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A Metronidazole-Modeled Central Composite Design Strategy for *Plantago ovata* Seed Mucilage-Supported Floating Microspheres for *Helicobacter pylori* Eradication

Gopinath Subramaniyan[1], Rajesh Pavan Ampapuram[2,*], Ramakrishnan Subburaya Rajendran[3], Padamala Ramakrishna Reddy[4]

[1]Faculty of Pharmacy, Department of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, India
[2]Research Scholar, Department of Pharmacy, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, India
[3]Department of General Medicine, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, India
[4]Department of Pharmaceutics, Balaji College of Pharmacy, Rudram peta, Ananthapuramu-515001, AP, India

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Abstract Background of the research and the purpose: Instead of using costly synthetic excipients or polymers, the authors were in search of an economical, easily available, and plant-based excipient for making microspheres. *Plantago* ovata seeds have already been proven to have anti-ulcer and gastro-protective activity. The same mucilage is used in this study as a polymer that can aid in floating the microspheres. In this study, mucilage from *Plantago ovata* seeds was added to sodium alginate and used with Metronidazole as a treatment to eradicate *Helicobacter pylori*. Methodology: *Plantago ovata* seed mucilage (POSM) and sodium alginate were used to make microspheres. To check the effects of independent variables (POSM and SA levels) on entrapment efficiency (EE) and floating time (FT) as responses, this paper used a central composite design with a design expert.11 software. The microspheres were investigated for Metronidazole (MNZ) content, size, yield, buoyancy, and release during congeniality studies. Results: As POSM content increased in the microspheres, MNZ entrapment increased, and the floating time (FT) was greater in microspheres with more POSM. MNZ release was slightly reduced in microspheres with higher levels of POSM. Conclusion: According to the investigation, MNZ can be retained in the stomach for extended periods of time in order to eradicate *H. pylori,* and the floating assets were enhanced by POSM.

Keywords Design, Floating, Microspheres, Mucilage, Release

1. Introduction

More drugs are now available directly to patients through the oral route, which is a growing trend. It is easy to prepare and administer the gastro retentive microsphere, which is a common dosage form. The semi-synthetic macrolide antibiotic metronidazole is effective in treating *H. pylori* infection. *H. pylori* is a pathogenic bacterium that colonizes profoundly privileged the gastric mucosa[1].

An anaerobic bacterial and protozoal antimicrobial, metronidazole is a nitroimidazole. In the process of passive diffusion, the active ingredient penetrates bacterial cells and produces an active product after reducing the nitro group, resulting in DNA damage[2].

Gastric floating systems are effective in keeping dosage forms in the stomach for a longer period of time, depending on their density (<gastric fluid). However, oral medication may be more convenient for some patients[3, 4]. Since synthetic polymers are rare, expensive, and take a long time to develop, natural polymers are economically and widely accepted in formulations[5, 6]. The authors are looking for a polymer that acts as both a gastric protection agent and a potential release modifier [7]. In the study of gastroretentive floating microspheres, POSM (*Plantago ovata* seed mucilage) was utilized[8]. POSM has been shown to have a healing effect on stomach/peptic ulcers and is effective against *H. pylori*. Metronidazole Plantago ovata seed mucilage-supported Floating Microspheres (MPFM) aims to ensure continuous systemic availability. Because precision liberation systems are easy to apply, they provide a great solution for drugs that are short-acting or require incessant medicating[9].

Due to the difficulty of adjusting multiple variable at a time, traditional research methods focus on one at a time[10]. All these features cannot be examined simultaneously due to statistical limitations. All these factors will be interrelated, making the results unreliable. An important component of multivariate analysis is the design of experiments (DOE). In DOE, partial factors are taken into account. The objectives of DOE are screening and optimization. In A, FD, the factors are amalgamated in every possible way[11]. FD is either "high" (+1) or "low" (-1) in terms of the level. Using design expert software, floating microspheres of MNZ in this study were evaluated[12].

2. Materials and Methods

Abbott Pharma, Hyderabad, India, provided Metronidazole (MNZ). From the local market, we purchased *Plantago ovata* seeds. Sodium alginate (SA) and calcium chloride were from Merck, Hyderabad.

2.1. Experimental Design

A 9-run, central composite design (CCD) was conducted using Design-Expert software (11.0.5.0, Stat-Ease, Inc.). This design aimed to predict independent variables by following their key, boundary, and quadratic chattels[13].

$Y= B_0+B_1\ X_1+B_2X_2+B_{12}X_1X_2+B_1X_1^2 + B_2X_2^2$. 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 MPFM are drug entrapment efficacy (EE) and floating time (FT). The levels of X_1 (Sod. alginate) are 100 (-1), 150 (0) and 200mg (+1), the levels of X_2 (POSM) are 50 (-1), 75 (0) and 100mg (+1). The ingredients in various MPFMs are shown in Table 1.

2.2. Preparation of MPFM

With a syringe and needle, calcium chloride solution (2% v/v) was added as drops, and SA, MNZ, and POSM solutions were dissolved [with a three-bladed propeller stirrer (IKA-R1385), 500 rpm for 10 min] with constant stirring. During this process, gluteraldehyde solution was added dropwise and stirred for 15 min. After filtering (Whatman filter paper) and dehydrating (at 40°C for 2 h), the mixture was then filtered[14]. A vacuum desiccator has been used to store the MPFM.

2.3. Evaluation Parameters

The open capillary method was used to determine the melting point of MNZ which indicates the initial impression to its purity.

2.3.1. Drug excipient compatibility studies

The samples were mixed together (1:1) in the mini pan of DSC at 50-300°C and scanned between 50-300°C (Venchal Scientific-412105-USA). FTIR spectroscopy (Bruker) was used to investigate the synergy between MNZ and POSM at a 4000-400 cm^{-1} range.

2.4. Evaluation of Physical Properties

In order to determine the particle size (PS) of the MPFM, a stage micrometer was used. Measurements were conducted under an eyepiece micrometer using dry MPFMs placed on a hygienic glass slide. Each batch of MPFMs was counted at least 200 times.

Table 1. Composition of the MPFM

Component	Formulations								
	F-1	**F-2**	**F-3**	**F-4**	**F-5**	**F-6**	**F-7**	**F-8**	**F-9**
Metronidazole (MNZ)	250	250	250	250	250	250	250	250	250
P. ovata seed 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 (ml)	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
Deionized water (ml)	200	200	200	200	200	200	200	200	200

2.4.1. Production yield

In each trial, the weight of MPFM (W_1) was determined by the basis of dry weight (W_2). This yield was resolute by allocating each dry MPFM (W_1) by the basis of dry weight (W_2)[15].

$$\%\ \text{Yield} = \frac{\text{Weight of attained by microspheres}}{\text{Total weight of drug and polyme}} \text{X } 100$$

2.4.2. Entrapment Efficiency

Using 0.1 M HCl as a dispersant overnight, 100 mg of MPFMs were dissolved in the solution. The mixture was filtered and the filtrate was analyzed at 278 nm spectrophotometrically (Elico Spectrophotometer, SL-174). EE was calculated based on the ratio of the actual amount of MNZ in the formulation to the amount initially added[16].

$$\text{Entrapment efficacy} = \frac{\text{Practical drug yield}}{\text{Theoritical drug content}} \text{X } 100$$

2.4.3. % Buoyancy Study

900 ml of 0.1 N HCl containing 100 mg of MPFM were shuffled through a type II USP dissolution apparatus at 100 rpm and agitated for 8 h. In order to separate the buoyant layer, MPFM was pipetted and filtered. Filtration separated the sinking particulate layer from the buoyant layer. A desiccator was used to dry and weigh both types of particles and determine buoyancy based on the following formula[17]:

$$\%\ \text{Buoyancy} = \frac{\text{Initial weight of microspheres}}{\text{Weight of floating microspheres}} \text{X } 100$$

2.4.4. In Vitro MNZ Release Study

As a dissolution medium, 900 ml of HCl (0.1N HCl) was used with a stirring rate of 50±5 rpm at 37±0.5°C using the USP-II apparatus. At each break, 5 ml of dissolution media was restocked, and the same amount was introverted and spectrophotometrically measured at 278 nm[18].

2.4.5. Statistical optimization

Utilizing Design-Expert (v.11), which creates contour plots (2D) and response surface plots (3D), we can predict independent stimuli on the ripostes. By judging ANOVA values, it was able to validate polynomial intents statistically. The ANOVA bequest was used to create a statistical model for regulating model profusion and gifts (p-value < 0.05)[19].

3. Results and Discussion

By DSC analysis, MNZ produced a peak indicating endothermic purity. When combined with the excipients shifted to the left, this peak becomes broader. Based on DSC observations, MNZ does not interact with the excipients. Secondary amines, phenyl esters, and carboxylic groups appear in the drug spectrum. When it comes to peaks and stretches, the blend (1;1) is comparable each other. In a spectroscope study, the peak and stretch intervals of the MNZ were not obstructed by excipients. Using QbD, we examined how critical quality attributes (CQAs) i.e., DEE and FT affected responses to the MPFM. The MPFM has been recognized. It was scrutinized the belongings of the MNZ release and the FT on POSM and SA. PS was resolute to using optical microscopy for all microspheres. The MPFM particles ranged in size from 33.4±1.02 µm to 43.7±2.34µm (Figure 1), with F-6 particles being larger. MPFM yields ranged from 79.1±3.85 to 96.3±6.25%. In Figure 1, the maximum yields for F-4 (96.3±6.25%) and F-2 (94.7±2.36%) with the least amount are shown. A study was conducted on MPFM to govern its dissolution. The comparison of formulations F-6 showed 96.3±6.25% (Figure 1) of good MNZ release at the end of 10 h. As POSM concentration is increased, its release retarding properties become more evident. The F-1 formulation showed the lowest floating ability (82.4±5.1%) (Figure 1), while the F-9 formulation had the highest (93.2±3.8%). It was revealed that MPFMs with higher ratios of SA and POSM exhibited better flotation than those with lower ratios. The MPFM's small size may be answerable for their lower floating ability for having less density compared to other formulations.

The EE of MPFM ranged from 70.3±3.28 to 83.3±4.84. A lower POSM formulation resulted in good MNZ entrapment (Figure 2).

In 0.1M HCl solution, MNZ valuation was done using a UV-VIS spectrometer at 278 nm λ_{max} as the maximum wavelength. The calibration curve (Figure 2) represented that Beer's law was obeyed at a concentration of 0-10 g/ml.

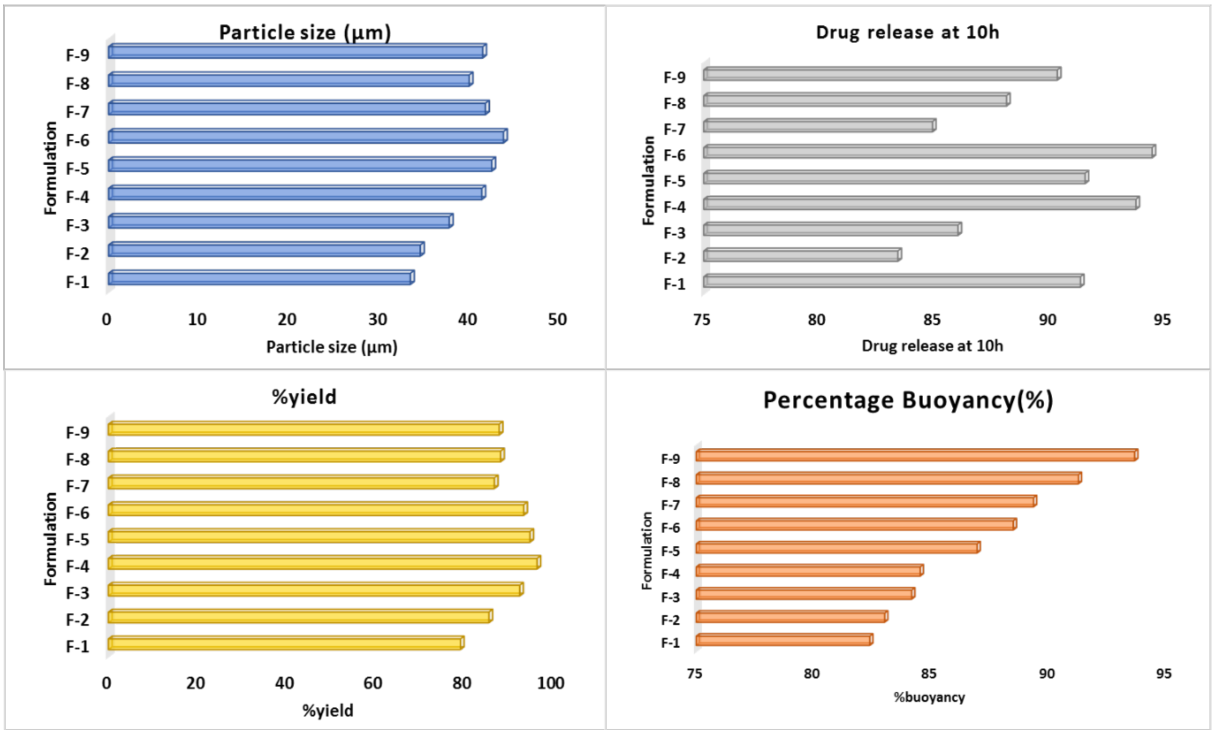

Figure 1. PS, % yield, drug release at 10h, and % buoyancy of MPFM

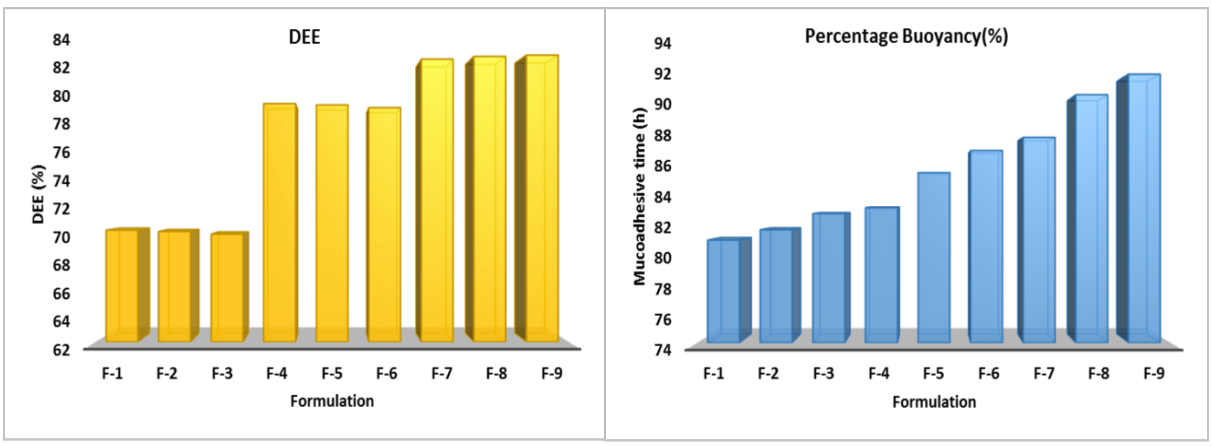

Figure 2. Drug entrapment efficacy and FT of MPFM

Table 2. Fit summary of the responses

Fit Summary for response 1: DEE (%)			
Source	**Sequential p-value**	**Adjusted R²**	**Predicted R²**
Linear	0.0002	0.9165	0.8698
2FI	0.8766	0.9003	0.7638
Quadratic	**< 0.0001**	**0.9997**	**0.9986**
Cubic		1.0000	
Fit summary for response 2: FT (h)			
Linear	< 0.0001	0.9697	0.9446
2FI	0.2642	0.9724	0.9233
Quadratic	**0.0020**	**0.9993**	**0.9975**
Cubic	0.8220	0.9985	0.9659

Table 3. ANOVA for the responses

ANOVA for the response 1: DEE (%)						
Model	270.43	5	54.09	5408.60	< 0.0001	significant
A-SA	0.0150	1	0.0150	1.50	0.3081	
B-POSM	253.50	1	253.50	25350.00	< 0.0001	
AB	0.0900	1	0.0900	9.00	0.0577	
A^2	0.0050	1	0.0050	0.5000	0.5305	
B^2	16.82	1	16.82	1682.00	< 0.0001	
Residual	0.0300	3	0.0100			
Cor Total	270.46	8				
ANOVA for the response 2: FT (h)						
Model	148.51	5	29.70	2167.41	< 0.0001	significant
A-SA	0.1350	1	0.1350	9.85	0.0517	
B-POSM	145.04	1	145.04	10584.12	< 0.0001	
AB	0.8100	1	0.8100	59.11	0.0046	
A^2	0.0272	1	0.0272	1.99	0.2535	
B^2	2.49	1	2.49	181.99	0.0009	
Residual	0.0411	3	0.0137			
Cor Total	148.55	8				

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

Model F-value is 5408.60, which indicates we are dealing with a significant model. An F-value of such a large value is unlikely to occur due to noise with a probability of 0.01%. Terms with p-values exceeding 0.0500 are considered significant. The three most significant model terms here are A, B, and B_2. The terms with a value of >0.1000 do not have any significance. It may be beneficial to reduce model terms (excluding those necessary to support the hierarchy) if there are a lot of insignificant terms.

This model has a significant F-value of 2167.41, which indicates it is significant. This large F-value occurs 0.01% of the time due to noise. Model terms with p-values of <0.0500 are significant. The model consists of three significant terms: A, B, and B_2. Model terms > 0.1000 are not significant. The model might be improved by eliminating insignificant terms (apart from those required to maintain the hierarchy). Entrapment can be expressed the following way:

$$\text{DEE (\%)} = +79.60-0.0500A+6.50B+0.1500AB-0.0500A^2-2.90B^2$$

The equation in terms of coded factors can be used to make predictions about the response to the given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. Using the coded equation, it is possible to compare the factor coefficients to identify the relative impact of the factors.

$$\text{\% Buoyancy} = +86.39+0.1500A+4.92B+0.4500AB+0.1167A^2+1.12B^2$$

It was found that the DEE was highly accurate (Figures 3A & B) after analyzing diagnostic plots. Around the normal probability line in the studentized residuals, a maximum of coloured points representing the DEE can be observed, indicating that the residuals are normal and that the response data should be analyzed as such based on the residuals (Figure 3A). Figure 3B shows that the values were far from the cook's line and supports the assumption of constant variance. 3D diagnostic plots (Figures3C & D) were used to evaluate the goodness-of-fit of the FT. According to the plots of the normal likelihood of superficially studentized residuals, the FT could be seen around the normal probability line, supporting the conclusion that the residuals were normal and the analysis of responses was appropriate. Figure 3A illustrates that residuals follow a normal distribution since they are straight lines and all points showed steady running (Figure 3D) and none of the points were extreme.

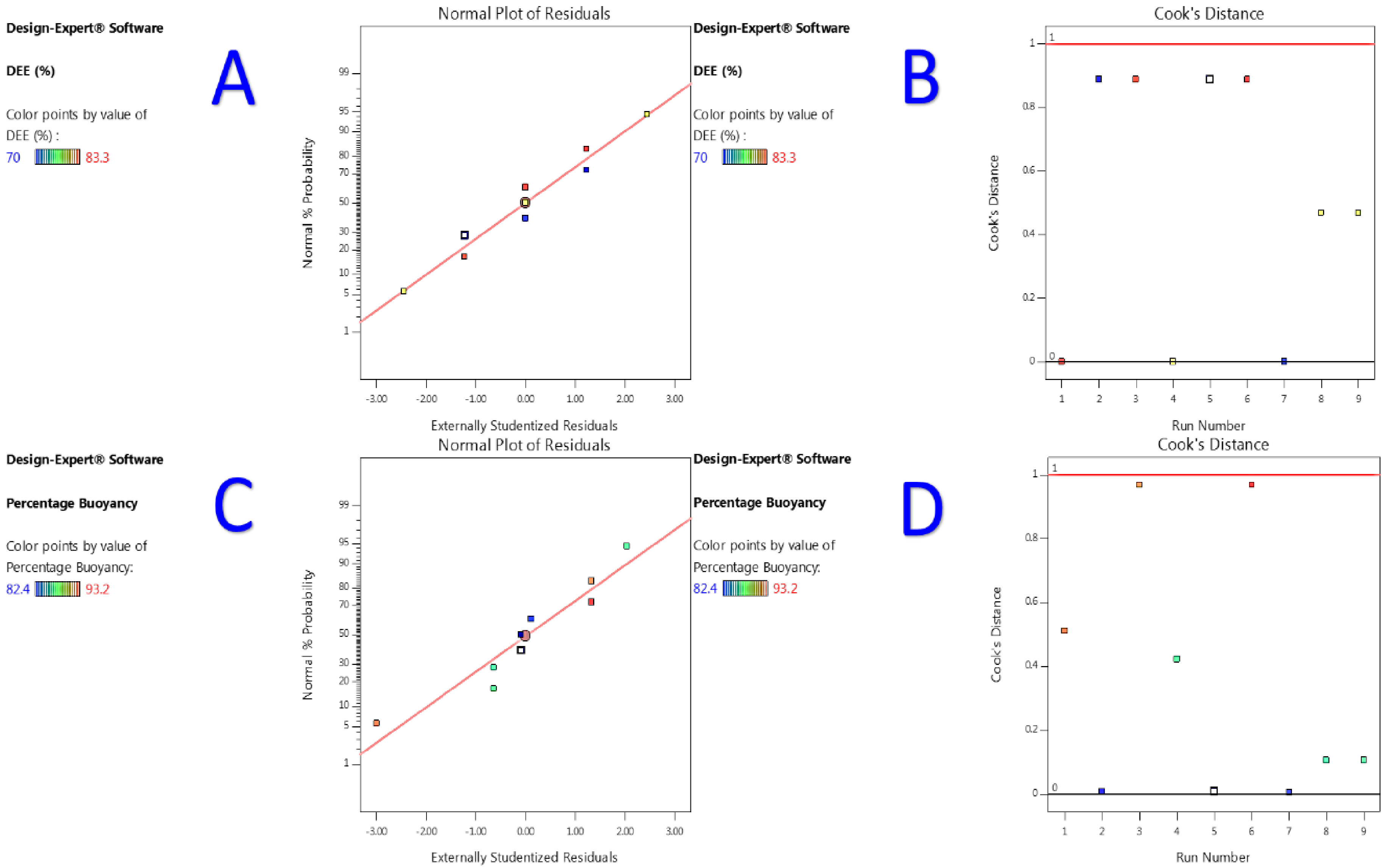

Figure 3 A-D. Plots showing the interaction effect of polymers on the drug's EE (A&B) and the FT (C&D) for MPFM

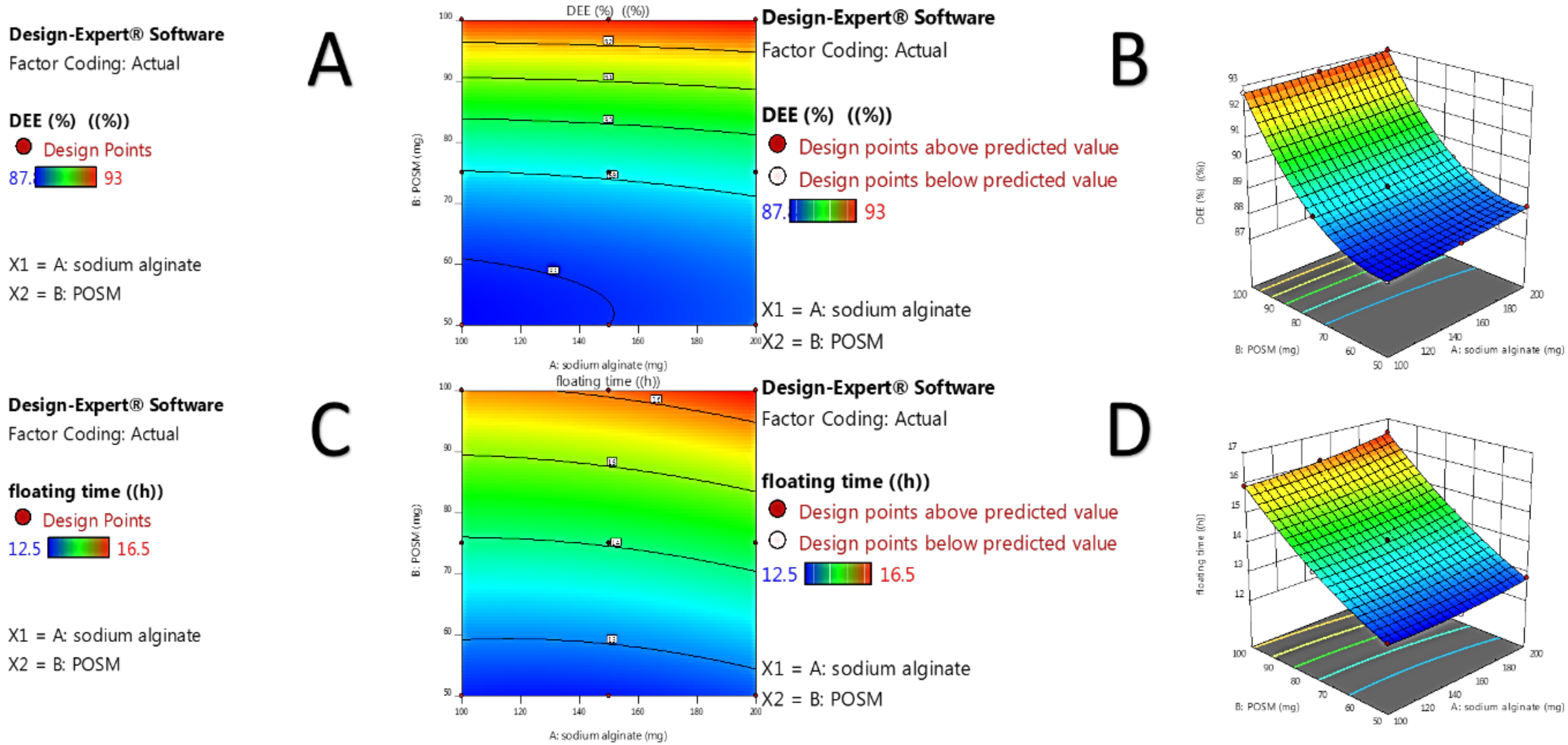

Figure 4. Contour plot and 3D response plot for DEE (A&B) and FT (C&D)

Figure 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. The floating time of MPFM was found to increase with the increase in the concentration of sodium alginate and POSM. The relation can be seen in Figure 4.

4. Conclusions

A floating polymer in the formulated microspheres controls the release of metronidazole (MNZ). Combining sodium alginate and Plantago ovata seed mucilage (POSM) made the formulations float better and release MNZ more readily. When compared to other formulations, F-9 was more effective at retaining MNZ. Formulas F-1 to F-3 have more entrapment as their POSM quantities are smaller. The study concludes that as POSM content increased in the microspheres, MNZ entrapment increased, and the floating time (FT) was greater in microspheres with more POSM. The MNZ release was slightly reduced in microspheres with higher levels of POSM. According to the investigation, MNZ can be retained in the stomach for lengthy periods to eradicate *H. pylori* and the floating assets were boosted by POSM. The study summarizes that POSM can be used in combination with metronidazole in effective eradication of *H. pylori* with added advantage of gastric protection.

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