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Research Article

FORMULATION AND *IN VITRO* EVALUATION OF FURAZOLIDONE MUCOADHESIVE MICROSPHERES

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ABSTRACT

The purpose of this research was to formulate and systematically evaluate *in vitro* performances of mucoadhesive Furazolidone microspheres for the potential use of treating gastric and duodenal ulcers, which were associated with *Helicobacter pylori*. Furazolidone mucoadhesive microspheres were prepared by simple emulsification phase separation technique using Eudragit RS100 as matrix and Carbopol 974P and Hydroxy propyl methyl cellulose K4M as mucoadhesive polymer.The prepared microspheres were evaluated with respect to the particle size, encapsulation efficiency, shape and surface properties, mucoadhesive property, *in vitro* drug release and suitability for anti *Helicobacter pylori* effect. The best batch exhibited a high drug entrapment efficiency of 82.12 % and percentage mucoadhesion after 1 h was 93.35 %. The drug release was also sustained up to 12 h. The preliminary results show great promise for this delivery strategy in the treatment of *H. Pylori* infection.

Keywords*: Helicobacter pylori*, Furazolidone, mucoadhesive, microspheres

INTRODUCTION

Helicobacter pylori (*H.pylori*) infection is associated with many upper gastrointestinal diseases, such as chronic gastritis, peptic ulcer, gastric carcinoma and mild malignant mucosa-associated lymphoid tissue lymphoma (MALToma)[1-3].The ideal treatment regimen for *H.pylori* eradication[4-6] should present a higher than 80%. Amoxicillin and clarithromycin most commonly used antibiotic for *H.pylori* eradication therapy. Furazolidone emerges as an alternative for therapeutic regimens in developing countries due to its low cost and prevalence of resistant strains. This antimicrobial is a monoamine oxidase inhibitor usually utilized in the treatment of giardiasis. There are studies demonstrating its efficacy and safety in several developing countries. The drug has been used in *H pylori* treatment regimens since 1990[7-11].

However, some other reports and clinical trials indicate that the H.*pylori* eradication therapies cannot bring out complete eradication of *H. pylori* and suggest that the therapeutic effect needs more investigation[12,13]. 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[14,15].

It is therefore necessary to design drug delivery systems that cannot only alleviate the shortcomings of conventional delivery vehicles but also deliver the antimicrobials to the infected cell lines. The absorption of an antibiotic into the mucus through the mucus layer (from the gastric lumen) is believed to be more effective for *H.pylori* eradication than absorption through the basolateral membrane (from blood). A preparation that spreads out, adheres to the gastric mucosal surface, and continuously releases antibiotic should be highly effective against *H. pylori* [16]. Bioadhesive microspheres have advantages such as efficient absorption and enhanced bioavailability of drugs owing to a high surface-to-volume ratio, a much more intimate contact with the mucus layer, and specific targeting of drugs to the absorption site[17-20].

The purpose of this study was to design mucoadhesive microspheres containing Furazolidone as an anti-*H. pylori* agent and to evaluate the effectiveness of the mucoadhesive microspheres for *H. pylori* eradication therapy.

MATERIALS AND METHODS

Materials

Furazolidone was obtained from Richy pharmaceuticals, Mumbai, Hydroxypropyl methyl cellulose K4M was obtained as gifts from Colorcon Asia Pvt. Ltd., Mumbai, India and Carbopol 974P was a gift from BF Goodrich Co., Germany. Eudragit RS 100 was a gift sample from Microlabs, Bangalore. All other reagents and chemicals used were of analytical grade.

Preparation of microspheres

Microspheres were prepared by a solvent evaporation method. The solvent system acetone/liquid paraffin was used. Agglomeration of microspheres was prevented by using 1% w/v Span80. Eudragit RL 100 was used to form a matrix of microspheres and mucoadhesive polymer were chosen to produce mucoadhesion is Carbopol 974P and Hydroxypropyl methyl cellulose K4M. Eudragit RS 100 and Furazolidone were dissolved in acetone and weighed quantity of Carbopol 974P and Hydroxypropyl methyl cellulose K4M were dispersed it. The total volume of acetone was 12 ml. This homogeneous final dispersion was cooled to 5 °C and poured slowly with stirring (700 rpm) into 80 ml of liquid paraffin containing 1% 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 petroleum ether and dried in vacuum at room temperature overnight.

Table 1: Formulation composition of mucoadhesive microspheres of Furazolidone

Formulation Code	Eudragit RL 100(%w/v)	Carbopol 934P(%w/v)	HPMC K4M* (%w/v)
F1	3	1.0	1.0
F2	5	1.0	1.0
F3	7	1.0	1.0
F4	5	0.5	0.5
F5	5	0.75	0.75
F6	5	1.5	1.5

HPMC = Hydroxypropyl methyl cellulose

Scanning electron microscopy

Scanning electron photomicrograph of Furazolidone loaded mucoadhesive microspheres were taken. A small amount of microspheres was spread on glass stub. Afterwards, the stub containing the sample was placed in the scanning electron microscope (JSM 5610 LV SEM, JEOL, Datum Ltd, Tokyo, Japan) chamber. Scanning electron photomicrograph was taken at the acceleration voltage of 20 KV, chamber pressure of 0.6 mm Hg, at different magnification. The photomicrograph of batch F6 is depicted in Fig. 1.

Particle size measurement

The prepared microspheres were sized by using a Malvern 2600 Laser Diffraction Spectrometer. The size of the microspheres was determined in n-hexane as a non-dissolving dispersion medium and the particles were suspended mechanically by magnetic stirring during the measurement.

Determination of drug encapsulation efficiency

To determine the total drug content of microspheres a known amount of microspheres wereground to fine powder. Accurately weighed (50mg) grounded powder of microspheres weresoaked in 50 ml of distilled water and sonicated using probe sonicator for 2 h. The whole solution was centrifuged using a tabletop centrifuge to remove the polymeric debris. Then the polymeric debris was washed twice with fresh solvent (water) to extract any adhered drug. The clear supernatant solution was filtrated through a 0.45 µm syringe filter then analyzed for Furazolidone content by UV/Vis spectrophotometer at 365 nm.

In vitro evaluation of mucoadhesiveness[21]

A strip of goat intestinal mucosa was mounted on a glass slide and accurately weighed mucoadhesive microspheres in dispersion form was placed on the mucosa of the intestine. This glass slide was incubated for 15 min in a desiccator at 90% relative humidity to allow the polymer to interact with the membrane and finally placed in the cell that was attached to the outer assembly at an angle 45^0. Phosphate buffer saline (pH 6.4), previously warmed to 37 ± 0.5^0C, was circulated to the cell over the microspheres and membrane at the rate of 1 ml/min with the help of pump. Washings were collected at different time intervals and microspheres were separated by centrifugation followed by drying at 50^0C. The weight of microspheres washed out was taken and percentage mucoadhesion was calculated by

$$\text{Percentage mucoadhesion} = \frac{Wa - Wl \times 100}{Wa}$$

where Wa = weight of microspheres applied; W1 = weight of microspheres leached out.

In vitro drug release studies

Release of Furazolidone from the microspheres was studied in 0.1N HCL (900 mL) using a USP XXIII paddle method Dissolution Rate Test Apparatus (Dissco 2000, Labindia) with a rotating paddle stirrer at 50 rpm and 37° ± 1°C. A sample of microspheres equivalent to 25 mg of Furazolidone was used in each test. Samples of dissolution fluid were withdrawn through a filter (0.45 µm) at different time intervals and were assayed for drug release by UV/Vis spectrophotometer at 365nm. The drug release experiments were conducted in triplicate (n = 3).

RESULTS AND DISCUSSION

The mucoadhesive microspheres of Furazolidone prepared in this study were well-rounded spheres with the size ranging approximately from 225 to 356 µm. The study of in vitro bioadhesion revealed that all the batches of prepared microspheres had good bioadhesive property ranging from 83±1.148 % to 94±1.357%. On increasing the mucoadhesive polymer concentration, the bioadhesive property of the microspheres also increased. The formulation F6 showed the highest bioadhesive property (94±1.357%). 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.

Table 2: Physico-chemical characteristics of the Furazolidone loaded mucoadhesive microspheres

S.No	Formulation code	Mean particle size (µm)	Drug entrapment (%) ±S.D (n=3)	Mucoadhesion (%) ±S.D* (n=3)
1	F1	224	80± 1.25	83±1..458
2	F2	276	89±2.75	86±1.123
3	F3	358	92±0.15	86±1.147
4	F4	254	92±2.20	82±1.258
5	F5	325	87±1.81	91±0.987
6	F6	356	82±1.24	93±1.357

S.D = Standard deviation

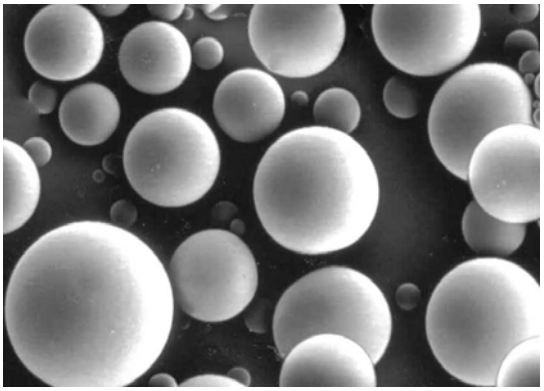

Fig. 1: SEM photograph of furazolidone loaded mucoadhesive microspheres

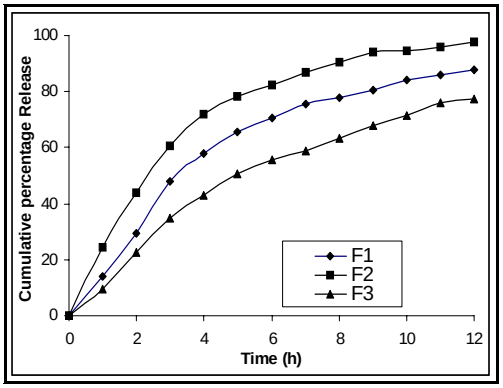

Fig. 2: Effect of EudragitRL100 on the inv release of Furazolidone in 0.1 N HCL

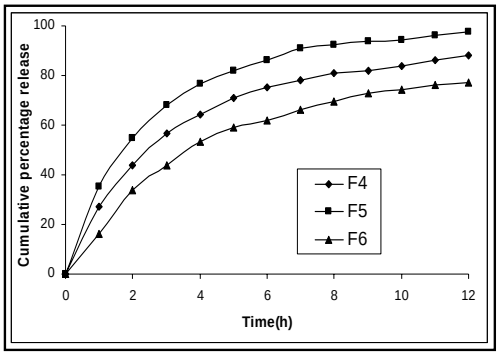

Fig. 3: Effect of Mucoadhesive polymers on the *in vitro* release of Furazolidone in 0.1 N HCL

From the result of the *in vitro* release test, the effect of Eudragit RL100 concentration on Furazolidone release from different batches of microspheres is shown in Fig. 2. A significant decrease in the rate and extent of drug release was observed with the increase in polymer concentration in microspheres and could be attributed to increase in the density of the polymer matrix and also increase in the diffusional path length which the drug molecules have to traverse. Similarly, Fig.3 indicates the effect of mucoadhesive polymers concentration on release properties of Furazolidone from microspheres. An increase in mucoadhesive polymers concentration caused retardation in drug release from the microspheres because of an increase in the viscosity of polymer solution and formation larger size microspheres.

CONCLUSION

H. *pylori* colonize the gastric mucosa leading to gastritis, gastric ulcer, and gastric carcinoma. To increase the efficacy of eradicating the infection, a localized delivery system of anti-*H*. *pylori* agents in the stomach is required. Furazolidone formulation was prepared to increase the local concentration of the drug in the stomach and, thus eradicate *H*. *pylori* infection. *In vitro* studies clearly indicates that the prepared formulations possess good bioadhesive properties. These properties enable the microspheres to adhere to the gastric mucosal surface and stay in stomach for prolonged periods and could ensure the stability of Furazolidone in gastric environment, which eventually resulted in better eradication of *H. pylori* than the conventional dosage forms. Further studies are planned to examine the gastric residence time of the microsphere formulation and the efficacy in eradicating *H*. *pylori* infection in suitable animal model.

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