150	150	150
HPMC K4M (mg)	---	---	150	150	---	---	---	---	150	150	---	---
HPMC K100M (mg)	----	----	----	----	150	150	----	----	----	----	150	150
Ethanol (ml)	40	40	40	40	40	40	40	40	40	40	40	40
Dichloromethan (ml)	40	40	40	40	40	40	40	40	40	40	40	40
Liquid paraffin (ml)	200	200	200	200	200	200	200	200	200	200	200	200
RPM	1000	750	1000	750	1000	750	1000	750	1000	750	1000	750
Time (min)	45	60	45	60	45	60	45	60	45	60	45	60

highest adhesion property and % drug entrapment .The optimized batch of capsules containing CF5 and OF5 batches passes the weight variation test and shows 89.59% of clarithromycin and 92.71% of rabeprazole. The CF5 and OF5 batches shows 92.38% and 94.71% cumulative drug release in 08 hrs of study.

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Table No.02: Micromeritics studies of clarithromycin microspheres.

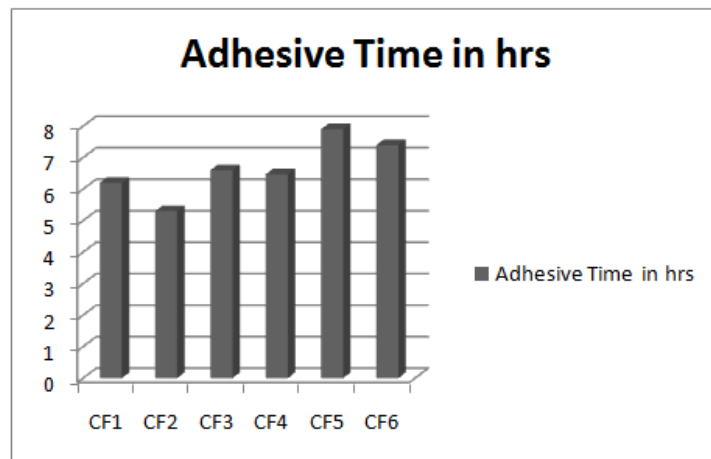
Batches	Average particle size(μm)	Tapped density (g/cm^3)	Angle of repose (θ)	Carr's Index
CF1	369.14	0.50	30 ° 23	12.76
CF2	325.65	0.45	29 ° 75	10.41
CF3	314.05	0.46	29 ° 77	14.58
CF4	282.66	0.47	27 ° 31	8.51
CF5	256.24	0.44	30 ° 32	10.20
CF6	249.16	0.41	30 ° 11	10.20

Table No.03: Micromeritics studies of rabeprazole microspheres.

Batches	Average particle size(μm)	Tapped density (g/cm^3)	Angle of repose (θ)	Carr's Index
OF1	325.64	0.49	29. ° 21	10.20
OF2	311.05	0.48	27 ° 23	8.51
OF3	313.13	0.47	29 ° 69	14.58
OF4	273.81	0.49	28 ° 76	12.76
OF5	281.50	0.46	26 ° 24	11.10
OF6	256.20	0.48	26 ° 22	10.33

Table No.04: Adhesive time study of clarithromycin

Batch code	Adhesive Time in hrs
CF1	6.2
CF2	5.3
CF3	6.6
CF4	6.47
CF5	7.9
CF6	7.40

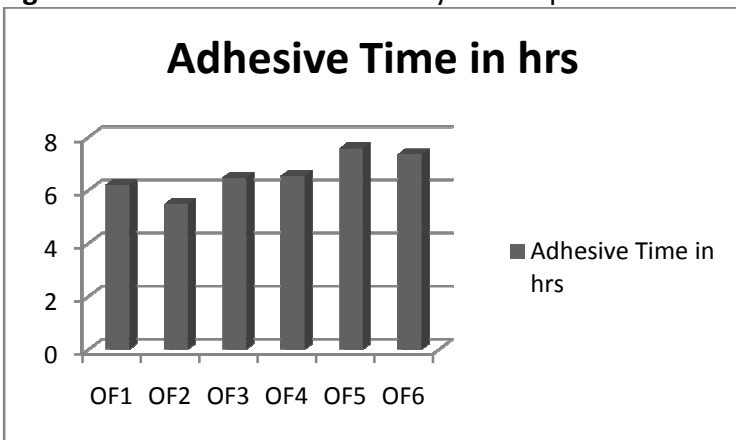
**Figure No.01:** Adhesive time study of clarithromycin**Table No.05:** Adhesive time study of rabeprazole

Batch code	Adhesive Time in hrs
OF1	6.2
OF2	5.47
OF3	6.47
OF4	6.54
OF5	7.57
OF6	7.35

CF4	75.0
CF5	83.0
CF6	79.0

Table No.07: Drug entrapment efficiency of rabeprazole

Batch No.	% Drug entrapment of rabeprazole
OF1	51.3
OF2	65.2
OF3	70.0
OF4	73.6
OF5	95.22
OF6	77.12

Figure No.02: Adhesive time study of rabeprazole**Table No.08:** Formulation of capsule of optimized batch

Batches	Microsphere equivalent to drug dose(mg)	Capsule size
CF5	20	0
OF5	20	0

Table No.06: Drug entrapment efficiency of clarithromycin

Batch No.	% Drug entrapment of clarithromycin
CF1	69.2
CF2	69.8
CF3	74.0

Table 09: General appearance of capsule

Evaluation Parameter	Observation
Texture	Fine and smooth
Pinholes	None
Deformity	None
Appearance	Satisfactory

Table No.10: Weight variation test

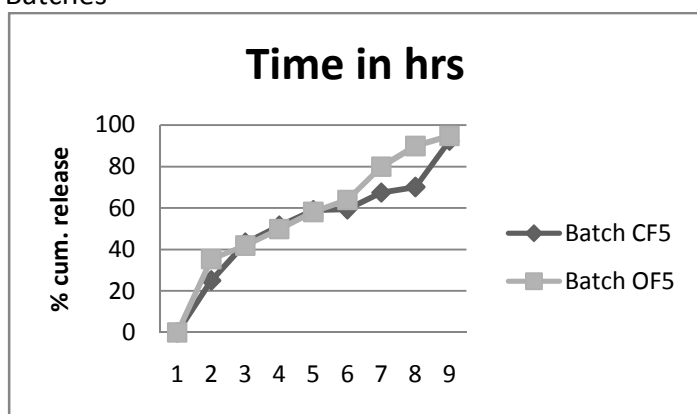
Average weight (mg)	Permitted limit (mg)	Range of all capsule (mg)	Permitted limit of variation	Pass/ fail
649.42	617.91-682.95	630.43-661.55	±.5%	Pass

Table No.11: Content uniformity test

Batches.	Amount of drug (mg)	% Drug content
CF5	224.09	89.59
OF5	18.55	92.71

Table No. 12: Cumulative drug release of optimized batches of capsule

Time(hrs)	Capsule of CF5 batch	Capsule of OF5 batch
0	00.00	00.00
1	25.02	35.32
2	43.24	41.89
3	51.43	49.83
4	58.93	58.09
5	59.34	63.9
6	67.44	79.90
7	70.09	89.89
8	92.38	94.71

Figure No.0 3: Cumulative release of CF5 and OF5 Batches**REFERENCES**

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