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Preparation and Characterization of Mucoadhesive Microspheres for Gastroretention using 3^3 Factorial Design

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Abstract

In Present research work mucoadhesive microspheres of Famotidine were prepared for the management of gastric ulcer and its associated diseases. The formulations were prepared by emulsion-solvent evaporation technique using carbopol-934P as mucoadhesive polymer and ethyl cellulose as carrier polymer. Total 27 formulations were prepared using 3^3 factorial design using Design Expert software. The 3^3factorial design was employed to study the effect of independent variables, drug-to-polymer-to-polymer ratio (X1), Emulsifying agent (X2) and stirring speed (X3) on dependent variables, drug entrapment (Y1) and particle size (Y2). The results of Study showed that amount of drug-polymers ratio, emulsifying agent and stirring speed affect the characteristics of microspheres. The microspheres were found to be discrete, spherical, free flowing with the good percentage of drug entrapment efficiency. An *in-vitro* mucoadhesive test showed that mucoadhesive microspheres adhered more strongly to the gastric mucous layer and could retain in the gastrointestinal tract for an extended period of time. The best batch exhibited good drug entrapment efficiency of 69% and *In vitro* mucoadhesion after 1 h was 84%. A sustained drug release was obtained for more than 12 h. *In vitro* release test showed that Famotidine released slightly faster in pH 1.2 hydrochloric acid than in pH 6.8 phosphate buffer. The drug-polymer and polymer-polymer ratio had a more significant effect on the dependent variables. In conclusion, the prolonged gastrointestinal residence time and enhanced Famotidine stability resulting from the mucoadhesive microspheres of Famotidine might make a contribution to management of famotidine.

Key-Words: Microspheres, H_2 Antagonist, Mucoadhesive, Famotidine

Introduction

Oral ingestion is the most convenient and commonly used method of drug delivery. 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[1].

The dosage forms that can control the release rates and target drugs to a specific body site have an enormous impact in the development of novel drug delivery systems. Among various novel drug delivery system microspheres as a drug delivery systems made from the naturally occurring biodegradable polymers have attracted considerable attention for several years in sustained drug delivery[2].

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Although, the release from the microspheres is limited, because of their short residence time at site of absorption. So, it would, be advantageous to have means for providing an intimate contact of the drug delivery system with the absorbing membranes. This can be achieved by using mucoadhesion properties to microspheres and developing mucoadhesive microspheres.

This mucoadhesive drug delivery system shows the advantage 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[3]. Carbopol-934P as an anionic polymer is used in mucoadhesive systems by several researchers[4]. Carbopol-934P was used as a polymer in the preparation of mucoadhesive microspheres owing to its good mucoadhesive and biodegradable properties and ethyl cellulose as carrier polymer for microspheres. Famotidine is a histamine-2 blocker. Famotidine works by decreasing the amount of acid the stomach produces.Famotidine is used to treat and prevent ulcers

in the stomach and intestines. It also treats conditions in which the stomach produces too much acid, such as Zollinger-Ellison syndrome. Famotidine also treats gastroesophageal reflux disease (GERD) and other conditions in which acid backs up from the stomach into the esophagus, causing heartburn.

Famotidine a histamine H2 receptor antagonist produces competitive blockade at histamine H2 receptors. Used for the treatment of gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome and gastroesophageal reflux disease. Famotidine is given in a dose of 20-40 mg daily and half-life of Famotidine is about 3 h and has only 40-45 % absolute bioavailability after oral administration due to incomplete absorption. This aspect make the drug suitable forgastroretentive drug delivery and its formulation into dosage forms may improve its absorption from the proximal small intestine due to prolongation of residence time in the stomach

None of various combinations against H. pylori, have shown complete eradication of bacterium. The incomplete eradication of H. pylori may be due to sub-bactericidal concentration of antibiotics in the gastric mucosal region, both from the lumen of the stomach and from the gastric supply.

Hence local diffusion of drug into gastric mucosa is essential for therapeutic efficacy. Various delivery systems of Famotidine have been prepared in recent time for increasing its local availability and efficacy such as polymer matrix tablets, gastroretentive floating systems,most of these studies emphasized on increasing the retention time of drug in the stomach and increasing the stability of antibiotics in acidic environment of stomach. But these systems could not assist in the complete eradication of bacterium.

These systems provide an intimate contact with mucus membrane due to polyvalent adhesive interaction or electrostatic attraction, H-bond formation, Vander-Waal forces and other. The system has an additional advantage of protecting acid sensitive drugs against acid degradation and offers effective drug diffusion across the mucus layer. [5]

Mucoadhesion helps in increasing the gastric residence time of particles, also thick viscoelastic mucosal gel do not allow antimicrobial drugs to penetrate through it easily . The Swelling of the polymer hinders docking it in gastric mucus and strong mucoadhesion decrease the mobility and thus interpenetrate penetrability in to mucus. In addition, gastric motility and proteolytic activity make mucus turnover intense there by make gastric residence of formulation shorter . Hence efficient adherence to mucus could make the system incapable of penetrating across the mucus layer and entering the underlying epithelia [6].

To overcome these limitations, the particulate system, are required to penetrate the mucus membrane and deliver the drug close proximity to the site of H. pylori infection. Many researchers reported the preparation of particulate systems capable of penetrating mucus membrane.

The present work is an attempt to develop a novel bi-specific, biodegradable, mucopenetrating system for delivery of Amoxicillin to deep mucus layers near the sanctuary of the H. pylori.

In context of the above principles, a strong need was felt to develop a dosage form that delivered amoxicillin in the stomach and would increase the efficiency of the drug providing sustained action. Thus, an attempt was made in the present investigation to use carbopol-934P as a mucoadhesive polymer and ethyl cellulose as a carrier polymer and prepare mucoadhesive Famotidine microspheres.

Material and Methods

Famotidine was obtained as a gift sample from Cipla (Baddi) and Carbopol 934 was obtained as a gift sample from Loba chemie. All other chemicals used were of analytical grade.

Preparation of Mucoadhesive Microspheres

Mucoadhesive microspheres of Famotidine 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, 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-ethyl cellulose-carbopol-934P) ratio in 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 three hours. 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.

For optimization Factorial design approach was used, total twenty seven formulations were prepared, the drug-to-polymer-to-polymer ratio, concentration of emulsifying agent and stirring speed were varied and all other parameters were kept same . The Microspheres prepared were filtered and washed several times with petroleum ether (80:20) to remove traces of oil. The microspheres were then dried at room

temperature (25°C and 60 % RH) for 24 hrs. Formulated Batches are shown in Table 1.

Table 1: Factorial Design Batches of Famotidine

Batch Code	Variables in coded form			Y_1	Y_2
	X_1	X_2	X_3	Drug Entrapment (%)	Particle Size (µm)
	Polymer Conc	Emulsifying agent	Stirring Speed		
F1	-1.00 1.00	-1.00	-	28	35
F2	0.00 1.00	-1.00	-	48	38
F3	1.00 1.00	-1.00	-	63	54
F4	-1.00 1.00	0.00	-	31	39
F5	0.00 1.00	0.00	-	39	42
F6	1.00 1.00	0.00	-	51	58
F7	-1.00	1.00		36	41
F8	0.00	1.00		48	52
F9	1.00	1.00		56	57
F10	-1.00	-1.00		35	34
F11	0.00	-1.00		51	48
F12	1.00	-1.00		56	63
F13	-1.00	0.00		41	50
F14	0.00	0.00		65	51
F15	1.00	0.00		61	47
F16	-1.00	1.00		45	54
F17	0.00	1.00		62	62
F18	1.00	1.00		61	58
F19	-1.00	-1.00		52	45
F20	0.00	-1.00		51	47
F21	1.00	-1.00		54	51
F22	-1.00	0.00		54	57
F23	0.00	0.00		57	59
F24	1.00	0.00		64	43
F25	-1.00	1.00		69	47
F26	0.00	1.00		58	64
F27	1.00	1.00		54	76

Coded Values	Actual Values		
	X_1(mg)	X_2 (%)	X_3 (rpm)
Low (-1)	100	1%	8
Medium (0)	200	2%	10
High (1)	300	3%	12

Where, X_1= Polymer Concentration, X_2= Emulsifying Agent, X_3= Stirring Speed.
Further Batch no: F14, F24 and F25 were selected for further study on the basis of good drug entrapment efficieny.

Optimization of Mucoadhesive Microspheres using 3^3 Factorial Design

A response surface design model with 3 factors, 3 levels and 27 runs was selected for the optimization study. The polynomial equation generated by this experimental design (using Design Expert 7.1.6) was as follows:

$$Y= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1 X_2 + b_{13}X_1 X_3 + b_{23}X_2 X_3 + b_{11}X_1 X_1 + b_{22}X_2 X_2 + b_{33}X_3$$

Where, Y is the dependent variable; b_0 is the intercept; b_1 to b_{33} are the regression coefficients; and X_1, X_2 and X_3 are the independent variable that was selected from the preliminary experiments.[7]

On the basis of the preliminary trials a 3^3 full factorial design was employed to study the effect of independent variables, i.e. Polymer Concentration (X1) , Emulsifying cagent concentration (X2) and the stirring speed (X3) on dependent variables drug entrapment and Particle size. Further evaluations were performed on the selected formulations.

Drug entrapment efficiency

Two hundred milligrams of accurately weighed microspheres were crushed in a glass mortar-pestle and the powdered microspheres were suspended in 10 mL phosphate buffer (pH 7.8). After 24 hrs the solution was filtered and the filtrate was analysed for the drug content. The drug entrapment efficiency was calculated using the following formula: Practical drug content/Theoretical drug content × 100. The drug entrapment efficiency for trial batches is reported in Table 1.

Particle size of microspheres

The particle size of the microspheres was determined by using optical microscopy method. Approximately 300 microspheres were counted for particle size using a calibrated optical microscope (SCOPE, Indore). The particle size of microspheres of trial batches is reported in Table 1.

***In-vitro* wash-off test for microspheres**

The *in-vitro* wash-off test as reported by Lehr et al [8] was used for the evaluation of percent mucoadhesion. A 1x1 cm piece of rat stomach mucosa was tied onto a glass slide (-3 inch-by-1inch-) using thread. Microspheres were spread (~50) onto the wet rinsed tissue specimen and the prepared slide was hung onto one of the groves of a USP tablet disintegrating test apparatus with continuous oxygen supply. The disintegrating test apparatus was used where the tissue specimen was given regular up and down movements in the beaker of the disintegration apparatus, containing gastric fluid (pH 1.2), for 10 hrs, the number of microspheres still adhering onto the tissue was counted. The results of *in-vitro* wash-off test after 10 hrs of trial batches are shown in Table 2.

Table 2: Percent Mucoadhesion of selected batches

Batch no	1h (%)	5h (%)	10hr (%)
F14	78	67	60
F24	81	72	66
F25	84	78	74

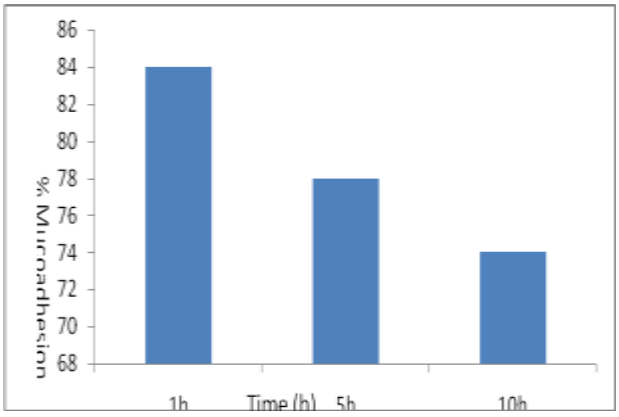

Figure 1: % Mucoadhesion of Batch F25

Figure 2*: In-vitro* mucoadhesion test assembly

Scanning electron microscopy

Scanning electron photomicrographs of drug-loaded carbopol-934P mucoadhesive microspheres were taken. A small amount of microspheres was spread on glass slide. Afterwards, the slide 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 [9-11].

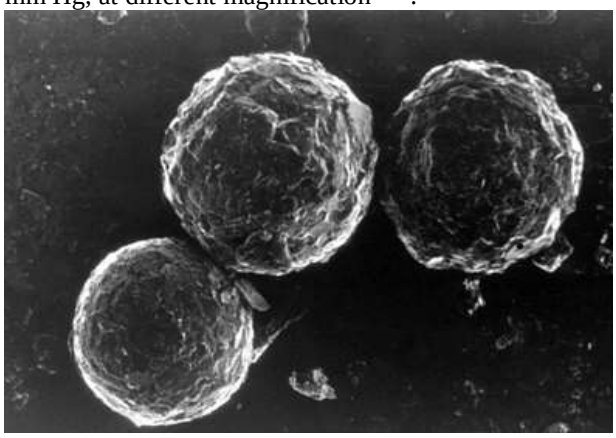

Figure 3: SEM (scanning electron microscopy) of Batch F25

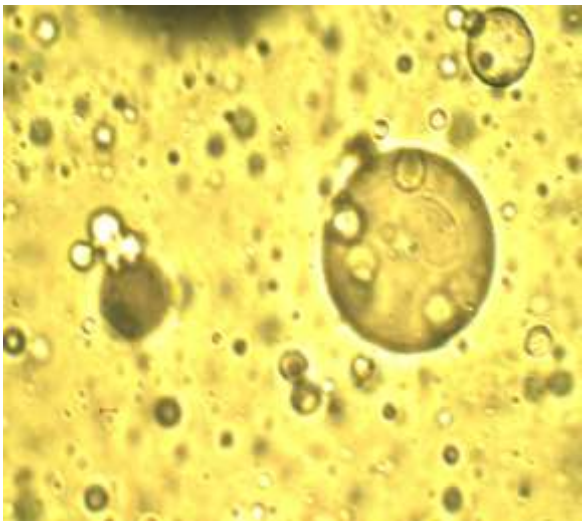

Figure 4: Optical Microscopic Photograph of Batch F25

Swelling index

50 mg of microspheres were allowed for swelling in SGF (pH 1.2) for 4 h, the excess adhered liquid was removed by blotting with filter paper and weighed.

The swelling Index was calculated using the following equation:

$$\text{Swelling Index} = \frac{X_s - X_0}{X_0} \times 100$$

Where,

X_s is the weight of the swollen microspheres after time t,

X_0 is the initial weight at zero time.

The swelling index was found in range of 1.5

Figure 5: Swelling Index of microspheres initially and after 4 hrs

Drug release study

The drug release study was carried out using USP XXIV basket apparatus (Electrolab, TDT-06T, India) at 37°C±0.5°C and at 100 rpm using 900 ml of 0.1 N HCl as a dissolution medium (n=5) as per USP XXVI dissolution test prescribed for tablets. Microspheres with the weight equal to 100 mg of were used for the test. 5 ml of sample from solution was taken at predetermined time intervals and were filtered through a 0.45 µm membrane filter, properly diluted, and analyzed spectrophotometrically. An equal amount of fresh dissolution medium was added immediately after taking of the test sample. Dissolution rate of drug dissolved at various intervals was calculated.

Table 3: *In-vitro* Drug release profile (Famotidine)

S. No	Time	% Cumulative Drug Release (pH-1.2)
1	0 min	0
2	30 min	10.31±0.45
3	1hr	13.42±0.34
4	2 hrs	18.25±0.23
5	3 hrs	25.01±0.56
6	4 hrs	33.76±0.23
7	5 hrs	49.00±0.87
8	6 hrs	57.60±0.67
9	7 hrs	68.13±0.53
10	8 hrs	83.04±0.37
11	9 hrs	87.68±0.31
12	10 hrs	92.03±0.73

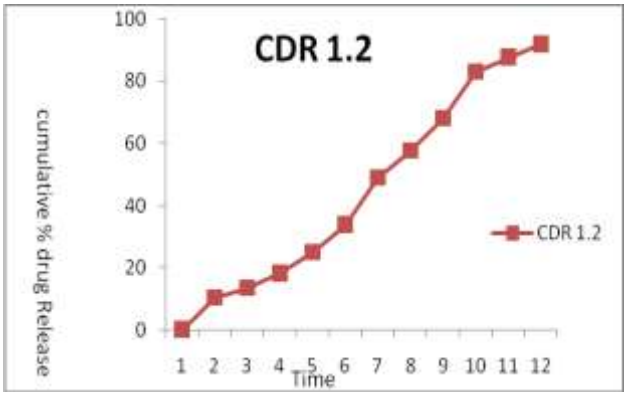

Figure 6: *In Vitro* drug release profile of formulation F25

Kinetics of Drug Release

The kinetics of drug release from the gastro retentive microspehers were established using the formula given by peppas, used to study the drug release behavior from the polymeric drug delivery systems. The release kinetic parameters were calculated according to peppas equation.

$$Mt/Mw = k\,t^n$$

Where,

Mt/Mw is the fractional release of the drug, t denotes the release time, k represents a kinetic constant,

incorporating structural and geometrical characteristics of the controlled release device, and n is the diffusional exponent and characterizes the type of release mechanism during the dissolution process. For non-Fickian release, the value of n falls between 0.5 and 1.0.

Table 4: Release Kinetics Profile of Formulation F25 in pH 1.2.

S.No	Time (hr)	Root T	Log T	Cum. (%) drug release	Cum. (%) drug retained	Log cum. (%) drug release	Log cum. (%) drug retained
1	30 min	0.54	-0.5	10.31	89.69	1.01	1.9
2	1	1	0	13.42	86.58	1.1	1.9
3	2	1.41	0.301	18.25	81.75	1.2	1.9
4	3	1.73	0.447	25.01	74.99	1.3	1.8
5	4	2	0.602	33.76	66.24	1.5	1.8
6	5	2.23	0.698	49.00	51	1.6	1.7
7	6	2.44	0.778	57.60	42.40	1.7	1.7
8	7	2.64	0.845	68.13	31.87	1.8	1.4
9	8	2.82	0.903	83.04	16.96	1.9	1.3
10	9	3	0.954	87.68	12.32	1.9	1.3
11	10	3.16	1	92.03	7.97	1.9	0.90

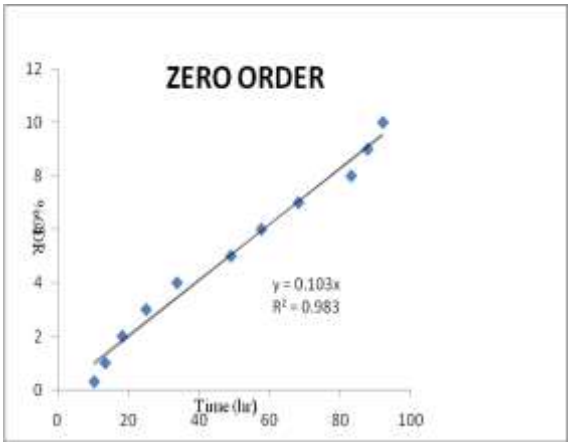

Figure 7: Zero order release of F25 in pH 1.2

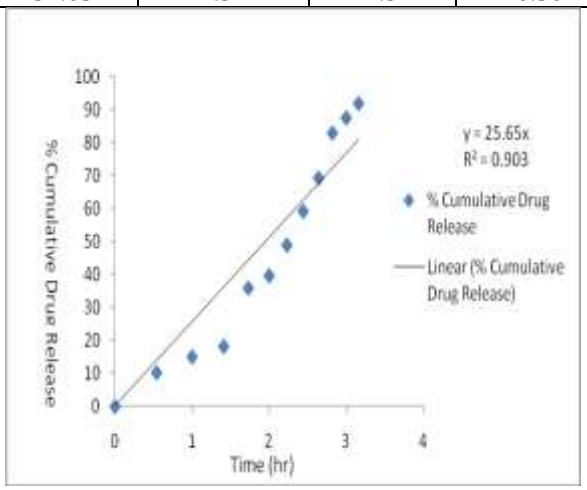

Figure 9: Higuchi release of F25 in pH 1.2

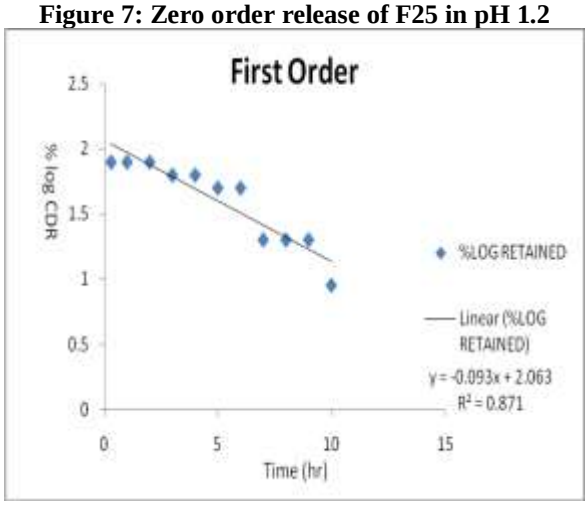

Figure 8: First order release of F25 in pH 1.2

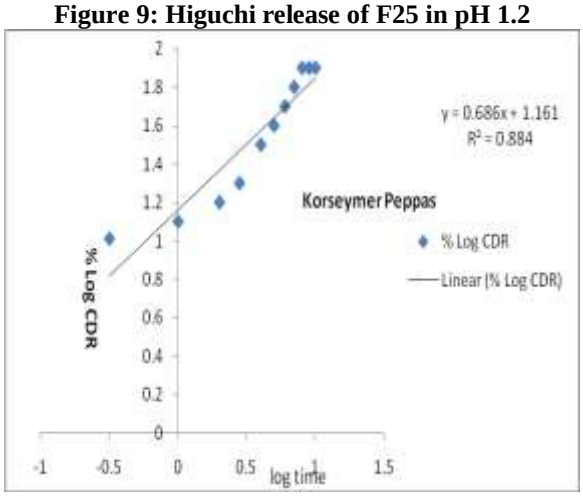

Figure 10: Korseymer Peppas release of F25 in pH 1.2

Table 5: Mathematical model used to describe the drug release

	Zero-order	First order	Higuchi Kinetics	korsemeyer Peppas
Regression Coefficient (R^2)				
pH 1.2	0.9835	0.8719	0.9038	0.884

Results and Discussion

The mucoadhesive microspheres of Famotidine were prepared by emulsion-solvent evaporation technique using carbopol-934P and ethyl cellulose. The Carbopol-934P was used as a polymer for the preparation because of its biodegradable and mucoadhesive properties. Ethyl cellulose was used as carrier polymer.

Further 3^3 factorial design was used to study the effect of independent variables (polymer concentration [X_1] , Emulsifying agent concentration [X_2] and stirring speed [X_3]) on dependent variables particle size, drug entrapment efficiency. The results clearly shows that all dependent variables are affected by the independent variables. The polynomial equations for each response with their high magnitude of the coefficients and mathematical sign indicate about the fit of the model.

Factorial Equation for Particle Size

$Y = 56.07 + 5.67X_1 + 2.83\ X_2 + 0.56\ X_3 + 0.42X_1\ X_2 - 2.08\ X_1\ X_3 - 1.75X_2 - 1.92X_1\ X_1 + 2.11X_2\ X_2 - -1.72X_3\ X_3$

The Model F-value of 3.61 [Table 4] implies the model is significant. There is only a 0.10% chance that a "Model F-Value" this large could occur due to noise.

ANOVA of dependent variables

Table 6: ANOVA of dependent variables

for drug entrapment					
	df	***SS***	***MS***	***F***	**R^2**
Regression	***9***	1463.31	**162.59**	**8.44**	***0.8170***
Residual	**17**	**327.66**	**19.27**		
Total	**26**	**1790.96**			
for particle size					
	df	***SS***	***MS***	***F***	**R^2**
Regression	**9**	**980.03**	**108.89**	**3.37**	**0.8250**
Residual	**17**	**549.60**	**32.33**		
Total	**26**	**1529.63**			

Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant.If there are many insignificant model terms (not counting those required to support hierarchy),model reduction may improve your model.

Results of the polynomial equation indicate that the effect of X_1 (drug polymer ratio) is positive and more significant than X_2(Emulsifying agent) and X_3 (stirring speed) i.e., as the drug polymer ratio was increased there was an increase in the polymer concentration, which lead to increased particle size, whereas as with the increase in stirring speed particle size was decreased.

Factorial Equation for Drug Entrapment

$Y = 53.07 + 7.06X_1 + 1.61\ X_2 + 4.39\ X_3 - 2.00\ X_1\ X_2 - 2.08\ X_1\ X_3 - 2.17\ X_2\ X_3 - 0.39\ X_1\ X_1 + 1.28\ X_2\ X_2 - 1.06X_3\ X_3$

The Model F-value of 14.02 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A, B, C, AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Both variables have significant efffect up to a level afterwards with the increase in both parameters the entrapment efficiency reduced.

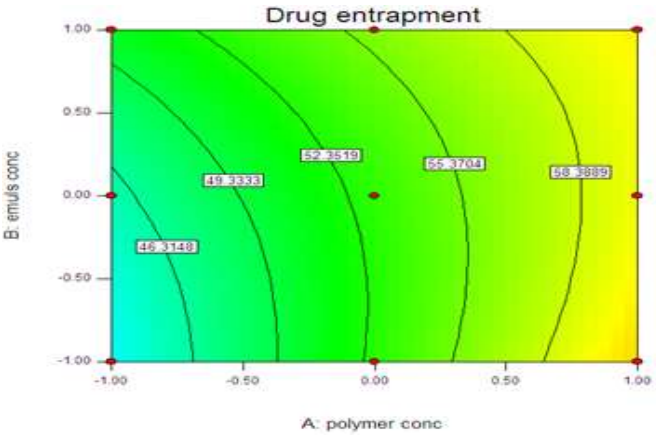

Figure 11: Contour Plot of Drug Entrapment

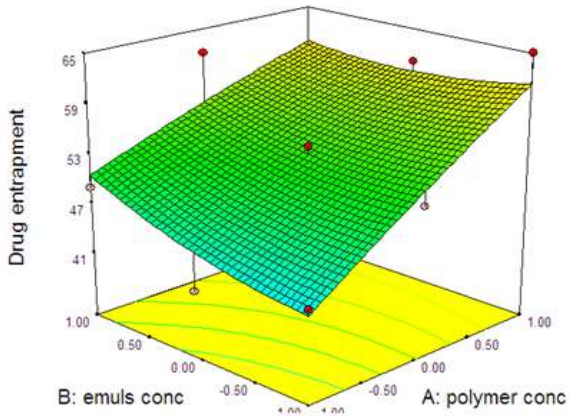

Figure 12: 3D surface plot of Drug Entrapment

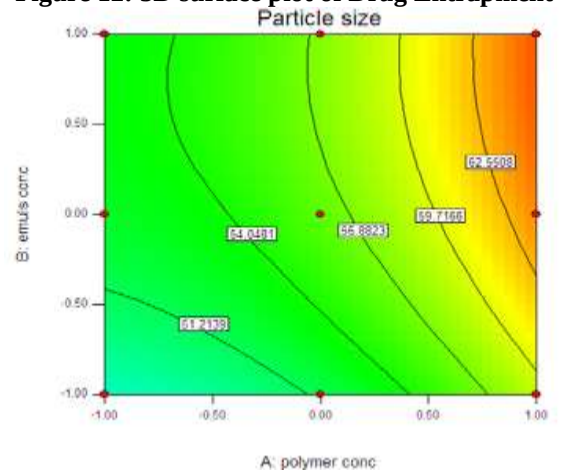

Figure 13 : Contour Plot of Particle size

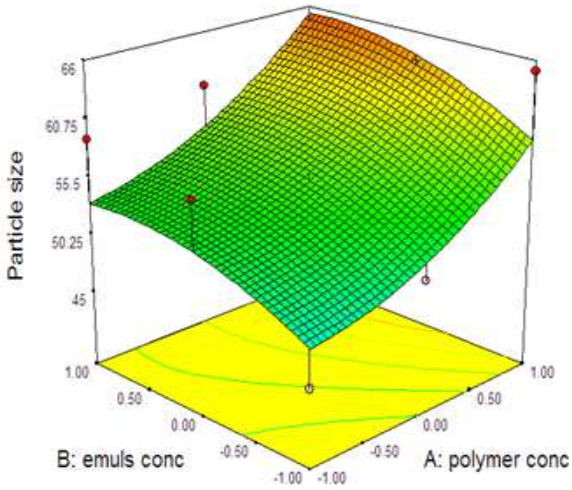

Figure 14: 3D surface plot of Particle Size

The results of a 3^3 full factorial design revealed that the polymer to-drug ratio, emulsifying agent and stirring speed significantly affected the dependent variables percentage drug entrapment efficiency and particle size. The microspheres of the best batch exhibited a high mucoadhesion of 74% after 10h, 69% drug entrapment efficiency. The *in vitro* release studies indicate that the mucoadhesive microspheres of Amoxicillin could sustain the release of the drug for more than 24 h.

Conclusion

The mucoadhesive microspheres of Famotidine can be successfully prepared by emulsion solvent evaporation technique using 3^3 optimization techniques. The microspheres showed the high percentage of mucoadhesion and good entrapment efficiency. Drug to polymer ratio, stirring speed and concentration of emulsifying agent showed a significant influence on percentage mucoadhesion, drug entrapment efficiency, particle size and t80. The best formulation showed more effective activity which might indicate a potential use of mucoadhesive Famotidine microspheres in treating H. pylori infection.

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