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PREPARATION, CHARACTERIZATION AND IN VITRO EVALUATION OF MUCOADHESIVE MICROSPHERES OF AMOXICILLIN

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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 are 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. The aim of the present investigation was to formulate and evaluate the mucoadhesive microsphere of amoxicillin using hydroxyl propyl methyl cellulose K15M, hydroxyl propyl methyl cellulose K100M and carbopol 974p. 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 F5 showed the highest adhesion property 7.7 hr. Batch F5 also showed highest 85.77% drug entrapment and 91.35% cumulative drug release and declared as an optimized batch.

KEYWORDS : *Mucoadhesive, Microspheres, Gastro retentive, Helicobacter pylori,Oral drug delivery system*

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INTRODUCTION

Microspheres carrier system made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery. Recently, dosage forms that can precisely control the release rates and

target drugs to a specific body site have made an enormous impact in the formulation and development of novel drug delivery systems. Microspheres form an important part of such novel drug delivery systems[1].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 [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]. Amoxicillin is a moderate-spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicllin is used in the treatment of a number of infections including acute otitis media, streptococcal pharyngitis, pneumonia, skin infections, urinary tract infections, salmonella, lyme disease, and chlamydia infections. It is used to prevent bacterial endocarditis in high risk people who are having dental work done, to prevent Strep. pneumococus infections in those without a spleen, and for both the prevention and treatment of anthrax. It is also a treatment for cystic acne [8]. H.pylori are a gram-negative bacillus responsible for one of the most common infections found in humans worldwide [9,10,11]. *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[12,13]. The purpose of this study was to formulate, characterize and evaluate the mucoadhesive microspheres containing amoxicillin as an anti-*H. pylori* agent [14,15,16].

MATERIALS AND METHODS

Amoxicillin was a kind gift from Ajanta Pharmaceutical Ltd. Mumbai India. Carbopol 974, HPMCK 15M, 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

Briefly, amoxicillin 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 ^{o}C) until free from oil. The collected microspheres were dried for 1hr at room temperature and subsequently stored in desiccators over fused calcium.

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 [17] given below,

$D_o = M / Vp$

Where,

D_o = bulk density

M = weight of samples in grams

Vp = final tapped volumes of granules in cm^3

3) Angle of repose

The angle of repose was determined by the fixed funnel method. Accurately weighed microparticles were taken in funnel. The height of funnel was adjusted in such a way that the tip of the funnel just touched to apex of the heap of the microparticles. It is the maximum angle possible between the surface of pile of microparticles and horizontal plane. The microparticles was allowed to flow through the funnel freely onto the surface. The diameter of microparticles cone was measured. The angle of repose was calculated by using the following equation, [18]

$\tan \theta = h/r$

Where,

θ = Angle of repose

h = Height of pile

r = Radius of pile.

4) Carr's index

The percentage compressibility of microspheres was calculated according to equation [19] given below,

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

Where,

D_f = Bulk density

Do = 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 40^0 relative to the horizontal plane and 0.1N HCl warmed at 37 ^{0}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. [20, 21]

6) Drug entrapment efficiency

Microspheres equivalent to 20 mg of the drug amoxicillin 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 272 nm against 0.1N HCl as a blank. The amount of drug entrapped in the microspheres was calculated by the following formula [22],

$$\% \text{ Drug entrapment} = \frac{\text{Calculated drug concentration}}{\text{Theoretical drug concentration}} \times 100$$

7) *In-vitro* drug release

The release rate of drug from microsphere 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 ± 0.5^0C 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 8hr, and the samples were replaced with fresh dissolution medium to avoid sink condition. The samples were filtered through Whatman filter paper no. 41. Absorbances of these solutions were measured at 272 nm.

RESULT AND DISCUSSION

The various batches have the average particle size in the range of 256µm to 366µm. The particle size was increased as the stirring time and stirring speed was decreased. The tapped density was found in between 0.44-0.52g/cm^3, Carr's index in between 10-14%. A faster stirring speed 1000 rpm gave much smaller but cohered particles. At lower speed (750rpm), the mean particle diameter and size distribution of the microparticles increased considerably. The percent drug entrapment of amoxicillin in all formulation was found to be good i.e. above 65%. The microsphere of batch F5 formulation showed highest adhesion property i.e. 7.7 hrs. Drug release was almost linear with time and batch F5 showed highest release of drug among all batches. Finally it was concluded that amoxicillin mucoadhesive microspheres were prepared successfully by using non-aqueous emulsification solvent evaporation method. The method followed was economical to get reproducible microspheres. Depending up on

adhesion property, drug entrapment efficiency and % cumulative drug release, batch F5 was declared as an optimized batch.

Table 1: Formulation of microspheres

Batches	F1	F2	F3	F4	F5	F6
Amoxicillin (mg)	300	300	300	300	300	300
Carbopol 974p (mg)	150	150	150	150	150	150
HPMC K15M (mg)	---	---	150	150	---	---
HPMC K100M (mg)	----	----	----	----	150	150
Ethanol (ml)	40	40	40	40	40	40
Dichloromethan (ml)	40	40	40	40	40	40
Liquid paraffin (ml)	200	200	200	200	200	200
RPM	1000	750	1000	750	1000	750
Time (min)	45	60	45	60	45	60

Table 2: Micromeritics studies of amoxicillin microspheres

Batches	Average particle size(μm)	Tapped density (g/cm^3)	Angle of repose (θ)	Carr's Index
F1	365.22	0.51	$27^{o}31$	10.55
F2	325.75	0.49	$28^{o}57$	13.44
F3	312.45	0.44	$26^{o8}7$	13.58
F4	282.65	0.47	$25^{o}85$	11.51
F5	262.55	0.50	$29^{o}38$	12.29
F6	256.61	0.52	$30^{o}11$	11.90

Table 3: Adhesive time study of amoxicillin

Batch code	Adhesive Time (hr)
F1	5.7
F2	6.2
F3	6.5
F4	6.4
F5	7.7
F6	7.4

Table 4: Drug entrapment efficiency of amoxicillin

Batch No.	% Drug entrapment
F1	68.21
F2	66.89
F3	76.57
F4	73.31
F5	85.77
F6	81.17

Table 5: Drug release pattern of batches F1 to F6

Time (hr)	Percentage cumulative release of drug					
	F1	F2	F3	F4	F5	F6
0	00.00	00.00	00.00	00.00	00.00	00.00
1	10.92±0.3	3.12±0.3	14.33±0.15	11.63±0.57	22.01±0.28	19.98±0.33
2	12.31±0.32	8.95±0.7	22.61±0.18	18.13±0.51	43.23±0.68	34.77±0.43
3	17.46±0.17	13.81±0.10	25.22±0.26	27.46±0.33	49.42±0.48	44.18±0.22
4	21.42±0.33	16.39±0.31	34.86±0.33	39.63±0.50	58.92±0.50	53.66±0.49
5	27.73±0.35	27.09±0.28	45.60±0.55	48.24±0.69	61.39±0.52	66.33±0.44
6	31.93±0.39	34.98±0.32	55.84±0.53	59.29±0.44	74.81±0.63	74.11±0.33
7	42.84±0.38	41.98±0.21	63.46±0.99	67.43±0.21	82.63±0.49	81.36±0.22
8	57.70±0.40	55.94±0.75	72.22±0.71	77.56±0.70	91.35±0.53	86.34±0.52

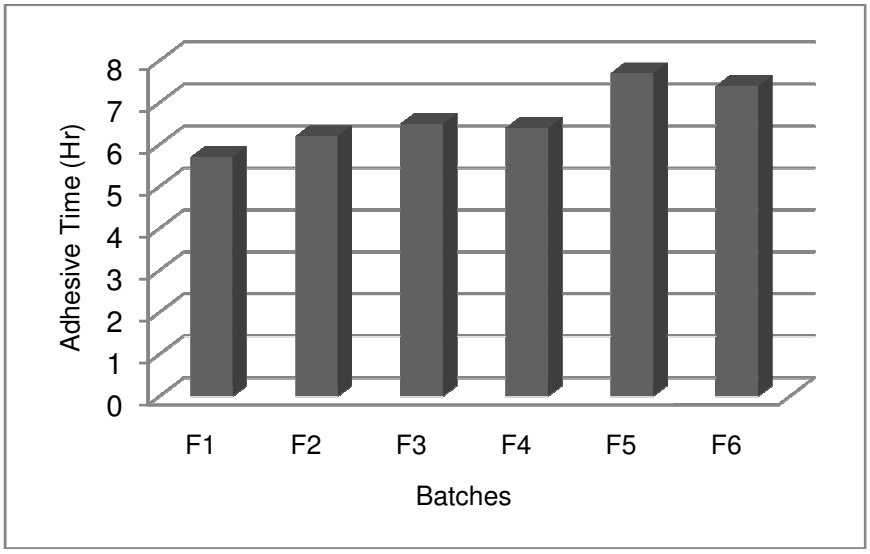

Fig. 1: Adhesive time study of amoxicillin

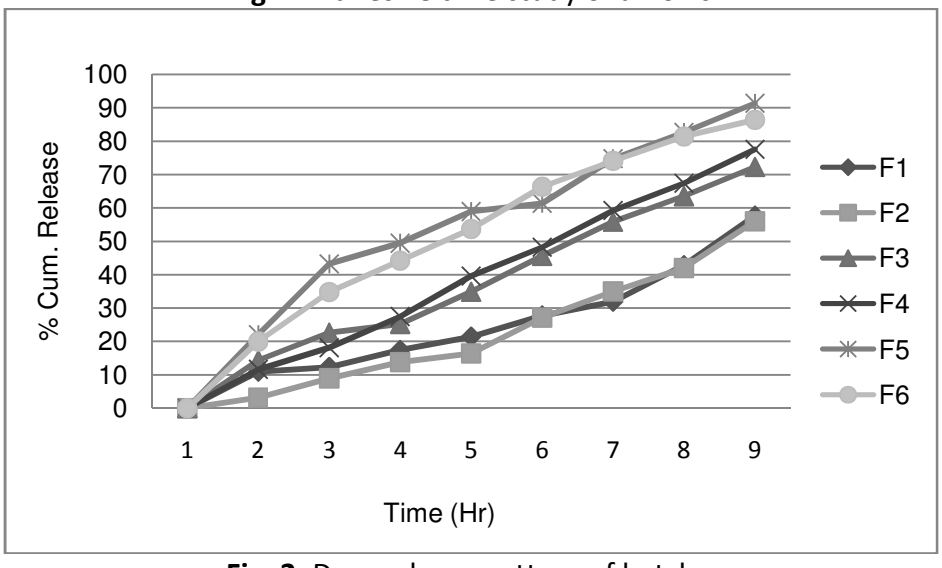

Fig. 2: Drug release pattern of batches

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