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Research Article

3² Factorial Design Screened *Abelmoschus esculentus* Fruit Mucilage abetted Floating Microspheres of Clarithromycin for Exterminating *Helicobacter pylori*

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ABSTRACT

The study aims to investigate the floating possessions of *Abelmoschus esculentus* fruit mucilage by incorporating it into floating microspheres with Clarithromycin. Nine interpretations of floating microspheres were made with sodium alginate (SA) and varying proportions of *A. esculentus* fruit mucilage (AEFM). A central composite design with design expert software to check the impact of independent variables (AEFM and SA levels) on entrapment efficacy and floating time (FT). As part of congeniality studies, the microspheres were scrutinized for clarithromycin (CMN) content and its release. The research discovered that CMN entrapment increased with an increase in AEFM levels in the formulations and that the FT was greater in formulations with higher AEFM levels. In formulations containing higher levels of AEFM, the drug release is slightly reduced. The study revealed that Clarithromycin is capable of good stomach-specific drug delivery by formulating it with SA as floating microspheres and the assets were enhanced by AEFM.

INTRODUCTION

More drugs are now available directly to patients through the oral route, which is a growing trend.^[1] It is easy to prepare and administer the gastro retentive microsphere, which is a common dosage form. The semi-synthetic macrolide antibiotic clarithromycin is awfully effective in treating *Helicobacter pylori* infection.^[2] *H. pylori* is a pathogenic bacterium that colonizes profound privileged the gastric mucosa. Clarithromycin enters the bacterial cell wall and binds reversibly to domain V of the 23S ribosomal RNA of the 50S subunit of the bacterial ribosome, averting aminoacyl transfer-RNA and polypeptide synthesis. *H. pylori*, regardless of its rapid absorption throughout the gut.^[3] Small amounts of water are soluble in the trihydrate form, resulting in slight hygroscopic behavior. Gastric

floating systems are effective in retaining dosage forms in the stomach for a more time, depending on density (<gastric fluid). For many patients, taking medicine by mouth is more convenient. Incorporating natural polymers into the formulation is economical and widely accepted by patients and formulation experts since synthetic polymers are rare, expensive, and require more time to develop. The authors are searching for a natural polymer used (probable) as a gastric protection agent as well as a release modifier. The authors employed *Abelmoschus esculentus* fruit mucilage (AEFM) in the study of gastro retentive floating microspheres.^[4,5] AEFM has been shown to have a healing effect on stomach/peptic ulcers^[6] and effective against *H. pylori*. Systemic availability at steady state is the aim of computer-aided facilities management

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(CAFMs). For short-acting drugs and those that require incessant medicating, precision liberation systems are a great solution since they are easily applied.

In traditional research methods, one variable at a time is focused on because it is easiest to control. It is statistically impossible to examine all these features concurrently. These factors will be interrelated, leading to unreliable results. The design of experiments (DOE) is central to multivariate analysis.^[7,8] In DOE, partial factors are included in a treaty. Screening and optimization are the objectives of DOE. A, FD incorporates every possible amalgamation of the factors. A high level is either (+1) or 'low' (-1) in FD, and these two levels are called FD. The study findings were based on the use of design expert software to judge the response of floating microspheres of clarithromycin (CMN).^[9,10]

MATERIALS AND METHODS

Mancare Pharmaceuticals Private Ltd., Mumbai, India, provided Clarithromycin (CMN) From the local market, we purchased *A. esculentus* fruits. Sodium alginate (SA) and calcium chloride were from Merck, Hyderabad.

Experimental Design

The CAFMs were screened with a 9 run, central composite design (CCD) using Design-Expert software (11.0.5.0, Stat-Ease Inc.). In the design, the goal was to govern the key, boundary, and quadratic chattels of independent variables on dependent variables using a quadratic model.^[11]

$Y = B_0 + B_1 X_1 + B_2 X_2 + B_{12} X_1 X_2 + B_1 X_1^2 + B_2 X_2^2$. Y is the dependent variable, X_1 and X_2 are the independent variables, and the regression coefficients are B_0 , B_1 and B_2 . The dependent variables/responses in CAFM were drug entrapment efficacy (DEE) and FT. The levels of X_1 (Sod. alginate) were 100 (-1), 150 (0) and 200mg (+1), the levels of X_2 (AEFM) were 50 (-1), 75 (0) and 100mg (+1). The ingredients in various CAFMs (Table 1).

Preparation of CAFM

In calcium chloride solution (2% v/v), a solution of SA, CMN, and AEFM were dissolved [using a three-bladed propeller stirrer (IKA-R1385), 500 rpm for 10 min] was added as drops with a syringe and needle with constant stirring. Glutaraldehyde solution was added dropwise, as part of this

procedure and 15 min of stirring, later filtered (Whatman filter paper) and oven-dehydrated (at 40°C for 2hours).^[12,13] Vacuum desiccators were used to store the CAFM.

Evaluation Parameters

The melting point of CMN was strongminded by using the open capillary method as it is a primary evaluation test to confirm the purity of the medicament.

Drug Excipient Compatibility Studies

CMN and AEFM in a 1:1 ratio mix (10 mg) were whispered in the mini pan of DSC and scanned between 50 to 300°C (Venchal Scientific-412105-USA).

The synergy between CMN and AEFM was scrutinized by FTIR spectroscopy (Bruker) by scanning at a 4000–400 cm^{-1} range.

Evaluation of Physical Properties

Stage micrometers were used to find particle size (PS) of the CAFM. Dry CAFM were placed on a hygienic glass slide and measured under an eyepiece micrometer. Per batch, a minimum of 200 CAFM were counted.^[14]

Production Yield

The production yield was judged by dividing the average weight of parched CAFM (W_1) recovered from each of three trials by the total weight of the initial dry weight (W_2).^[15]

Entrapment Efficiency

In 0.1-M HCl overnight, 100 mg of CAFMs were dispersed with intermittent shaking. At a wavelength of 272 nm, the mixture was filtered, and the filtrate was analyzed spectrophotometrically (Elico Spectrophotometer, SL-174). According to the ratio between the actual extent of CMN in the formulation and the amount initially added, entrapment efficiency was deliberated.^[16]

%Buoyancy Study

A total of 100 mg of CAFM was shuffled through a type II USP dissolution apparatus containing 900 mL of 0.1 N HCl. We agitated the medium for 8 hrs with a paddle rotating at 100 rpm. CAFM was later pipetted and filtered to separate the buoyant layer. Filtration separated the particles in the sinking particulate layer. Both types of particles were dried in a desiccator and weighed. The %buoyancy was judged as follows:^[17]

Table 1: Composition of the CAFM

Component	Formulations								
	CAF-1	CAF-2	CAF-3	CAF-4	CAF-5	CAF-6	CAF-7	CAF-8	CAF-9
Clarithromycin (CMN)	250	250	250	250	250	250	250	250	250
<i>A. esculentus</i> fruit mucilage (mg)	50	50	50	75	75	75	100	100	100
Sodium alginate (mg)	100	150	200	100	150	200	100	150	200
Calcium chloride (%)	2	2	2	2	2	2	2	2	2
Glutaraldehyde (minims)	4	4	4	4	4	4	4	4	4
Deionized water (mL)	200	200	200	200	200	200	200	200	200



In-vitro CMN Release Study

The dissolution of CAFM was investigated by using the USP-II apparatus at a stirring rate of 50 ± 5 rpm at a temperature $37 \pm 0.5^\circ\text{C}$ with 900 mL of HCl (0.1N HCl) as a dissolution medium. A 5 mL sample was introverted at different breaks and the volume of dissolution media was restocked. The samples were then spectrophotometrically analyzed at 272 nm.^[18,19]

Statistical Optimization

We estimated independent influences on the retorts using Design-Expert, which produced contour plots (2D) and response surface plots (3D). Statistically validating polynomial intentions were achieved by judging ANOVA foods. Using the ANOVA endowment, a statistical model was created to regulate model profusion and aptitude (an F-value with $p < 0.05$).

RESULTS AND DISCUSSION

Based on the DSC assessment of the purity of the CMN, it produced an endothermic peak. Combining this peak with excipients, which shifted left, broadens it. DSC observations indicate that CMN has no interface with the excipients.

On the CMN spectrum, secondary amines, phenyl esters, and carboxylic groups are evident. In terms of peaks and stretches, the blend (F-9) compares favorably with pure drug. A spectroscopy study with excipients revealed no obstructions in the CMN peak and stretch intervals.

Using QbD, we investigated the effect of CQAs (DEE and FT) on responses to the (CAFM). An CAFM has been identified. The effects of CMN release and FT on AEFM and SA were investigated.

Optical microscopy was used to decide PS for all formulations. CAFM arrays range in size from 35.62 ± 0.8 m to 50.55 ± 0.6 m (Fig. 1), with CAFM-9 particles having a larger size.

The %yield for CAFM was 80.25 ± 1.5 to 91.02 ± 1.1 , and the maximum yields for CAFM-8 and CAFM-9 (Fig. 1).

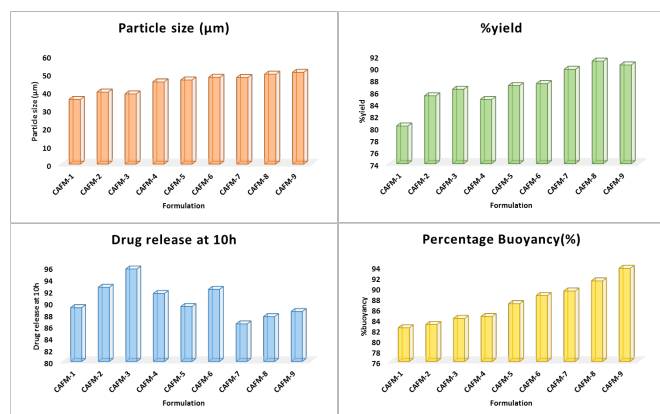


Fig. 1: PS, % yield, drug release at 10 hours and % buoyancy of CAFM

A study was conducted on CAFM to govern its dissolution. The comparison of formulations CAFM-3 and CAFM-2 shows $95.64 \pm 1.4\%$ and $92.54 \pm 1.9\%$ (Fig. 1) of good CMN release at the end of 10 hrs. AEFM began to exhibit release retarding properties as its concentration increased.

The floating ability (Fig. 1) of CAFM-1 formulation was lowest (82.38%) and it was highest for CAFM-9 formulation (93.64%). CAFM with a higher ratio of SA and AEFM were found to have better floating ability than those containing a lower ratio of these polymers. The lower floating ability of the CAFM may be attributed to their small size.

The drug entrapment of CAFM ranged from 76.5 ± 2.19 to 89.8 ± 1.84 . A lower AEFM formulation resulted in good CMN entrapment (Fig. 2).

The drug entrapment of CAFM ranged from 10.5 ± 0.82 to 13.9 ± 1.01 . The FT was proportional to the PS (Fig. 2).

In 0.1M HCl solution, we identified 272 nm λ_{max} as the maximum wavelength for CMN estimation using a UV-VIS spectrometer. The calibration curve followed Beer's law (three times) and fell between 0 to 10 µg/mL. Data like this can be secondhand to fix the uniformity of content. Table 2 shows the fit summary for DEE and FT (h)

The results of the ANOVA for DEE and FT are shown in Table 3.

11973.00 is the F-value for the model, indicating it is significant. There is only a 0.01% chance of such a large F-value occurring due to noise.

Model terms with p-values < 0.0500 are significant. There are three significant model terms in this case: A, B, and B². Model terms with values < 0.1000 are not significant. It may benefit from model reduction if there are numerous insignificant model terms (excluding those obligatory to support the hierarchy).

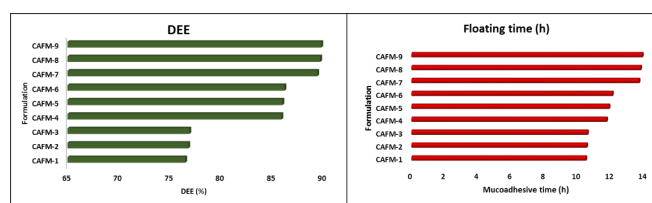
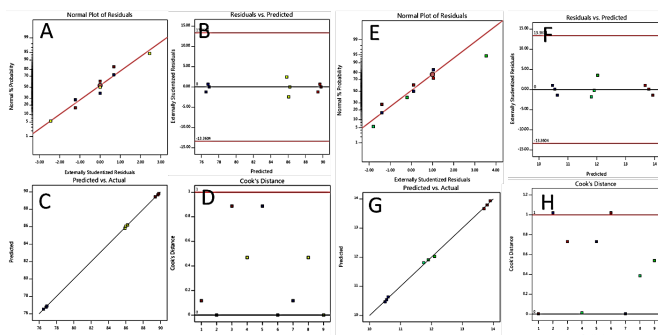


Fig. 2: Drug entrapment efficacy and FT of CAFM

Table 2: Fit summary of the responses

Fit summary for the response 1: DEE (%)			
Source	Sequential p-value	Adjusted R ²	Predicted R ²
Linear	0.0002	0.9185	0.8721
2FI	1.0000		
Quadratic	< 0.0001	0.9999	0.9996
Cubic	0.8660	0.9997	0.9932
Fit summary for the response 2: FT (h)			
Linear	< 0.0001	0.9876	0.9806
2FI	0.7825	0.9853	0.9652
Quadratic	0.0281	0.9977	0.9898
Cubic	0.1429	0.9999	0.9968



Figs. 3A-H: Plots showing the interaction effect of polymers on the drug's EE (A, B, C, D) and the FT (E, F, G, H) for CAFM

Table 3: ANOVA for the responses

ANOVA for the response 1: DEE (%)						
Model	266.07	5	53.21	11973.00	< 0.0001	significant
A-SA	0.2017	1	0.2017	45.38	0.0067	
B-AEFM	249.62	1	249.62	56163.37	< 0.0001	
AB	0.0000	1	0.0000	0.0000	1.0000	
A ²	0.0050	1	0.0050	1.12	0.3667	
B ²	16.25	1	16.25	3655.12	< 0.0001	
Residual	0.0133	3	0.0044			
Cor Total	266.08	8				
ANOVA for the response 2: FT (h)						
Model	16.05	5	3.21	707.52	< 0.0001	significant
A-SA	0.0704	1	0.0704	15.52	0.0291	
B-AEFM	15.84	1	15.84	3492.09	< 0.0001	
AB	0.0025	1	0.0025	0.5510	0.5117	
A ²	0.0001	1	0.0001	0.0306	0.8723	
B ²	0.1335	1	0.1335	29.42	0.0123	
Residual	0.0136	3	0.0045			
Cor Total	16.06	8				

A Model F-value of 707.52 indicates that the model is significant. This large F-value may occur due to noise only 0.01% of the time.

Model terms with p-values <0.0500 are significant. There are three significant model terms in this case: A, B, and B². Values > 0.1000 indicate that the model terms are not significant. It may improve the model by reducing insignificant terms (excluding those needed to support the hierarchy). The equation for entrapment can be expressed as:

$$DEE (\%) = +86.07 + 0.1833A + 6.45B + 0.0000AB - 0.0500A^2 - 2.85B^2$$

With the equation in terms of coded factors, it is possible to predict the response to different levels of each factor. By default, the factors with high levels are coded (+1) and those with low levels as (-1). It is possible to govern the relative impact of the factors by relating the coefficients of the coded equation. Whereas the

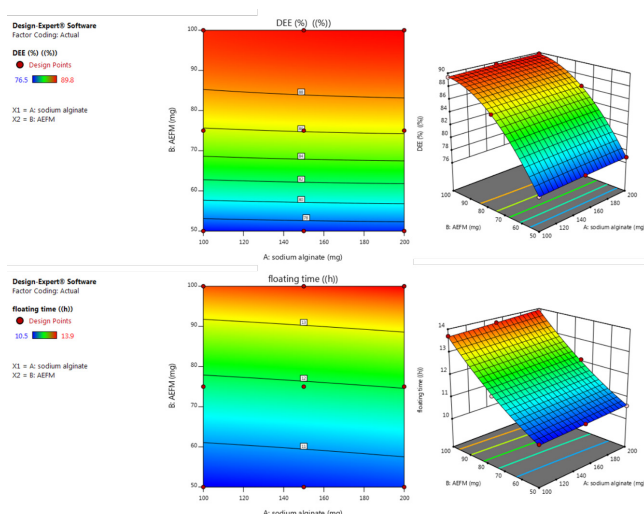


Fig. 4: Contour plot and 3D response plot for DEE (top) and FT (bottom)

final equation for floating time is $FT = +11.91 + 0.1083A + 1.62B + 0.0250AB + 0.0083A^2 + 0.2583B^2$

With the equation in terms of coded factors, it is possible to predict the response to different levels of each factor. By default, the factors with high levels are coded (+1) and those with low levels as (-1). It is possible to fix the relative impact of the factors by equating the coefficients of the coded equation.

Using diagnostic plots, we found the DEE to be highly accurate (Figs. 3A–D). A maximum of coloured points representing the DEE can be seen around the normal probability line in the studentized residuals, indicating that the residuals are normal and that the response data should be analysed as such. According to the residuals (Fig. 3A), there is a stratification around a straight line that is consistent with the normality hypothesis. According to the residuals plot versus predicted values, DEE does not seem to exceed its limits. Based on the distribution of studentized residuals, these plots support the assumption of constant variance (Fig. 3B). Through the plotting of residuals versus run number, we were able to decide the factors predicting DEE testing. The run did not yield any distant observations (Fig. 3C). This figure shows the predicted and actual DEE values in Fig. 3D.

The goodness of fit of the FT was evaluated using diagnostic plots (Figs. 3E–H). Based on normal likelihood plots of superficially studentized residuals, FT could be observed around the normal probability line, leading to a conclusion that residuals were normal, and the analysis of responses was appropriate. Fig. 3E shows that residuals are normally distributed because they are straight lines. FT was within its set limits when plotted against externally studentized residuals. The continuous variance postulation (Fig. 3F) is confirmed by Fig. 3F. There are variables that affect FT, as shown in the residuals and run numbers in the plot. Each point showed steady running



(Fig. 3G). No outliers were observed. FT experimentally perceived values agreed well with predicted values (Fig. 3H).

Fig. 4 is the representation of DEE with contour plots and FT with 3D response plots.

Graphs like this show the simultaneous influence of two factors. As the polymer content increases, the floating time increases in an equivalent manner (Fig. 4).

CONCLUSION

Floating polymers in the floating drug delivery system control the rate and quantity of CMN release. The combination of sodium alginate and AEFM resulted in better floating and CMN release from the formulations. Compared to other formulations, CAFM-3 showed better results. As the AEFM quantity is smaller in formulas CAFM-1 to CAFM-3, entrapment is improved. There was a negative correlation between an increase in floating time and a higher AEFM content in all batches. According to the study, floating microspheres with AEFM and sodium alginate might enhance CMN retention in the stomach.

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