Available online on 25.12.2017 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

IN VITRO AND *IN VIVO* EVALUATION OF MUCOADHESIVE MICROSPHERES FOR TREATMENT OF *HELICOBACTER PYLORI* USING FACTORIAL DESIGN

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ABSTRACT

The research involves characterization of *in vitro* and *in vivo* activity of mucoadhesive microspheres for *Helicobacter pylori* eradication. Amoxicillin and Famotidine were used as model drugs so that the dual therapy gives better *H. pylori* eradication. Preparation was carried by an emulsion-solvent evaporation method and 27 batches were prepared individually using 3^3 factorial designs to study the effect of independent variables on dependent variables. The *in vitro* mucoadhesion test and *in vivo* studies (Bacterial clearance study, *in vivo* mucoadhesion and *in vivo* ulcer index studies) were performed. A27 batch showed 66% mucoadhesion after 10 h and F24 showed 74% of mucoadhesion after 10 h. In the bacterial clearance studies, the mean bacterial count (log colony forming units) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58. The drug-loaded microspheres formulation exhibited better clearance from infection than plain drugs solution at the same doses. Drug microspheres formulation was found to be effective in the treatment of *H. pylori* infections effectively, and in *in vivo* mucoadhesion studies, the developed system was well taken up and processed by the cells of gastric mucosa of the stomach.

Cite this article as: Hardenia A, Gupta AK, Singh RP, In-vitro and in-vivo evaluation of mucoadhesive microspheres for treatment of helicobacter pylori using factorial design, Journal of Drug Delivery and Therapeutics. 2017; 7(7):65-68

INTRODUCTION:

More than 50% of drug delivery systems available in the market are oral drug delivery systems. These systems have the obvious advantages of ease of administration and patient acceptance. Attempts to develop a single-dose therapy for the whole duration of treatment have focused attention on controlled or sustained release drug delivery systems. Gastro retention helps provide a better availability of new products with new therapeutic possibilities and substantial benefits for patients. GRDFs greatly improve the pharmacotherapy of the stomach through local drug release drug concentrations at the gastric mucosa (eradicating *H. pylori* from the sub mucosal tissue of the stomach), making it possible to treat stomach and duodenal ulcers, gastritis, and esophagitis. This can be achieved by coupling bioadhesion characteristics to microspheres and developing characteristics to microspheres and developing mucoadhesive microspheres [1]. *H. pylori* is a major gastric pathogen in the worldwide distribution; these are spiral-shaped bacteria found in the stomach, which destroy stomach along with the duodenal tissue, Several treatment regimens are emerging for *H. pylori* infection from the early 1990s, when monotherapy was first recommended. Even though *H. pylori* is highly sensitive to most of the antibiotics, its elimination is not easy even with the best currently available therapy[2]. Therapeutic regimens directed against *H. pylori* infection will continue to evolve. Amoxicillin (-amino-hydroxybenzylpenicillin) is a semi-synthetic, orally absorbed, broad-spectrum antibiotic. It is now widely used in the standard eradication treatment of gastric and duodenal ulcers [3]. Famotidine is a histamine H2-receptor antagonist. It is mostly prescribed for gastric ulcers, duodenal ulcers, also for gastro esophageal reflux diseases. With low bioavailability (40-45%) and short biological half-life (2.5-4.0 h), it favors the development of a sustained release formulation [4]. Thus, an attempt was made in the present investigation to prepare mucoadhesive microspheres. The microspheres were characterized by *in vitro* and *in vivo* tests and factorial design was used to optimize the variables.

METHODS:

The mucoadhesive microspheres were prepared by emulsion solvent evaporation method using Carbopol 934 and ethyl cellulose as polymers. In the first step, ethyl cellulose was dissolved in 200 ml of ethanol and

then drug and polymer were dispersed in the solution of ethyl cellulose under stirring. The preliminary trial batches were prepared and optimized using 3^3 factorial design earlier by varying the drug-to-polymer-to-polymer (amoxicillin/famotidine-ethyl cellulose-carbopol-934P) ratio in the range of 1:3:1% to 1:3:3%. The final mixture was extruded through a syringe (gauge No. 20) in 500 ml of liquid paraffin (mixture of heavy and light, 1:1 ratio) containing Span 80 and stirring was carried out using a propeller stirrer (Remi, Mumbai, India) at 1000 rpm. The stirring was done for 3 h. In preliminary trial batches, the amount of emulsifying agent (1-3%), the drug: polymer concentration 1:3:1% to 1:3:3%, and stirring speed (500-1000 rpm) were varied

RESULTS AND DISCUSSION:

Optimization of amoxicillin-loaded microspheres

For preparation, a full factorial design was employed. A design model with 3 factors, 3 levels, and 27 runs was selected for the optimization study. The dependent variables obtained at various levels of the 3 independent variables (X1, X2, and X3) were subjected to multiple regression to yield a second-order polynomial equation obtained coefficient. The polynomial equation generated by this experimental design (using Design Expert 7.1.6) was as follows:

$$Y = b0 + b1X1 + b2X2 + b3X3 + b12X1X2 + b13X1X3 + b23X2X3 + b11X1X1 + b22X2X2 + b33X3$$

Where, Y is the dependent variable; b0 is the intercept; b1 to b33 are the regression coefficients; and X1, X2, and X3 are the independent variables. Response surface graphs for amoxicillin are shown in Figures 1-4 and for famotidine are shown in Figures 5-8.

In vitro Mucoadhesion

The *in vitro* mucoadhesiveness test was carried out among the factorial design batches based on good entrapment efficiency and the batch A27 and F24 showed that even after 10 h, 66% and 74% microspheres were adhered to the gastric mucous layer. The mucoadhesive microspheres were spherical and free flowing.

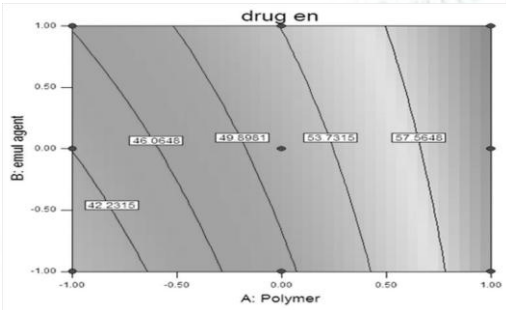

Figure 1: Contour plot for drug entrapment

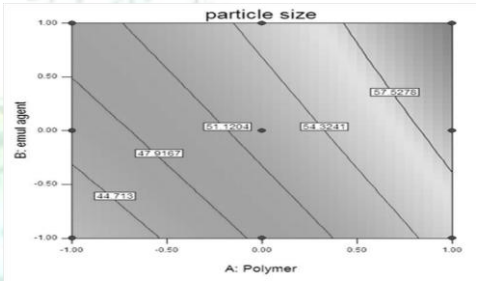

Figure 2: Contour plot for particle size

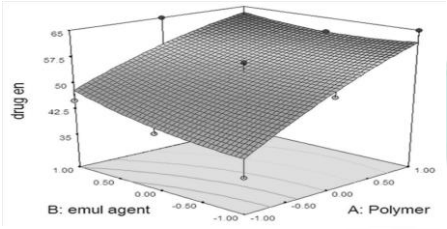

Figure 3: Response surface plot for drug entrapment

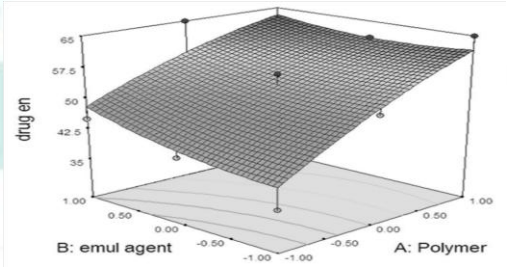

Figure 4: Response surface plot for particle size

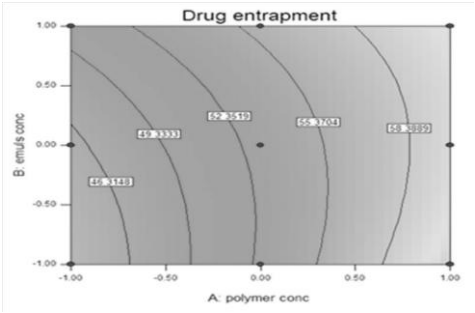

Figure 5: Contour plot for drug entrapment

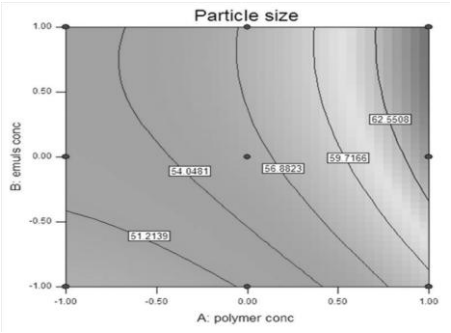

Figure 6: contour plot for particle size

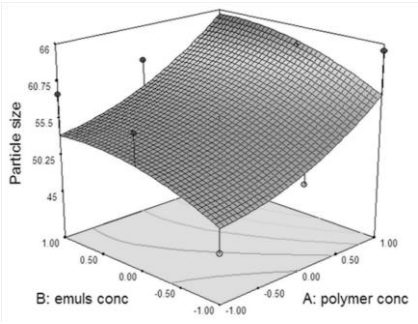

Figure 7: Response surface plot for drug entrapment

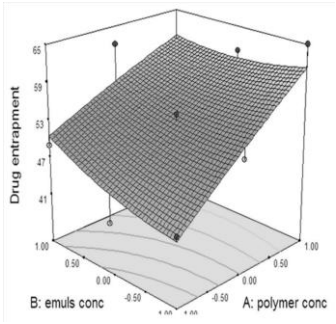

Figure 8: Response surface plot for particle size

In vitro drug release studies

A sustained drug release was obtained for more than 10h.

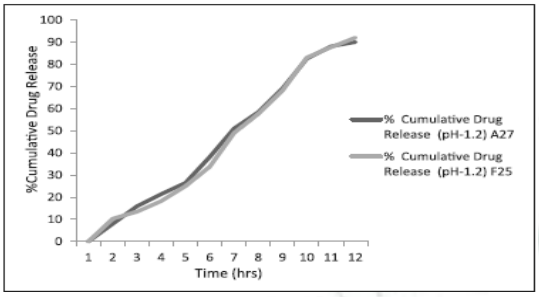

Figure 9: Dissolution profile

In vivo studies

Bacterial clearance count

Infected animal model, i.e, male albino rats with *H. pylori* was used for *in vivo* study. The control group of rat received only physiological saline. The mean bacterial count (log CFU) was found to be 9.64 ± 0.35. The mean bacterial count (log CFU) after oral administration of plain drugs solution (amoxicillin) was found to be 5.83 ± 0.23, which is due to unavailability of 100% drugs and short residence. The mean bacterial count (log CFU) after oral administration of drug-loaded microspheres was found to be 3.72 ± 0.58.

In vivo mucoadhesion study

The mucoadhesive behavior of the microspheres was determined after 2 h of incubation of films pieces in the stomach and intestine. This muco-adhesivity was also confirmed *in vivo* using FITC dye with the formulation. This shows that the system could attach the mucosal gel layer where *H. pylori* resides [Figures 10 and 11].

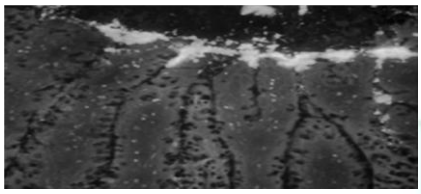

Figure 10: Plain fluorescence isothiocyanate solution

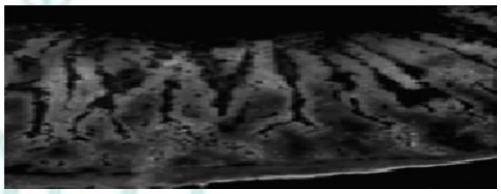

Figure11: Fluorescence isothiocyanate-labeled microspheres in gastric tissue (after 2 h)

In vivo ulcer index studies

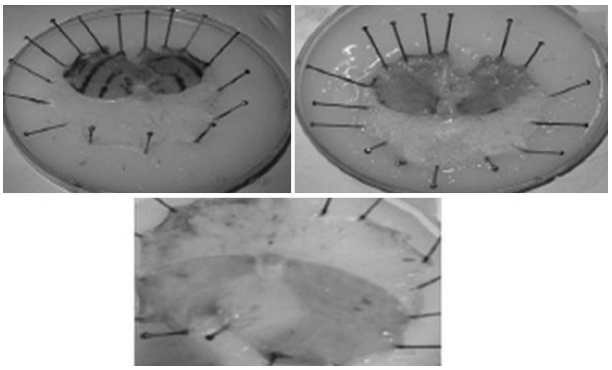

Figure 12: (a) Ulcer-induced rat stomach. (b) Famotidine suspension-treated. (c) Mucoadhesive microsphere-treated

Mucoadhesive microsphere dispersion showed a significant decrease in ulcer index (0.46 ± 0.011) when compared with the control group (3.61 ± 0.14) and famotidine suspension-treated animal (0.66 ± 0.035) (Figure 12).

CONCLUSION:

The mucoadhesive microspheres were developed using a 3^3 factorial design and showed a high percentage of mucoadhesion, drug entrapment efficiency, and exhibited a sustained release property. The formulation showed more effective *H. pylori* activity of mucoadhesive amoxicillin microspheres compared to amoxicillin and famotidine mucoadhesive microsphere dispersion showed a significant decrease in ulcer index when compared with the control group and famotidine suspension-treated animal, which might indicate a

potential use of mucoadhesive microspheres in treating *H. pylori* infection.

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