ORIGINAL ARTICLE

Development and Optimization of Metformin Hydrochloride Loaded Hydrogel Microspheres Prepared with Natural Polymers

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Abstract

Aim: Natural materials have advantages over synthetic materials as pharmaceuticals because they are nontoxic, less expensive, and easily available. Furthermore, they can be modified to obtain customized materials for drug delivery systems better or equivalent to synthetic products that are commercially available. The present investigation aimed at the optimization of controlled release hydrogel microsphere of metformin hydrochloride prepared with bora rice flour (BRF), mucilage of *Dillenia indica* fruits, and mucilage of *Abelmoschus esculentus* in combination. **Materials and Methods:** Physical, mucoadhesive, and *in vitro* drug release properties were studied. Validation of the optimization process, selection of optimized batch, and stability study of optimized batch were also among the objectives of this study. The response surface approach was used for optimization process. The experimental values were compared with the predicted values, and percentage errors were calculated. **Results and Discussion:** In the statistical optimization process, the models for the selected response variables were significant. It was observed that there was variable influence of the concentration of the independent variables (BRF and mucilage) on the responses. Mucilage exhibited pronounced effect on the properties of microspheres than BRF. However, the observed effect was the resultant effect of the influence of individual variables on microspheres. **Conclusion:** In this study, much deviation was not of the experimental values from the predicted values.

Key words: Bora rice, hydrogel, microspheres, mucilage, optimization

INTRODUCTION

Hydrophilic matrix systems are most popular because of the simplicity of formulation, ease of manufacturing, low cost, Food and Drug Administration acceptance, and applicability to drugs with a wide range of solubility.[1-5] Drug release from these systems is the sequence of controlled matrix hydration, followed by gel formation, change of textural/rheological behavior, matrix erosion, and/or drug dissolution and diffusion, the significance of which depends on drug solubility, concentration, and changes in matrix characteristics.[6]

Statistical experimental design methodologies are powerful, efficient, and systematic tools in the design of pharmaceutical dosage forms, allowing a rational study of the influence of formulation parameters on the selected responses with a shortening of the experiment time and an improvement in the research and development work.[6-8] The main objective of the experimental design strategies is to plan experiments to obtain maximum information regarding the considered experimental domain with the lowest number of experiments,[9] allowing a quick and efficient quantification and prediction of the effects of formulation changes on the considered significant responses.[10-13] Response surface method (RSM) designs help to quantify the relationships between one or more measured responses and the vital input factors. Goals might include meeting a set of specifications for several responses simultaneously.

The previous study demonstrated bora rice powder, mucilage from *Dillenia indica* (DI) fruits, and mucilage of *Abelmoschus*

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esculentus (AE) as potential raw material for drug delivery.[14-16] The present investigation aimed at the optimization of controlled release hydrogel microsphere of metformin hydrochloride (MH) prepared with bora rice flour (BRF), mucilage of DI fruits, and mucilage of AE in combination. The study aimed at the evaluation of various parameters such as particle size, liquid uptake capacity, drug entrapment efficiency (DEE), *in vitro* and *ex vivo* mucoadhesive properties, and *in vitro* drug release. Validation of the optimization process and selection of optimized batch of microsphere were also among the objectives of this study. The objective also covered stability study of the optimized batch. The results of the study are reported here.

MATERIALS AND METHODS

Materials

MH was received as a gift sample from Ozone Pharmaceutical Ltd., Assam, and India. The mucilage of DI and AE was extracted by acetone precipitation method.[17] All other chemicals used in this study were of analytical grade and were procured commercially. These were used as such without testing and purification. The intestinal portion of goat, used for mucoadhesive study, was procured from the local slaughterhouse. This was washed with phosphate buffer pH 7.4 to remove non-cellular materials. Design-Expert® 8 Application (Design-Expert version 8.0.6 Trial, Stat-Ease Inc., USA) was used for the design and optimization of the formulation, Microsoft Office Excel 2003 (Microsoft® Office 2003 version 11.0.5612, Microsoft Corporation, USA) was used for generation of graphical representation of the data of the experiments.

Methods

Experimental design

The response surface approach involving central composite design is a randomized full factorial design with rotatable alpha value (α = 1.41421), which creates a design that has the standard error of predictions equal at points equidistant from the center of the design, was employed with the help of Design-Expert® 8 Application. Maximizing the data of selected formulation of development batch of MH, ESF-6, keeping different ratio of the amount of BRF (a) and mucilage (mucilage of both DI and AE) (b) were selected as the factors (independent variables). The cumulative drug release (%) in 10 h (Rel_{10hr}), particle size (μm), and DEE (%) was taken as response variables. The amount of MH, revolution of mechanical stirrer and other processing variables were kept constant throughout the study.

Formulation

Aqueous dispersions (8.0 ml) of BRF, mucilage of DI fruits, and mucilage of AE pods in different amounts were dispersed together in water so that 8.0 ml of the dispersion when mixed with the 32.0 ml of the oil phase to form w/o emulsion with required concentration of the excipients, as shown in Table 1.

Evaluation

Physical properties

Evaluation of the microspheres was carried out for particle size, surface topography, liquid uptake capacity, DEE, and mucoadhesive property. *Ex vivo* mucoadhesive test was carried out in phosphate buffer (pH 7.4) using goat intestinal mucosa.[17-19] The *in vitro* drug release study was carried out in phosphate buffer (pH 7.4). The data of *in vitro* drug release study were fitted in various kinetic models to find out the release kinetics, and the mechanism of release was delineated by fitting the data in Korsmeyer–Peppas model. The best expression of release kinetics for the prepared batch of microspheres was ascribed to that in which the R^2 value was closest to one.

Optimization of data analysis and validation of optimization model

Various RSM computations for the current optimization study were performed employing design expert application.

Table 1: Composition of the factorial batch formulation of metformin hydrochloride loaded microspheres

Formulation code	Composition and formulation parameters							
	Bora rice flour (%)	Mucilage of *Dillenia indica* and *Abelmoschus esculentus* (%) (1:1)	Drug (%)	Ethyl cellulose (g)	Methanol (ml)	Dichloro-methane (ml)	Acetone (ml)	Stirring speed (rpm)
OP-ES-1	2	2	2.5	0.5	7.0	15.0	10.0	500
OP-ES-2	1	3	2.5	0.5	7.0	15.0	10.0	500
OP-ES-3	3	1	2.5	0.5	7.0	15.0	10.0	500
OP-ES-4	1	1	2.5	0.5	7.0	15.0	10.0	500
OP-ES-5	3	3	2.5	0.5	7.0	15.0	10.0	500
OP-ES-6	2.7	2.9	2.5	0.5	7.0	15.0	10.0	500
OP-ES-7	2.7	1.2	2.5	0.5	7.0	15.0	10.0	500
OP-ES-8	2.8	1.1	2.5	0.5	7.0	15.0	10.0	500
OP-ES-9	2.5	2.4	2.5	0.5	7.0	15.0	10.0	500

Polynomial models, including interaction and quadratic terms, were generated for all the response variables using multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented as follows:

$$Y = \beta_0+\beta_1A+\beta_2B+\beta_3AB+\beta_4A^2+\beta_5B^2+\beta_6AB^2+\beta_7A^2B+\beta_8A^2B^2 \quad (1)$$

Where, β_0 is the intercept representing the arithmetic average of all quantitative outcomes of nine runs; β_1–β_8 are the coefficients computed from the observed experimental response values of Y; and A and B are the coded levels of the independent variables. The terms AB and Ai^2 (i=1–2) represent the interaction and quadratic terms, respectively. The statistical validity of the polynomials was established on the basis of analysis of variance (ANOVA) provision in the design expert application. Subsequently, the feasibility and grid searches were performed to locate the composition of the optimized formulation.[20-22]

Two-dimensional (2-D) perturbation, actual versus predicted and three-dimensional (3-D) response surface plots were constructed based on the model polynomial functions using Design-Expert software to see the interaction effects on the factors and deviation corresponding responses from reference points.

Nine optimum checkpoints were selected to validate the chosen experimental design and polynomial equations. The factorial formulations corresponding to the checkpoints were prepared and evaluated for various responses as described under, and subsequently, the resultant experimental data of response properties were quantitatively compared with that of their predicted values.

Selection and comparison of the optimized batch formulation of MH

The optimized batch of MH loaded microsphere was selected on the basis of exhibited physical and *in vitro* drug release properties in relation to the predicted values. The cumulative amount of drug release (%) in 10 h (Rel_{10hr}), DEE, and particle size were considered as responses of the two variables. The formulation exhibiting the close value to the predicted and theoretical values of these properties was selected as the optimized formulation.

Stability study of the optimized batch formulation of MH

The stability study of the optimized formulation was carried out in accelerated condition[23] at 40 °C ± 2 °C temperature and 75 % ± 5 % RH. The relative humidity of 75 % ± 5 % was created using a saturated solution of sodium chloride. The formulation was tested at 3 time points, 0, 3, and 6 months, for changes in surface and drug release property.

The *in vitro* drug release data of the formulation pre- and post-stability study were applied for calculation of the similarity factor (f_2) and difference factor (f_1) as per SUPAC-MR (1997) using the following equations (Equation-2 and 3).[17,21-23] The similarity factor >50 (>50) indicates similarity of release profiles. The difference factor should be lower than 15 (<15).

$$f_2 = 50 \log\left\{\left[1+1/n\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5} \times 100\right\} \quad (2)$$

Where, n- number of sampling time points, Σ- summation over all time points, R_t- dissolution at time point "t" of the reference (unchanged drug product, i.e., pre-change batch), T_t- dissolution at time point "t" of the test (changed drug product, i.e., post-change batch).

The difference factor is given by

$$f_1 = \frac{\sum_{t=1}^{n}(R_t - T_t)}{\sum_{t=1}^{n}R_t} \quad (3)$$

Where, "n" is the number of sampling points; R_t and T_t are the percent dissolved of reference and test samples at time point "t," respectively.

RESULTS AND DISCUSSION

Physical properties

Particle size

The particle size of the factorial batch formulation of MH was found to be in the range of 120 ± 3.22–230 ± 1.22 µm (OP-ES-1 to OP-ES-9). The smallest particle size was found in formulation OP-ES-4 with BRF to mucilage ratio of 1:1, whereas in formulation OP-ES-5 with BRF to mucilage ratio 3:3, largest particle was detected [Table 2]. It was observed that the particles were larger in formulations where the BRF to mucilage ratio was more. The particle size of the formulations in descending order was OP-ES-5>OP-ES-6>OP-ES-9>OP-ES-8>OP-ES-2>OP-ES-3>OP-ES-7>OP-ES-1>OP-ES-4. It was observed that the increase in particle size was not in order with respect to either amount of BRF and mucilage alone, but was dependent on the sum total of the amount of both BRF and mucilage.

Surface topography

The scanning electron microscopy image of the OP-ES-5 of the factorial batch formulation showed the spherical shape and smooth surface.

Liquid uptake capacity

The liquid uptake capacity (%) of the factorial batch microspheres [Table 3] exhibited high uptake in a buffer (pH 7.4), and in water but low in 0.1 M HCl. This revealed that the uptake was affected by the pH of the medium. The uptake capacity of the microspheres in 0.1 M HCl exhibited almost opposite trend to the uptake in buffer (pH 7.4). On

Table 2: *In vitro* evaluation of factorial batch of metformin hydrochloride loaded microspheres

Formulation code	Mean particle size (μm)±SD *n*=100	Mean drug entrapment efficiency (%)±SD; *n*=3
OP-ES-1	128±3.77	78.42±0.58
OP-ES-2	145±0.98	84.67±1.53
OP-ES-3	140±2.15	70.37±1.07
OP-ES-4	120±3.22	72.75±1.07
OP-ES-5	230±1.22	83.22±0.70
OP-ES-6	210±0.95	81.14±1.53
OP-ES-7	140±1.64	76.3 ±1.14
OP-ES-8	155±2.37	74.28±1.67
OP-ES-9	165±2.58	81.18±0.98

SD: Standard deviation

Table 3: Liquid uptake capacity of the factorial batch of metformin hydrochloride loaded microspheres

Formulation code	Liquid uptake capacity in different media (%) ±SD; *n*=3		
	0.1 M HCl	Water	Phosphate buffer (pH 7.4)
OP-ES-1	30.34±0.36	51.05±0.02	55.12±0.52
OP-ES-2	20.52±0.49	58.08±0.66	62.24±0.43
OP-ES-3	36.26±0.47	48.14±0.69	42.18±0.03
OP-ES-4	26.22±0.87	41.62±0.26	46.32±0.13
OP-ES-5	24.74±0.33	54.55±0.08	60.16±0.69
OP-ES-6	21.28±0.47	56.52±0.84	59.14±0.22
OP-ES-7	31.38±0.15	47.63±0.78	52.66±0.65
OP-ES-8	33.63±0.46	43.87±0.26	48.68±0.45
OP-ES-9	31.28±0.21	53.88±0.35	58.08±0.76

SD: Standard deviation

the other hand, similar trend of uptake was observed in both water and buffer (pH 7.4) except the formulations OP-ES-4, in which the ratio of BRF to mucilage was 1:1. The uptake in phosphate buffer (pH 7.4) was highest in formulation OP-ES-2 followed by OP-ES-5, OP-ES-6, OP-ES-9, and OP-ES-1. This revealed that the uptake was dependent on the amount of mucilage incorporated.

DEE

The percentage DEE of the formulations OP-ES-1 to OP-ES-9 was found to the in the range of 70.37 ± 1.07–84.67 ± 1.53 [Table 2]. The formulations OP-ES-2, OP-ES-5, OP-ES-6, and OP-ES-9 exhibited more than 80.0 % entrapment of MH. The formulation OP-ES-2 exhibited highest entrapment (84.67 ± 1.53 %) of MH. Increased entrapment of MH was observed when higher amount of mucilage was incorporated into BRF.

Mucoadhesive property

The results of *in vitro* wash-off test carried out in phosphate buffer (pH 7.4) for a period of 4 h, as shown in Figure 1. It was observed that a large population of microspheres was washed away from the surface of the goat intestinal mucosa within 30 min. Thereafter, the removal of microspheres from the surface was relatively less. This was due to the fact that there was an increase in the swelling of microspheres after 30 min which led to higher adhesion force between the mucosal and microsphere surfaces, and less number of microspheres was washed away. It was observed that the formulations OP-ES-1, OP-ES-2, OP-ES-5, OP-ES-6, and OP-ES-9 exhibited more adhesion than formulations OP-ES-3, OP-ES-4, OP-ES-7, and OP-ES-8. The formulation OP-ES-2 exhibited highest adhesion in comparison to other formulations at 4 h.

The results of *ex vivo* mucoadhesive study are depicted in Table 4. It was observed that OP-ES-2 exhibited the highest force of adhesion in comparison to rest of the formulations. The formulations in descending order of adhesion force, measured after 30 min, were OP-ES-2, OP-ES-6, OP-ES-5, OP-ES-9, OP-ES-1, OP-ES-7, OP-ES-8, OP-ES-4, and OP-ES-3 [Table 4]. This demonstrated the influence of mucilage on the mucoadhesive property of the formulations.

Table 4: Summary of the experimental values of response variables of the formulations (OP-ES-1 to OP-ES-9)

Formulation Code	Mean particle size (μm)±SD; *n*=100	Mean drug entrapment efficiency (%) ±SD; *n*=3	Liquid uptake in phosphate buffer (pH 7.4)	No. of particles adhered on mucosal surface at 4th h (*in vitro* wash off test)	Detachment force (dyne.cm^{-2}) after 30 min (*ex vivo* mucoadhesive test)	Cumulative drug release (%) in 10 h	Release exponent (*n*) of Korsmeyer–Peppa model
OP-ES-1	128±3.77	78.42±0.58	55.12±0.52	5	6691.47	82.86	0.7239
OP-ES-2	145±0.98	84.67±1.53	62.24±0.43	8	7459.24	90.23	0.5774
OP-ES-3	140±2.15	70.37±1.07	42.18±0.03	2	5187.13	84.65	0.7239
OP-ES-4	120 ±3.22	72.75±1.07	46.32±0.13	3	5717.71	83.68	0.7041
OP-ES-5	230±1.22	83.22±0.70	60.16±0.69	6	7016.05	79.65	0.7466
OP-ES-6	210±0.95	81.14±1.53	59.14±0.22	5	7131.53	82.03	0.6171
OP-ES-7	140±1.64	76.3 ±1.14	52.66±0.65	4	6672.74	82.62	0.6359
OP-ES-8	155±2.37	74.28±1.67	48.68±0.45	4	6195.22	83.21	0.6912
OP-ES-9	165±2.58	81.18±0.98	58.08±0.76	5	6810.06	81.21	0.6438

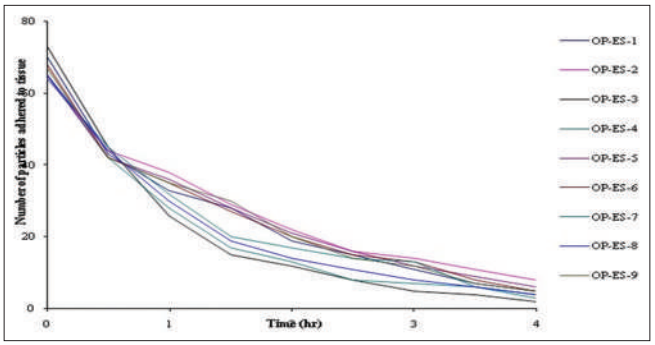

Figure 1: *In vitro* wash-off test of the optimized batch of microspheres (OP-ES-1 to OP-ES-9)

Kinetics of drug release

In vitro drug release data were fitted in the equation of kinetic models to obtain drug release profiles [Figure 2]. The *in vitro* drug release rate constant was calculated and the correlation coefficient (R^2) was determined for all the formulations [Table 5]. The *in vitro* drug release of the formulations OP-ES-1, OP-ES-4, OP-ES-8, and OP-ES-9 was best explained by the first-order equation with highest linearity. The correlation coefficient (R^2) of the above formulations was 0.9433, 0.9582, 0.9697, and 0.9782, respectively. On the other hand, formulations OP-ES-2, OP-ES-3, OP-ES-5, OP-ES-6, and OP-ES-7 with the correlation coefficient (R^2) 0.9236, 0.9557, 0.9596, 0.8906, and 0.913 followed Higuchi kinetics, which revealed that the drug diffused at a comparatively slower rate as the diffusional path length was increased. This is referred to as the square root kinetics (or Higuchi's kinetics).[24]

It was observed that OP-ES-5 and OP-ES-2 released the lowest and highest percentage of drugs in 10 h [Table 4], respectively. This might be due to erosion of the microspheres due to higher amount of BRF, because, the ratio of BRF and mucilage in OP-ES-5 was 3:3, whereas, it was 1:3 in OP-ES-2.

Mechanism of drug release

The drug release mechanism from controlled release devices is very complex and the prediction of the mechanism does not always resemble practical situations. Although some processes may be classified as either purely diffusional or purely erosion controlled, many others can only be interpreted as being governed by both. To evaluate the mechanism of drug release of controlled release hydrogel microspheres of MH, the *in vitro* drug release data at various time points were fitted in the Korsmeyer–Peppas equation.

The "R^2" and "*n*" values of various factorial batch formations are depicted in Table 5. The value of the correlation coefficient of the formulations 0.9498, 0.8963,

0.9498, 0.8608, 0.9698, 0.8288, 0.8483, 0.9756, and 0.9725 for OP-ES-1 to OP-ES-9, indicated good linearity between the "log cumulative amounts of drug release" versus "log time." The release exponent (n) value of the formulations was >0.45 and <0.89 (0.45<n<0.89), which indicated that the mechanism of drug release from the microspheres was non-Fickian, anomalous diffusion. Fickian diffusion is characterized by linear dependence of the release of drug with the square root of time, that is, concentration dependent. The fundamental principle of diffusion is based on Fick's laws, which describe the macroscopic transport of molecules by a concentration gradient. Anomalous diffusion of drug release mechanism signifies a coupling of the diffusion and erosion mechanism which indicates that the drug release is controlled by more than one process. Hence, the release of drug from the microspheres of OP-ES-1 to OP-ES-9 was controlled by both diffusion and erosion process in 10 h of *in vitro* drug release study.

Optimization of data analysis and validation of optimization model

To optimize the formulation design for the preparation of controlled release hydrogel microspheres of MH, the effect of the concentration was considered of BRF and mucilage (mucilage of DI and AE in 1:1 ratio) as two independent variables were considered on the properties of microspheres. The cumulative amount of drug release (%) in 10 h ($Rel_{10\,h}$), DEE and particle size were considered as responses of the two independent variables. Ideally, 28–30% of MH should be released within 1 h and about 90–100% should be released within 8 h. This theoretical consideration is based on the pharmacokinetic data of MH. However, these ideal release properties were not considered as response variables; instead, the release of MH within 10 h was only considered.

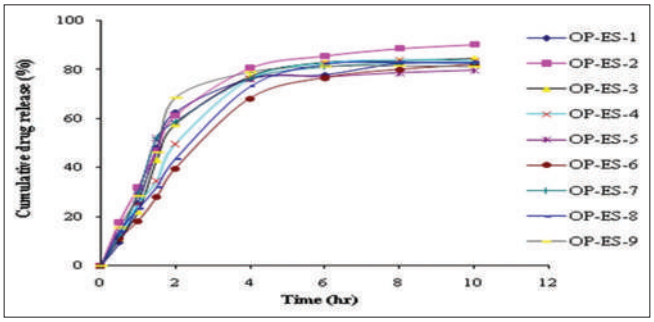

Figure 2: *In vitro* drug release profile of factorial batch metformin hydrochloride loaded microspheres (OP-ES-1 to OP-ES-9)

On the evaluation of the model, at 5 % alpha level to detect signal to noise ratios; it was found that there were no aliases in the design model. The standard errors for the independent variables A and B (BRF and mucilage) were same (0.35). The values of variance inflation factor were found to be ideal (1) indicating absence of multicollinearity. This was also supported by low Ri-squared (R^2) value (0.0) [Table 6].

The estimation of the significance of the model, ANOVA at a 5.0% level was determined. The model F and *P*-values for particle size were 10.21 and 0.0142; for entrapment efficiency was 9.07 and 0.0496, and for $Rel_{10\,h}$ was 119.40 and 0.0012. However, model was not significant in all combinations of the independent variables, as observed in Table 7. From *P*-values presented in Table 7, it was observed that for all the three responses the cross-product contribution (AB) was not significant. The linear (A) and quadratic (A^2) contribution of A (BRF) was not significant for the responses entrapment efficiency and $release_{10hr}$, but the linear contribution was significant for particle size. In case of mucilage (B), the linear (B) contribution was significant for all three responses, and quadratic (B^2) contribution of B was significant only for $release_{10hr}$. The quadratic contribution of both A and B was not considered for particle size as these two quadratic contributions resulted insignificant model. The results of ANOVA revealed that the model was significant

Table 5: Analysis of *in vitro* dissolution data of the factorial batch microspheres (OP-ES-1 to OP-ES-9)

Formulation code	Zero-order		First-order		Higuchi		Korsmeyer–Peppas	
	R^2	K_0	R^2	K_1	R^2	K_H	R^2	n
OP-ES-1	0.7096	11.34	0.9433	−0.573	0.8845	0.0228	0.9498	0.7239
OP-ES-2	0.7645	8.4799	0.9159	−0.2418	0.9236	0.0293	0.8963	0.5774
OP-ES-3	0.8691	11.128	0.9466	−0.2888	0.9557	0.0267	0.9498	0.7239
OP-ES-4	0.8106	10.652	0.9582	−0.2881	0.9428	0.0268	0.8608	0.7041
OP-ES-5	0.8994	11.322	0.9543	−0.2904	0.9596	0.0268	0.9698	0.7466
OP-ES-6	0.7152	9.0764	0.8317	−0.1981	0.8906	0.0287	0.8288	0.6171
OP-ES-7	0.752	9.6516	0.892	−0.2270	0.913	0.028	0.8483	0.6359
OP-ES-8	0.8976	11.034	0.9697	−0.2798	0.9675	0.0276	0.9756	0.6912
OP-ES-9	0.8871	11.189	0.9782	−0.3098	0.9683	0.027	0.9725	0.6438

R^2: Regression coefficient, K_0: Zero-order rate constant ($mg.ml^{-1}.min^{-1}$), K_1: First-order rate constant ($mg/ml/min^{-1}$), K_H: Higuchi dissolution rate constant, n: Release exponent, which characterizes the release mechanism of drug

Table 6: Design matrix evaluation for response surface quadratic model

Term	Standard error**	Variance inflation factor	Ri-squared	0.5 Standard deviation (%)	1 Standard deviation (%)	2 Standard deviation (%)
A	0.35	1.00	0.0000	8.1	17.2	49.0
B	0.35	1.00	0.0000	8.1	17.2	49.0
AB	0.50	1.00	0.0000	6.5	11.1	28.9
A^2	0.59	1.68	0.4050	9.5	22.6	63.1
B^2	0.59	1.68	0.4050	9.5	22.6	63.1

**Basis standard deviation=1.0

Table 7: Summarized values of test for significance from the analysis of variance study for the three responses

Source	Particle size		Entrapment efficiency		$Release_{10hr}$	
	F-value	*P*-value Prob>F	F-value	*P*-value Prob>F	F-value	*P*-value* Prob>F
A-BRF	6.39	0.0526	0.54	0.5166	6.06	0.0907
B-mucilage	23.38	0.0047	25.63	0.0149	488.15	0.0002
AB	0.86	0.3954	2.93	0.1852	3.53	0.1569
A^2			0.054	0.8312	1.00	0.3904
B^2			8.73	0.0598	49.13	0.0060

*Significant effect (P<0.05), BRF: Bora rice flour

and concentration of mucilage would affect the properties of the microspheres.

Effect of concentration of BRF and mucilage on particle size

The "model F-value" of 10.21 implies that the model is significant. There are only 1.42 % chances that a large "model F-value" could occur due to noise. Mucilage (B) is a significant model term and would affect the particle size.

The mathematical relationship generated using MLRA is expressed in Equations 4 and 5 in terms of coded and actual values.

Final equation in terms of coded factors:

$$\text{Particle size} = +146.67+12.03A+23.00B+6.25A \quad (4)$$

Final equation in terms of actual factors:

$$\text{Particle size} = +146.667 + 12.0267BRF + 22.9994 \text{ Mucilage} +6.250BRF.\text{Mucilage} \quad (5)$$

The positive sign in the mathematical expression indicated an increase of particle size on increasing the concentration of BRF and mucilage. The effect of mucilage alone on particle size would be more than that of both BRF and the combination of BRF and mucilage (cross-product combination). On the other hand, the effect of BRF on particle size would be higher than the cross-product combination (BRF and mucilage). However, at a given set of factor levels, the final result would be the net effect of all the coefficient terms contained in a polynomial.

3-D response surface, 2-D perturbation, and predicted versus actual plots were constructed based on the model polynomial functions using Design-Expert software, to see the interaction effects and deviation from reference corresponding responses are presented in Figures 3-5. The response surface plot exhibited a directly proportional relationship with both the variables.

The perturbation plot presented in Figure 4 compared the effect of the factors at midpoint (coded 0, and 0) in the design space. It can be observed from the plot that the effect of both the Factors A and B (BRF and mucilage) was linear and had similar pronouncing effect on particle size. The linear plot of predicted versus actual exhibited few scattered points and outside the line. More than 50.0 % of formulations maintained linearity to the predicted values of particle size.

Effect of concentration of BRF and mucilage on entrapment efficiency

P-value of the ANOVA model was observed to be 0.0496, which was little <0.05. This was because of the influence of the linear, quadratic, and cross-product contribution of the factors to the responses and can be observed in Table 7.

The mathematical relationship generated using MLRA is expressed in Equations 6 and 7 in terms of coded and actual values.

Final equation in terms of coded factors:

$$DEE = +74.00-0.83A+5.75B+2.75AB-0.44A^2+5.56B^2 \quad (6)$$

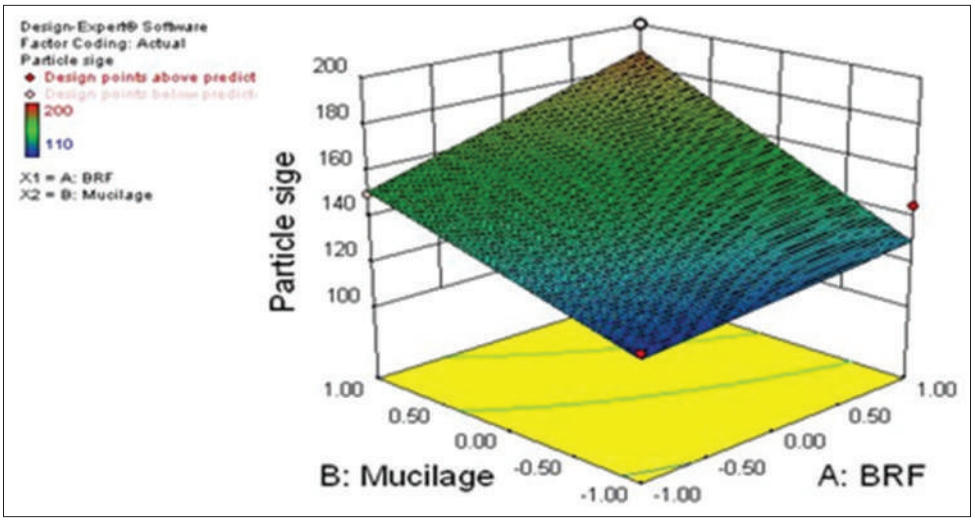

Figure 3: Three-dimensional response surface plot for particle size

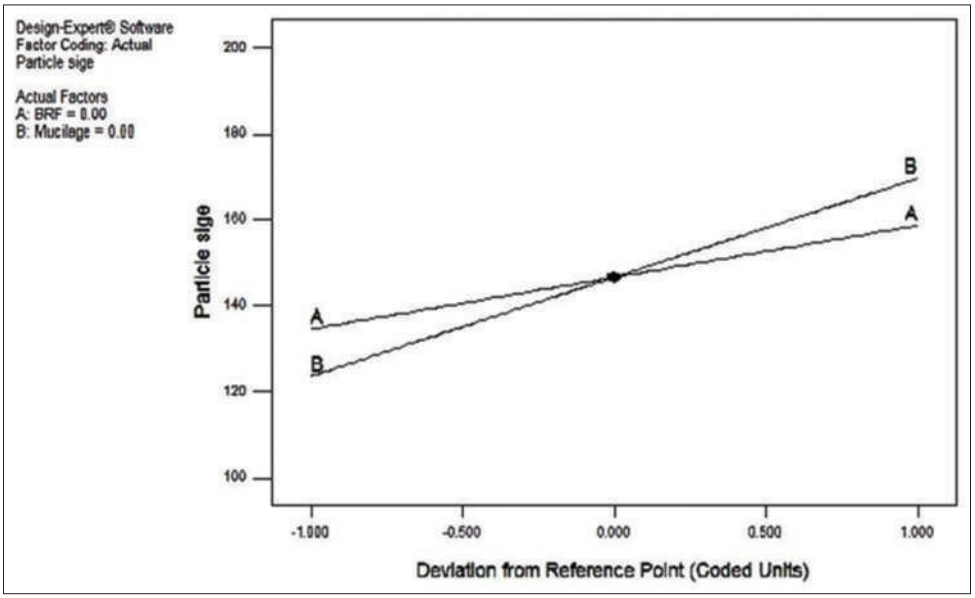

Figure 4: Perturbation plot for particle size

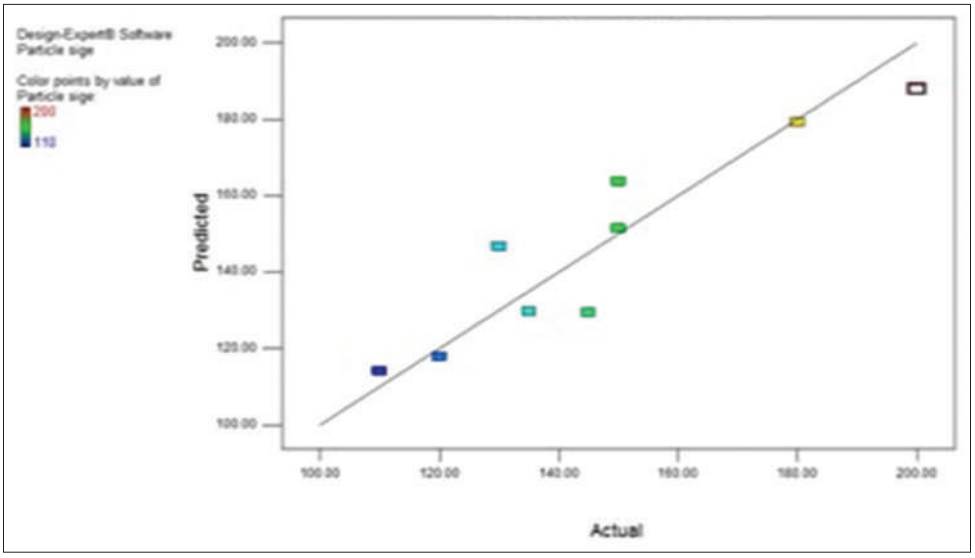

Figure 5: Predicted versus actual plot for particle size

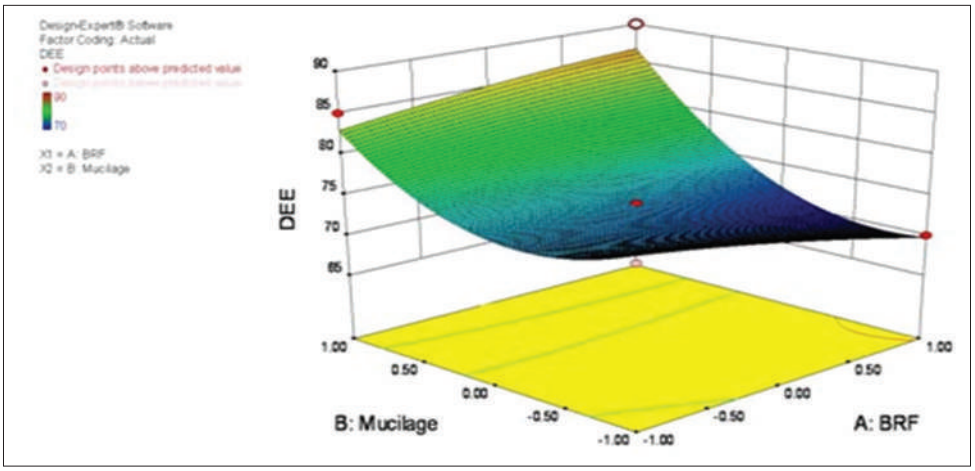

Figure 6: Three-dimensional response surface plot for drug entrapment efficiency

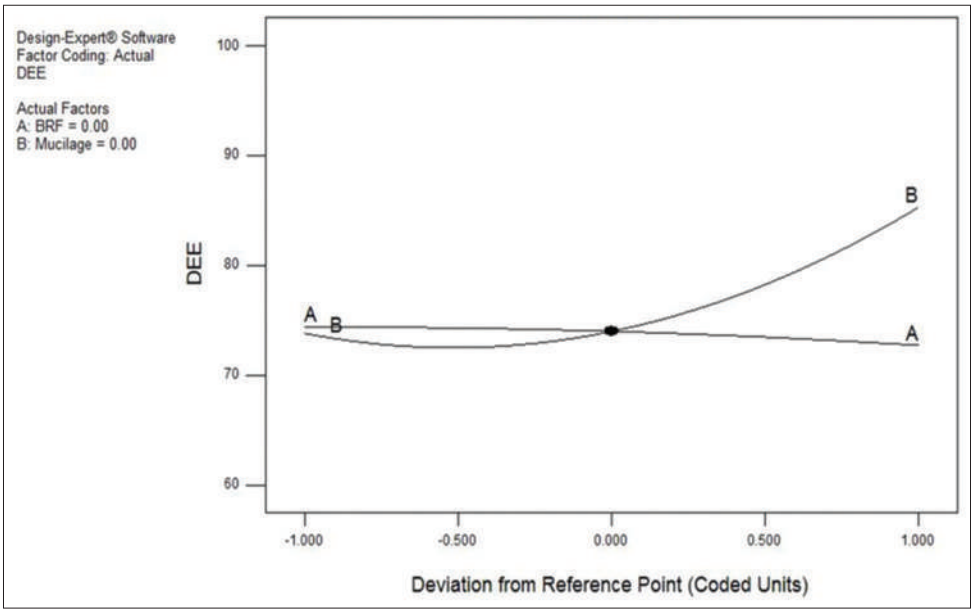

Figure 7: Perturbation plot for drug entrapment efficiency

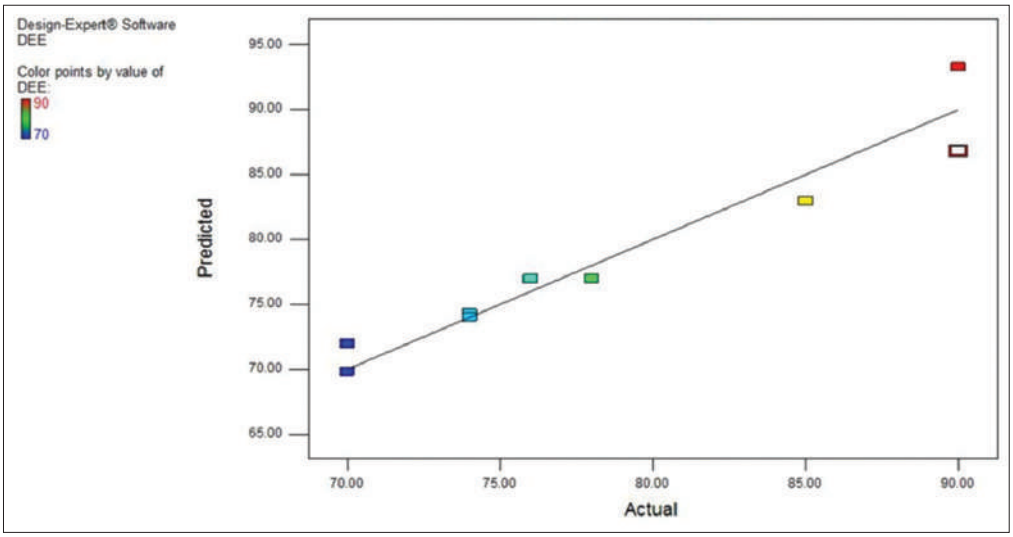

Figure 8: Predicted versus actual plot for drug entrapment efficiency

Final equation in terms of actual factors:

$$DEE = +74.00 - 0.83BRF + 5.75Mucilage + 2.75 BRFMucilage - 0.4375 BRF^2 + 5.56Mucilage^2 \quad (7)$$

The positive sign in the mathematical expression indicated an increase of particle size on increasing the concentration of BRF and mucilage in combination (AB). The linear and quadratic contribution of mucilage (B and B^2) was directly proportional to the entrapment efficiency; in contrast, similar contribution of BRF (A and A^2) was inversely proportional to the entrapment efficiency. The response surface, perturbation, and predicted versus actual plots are presented in Figures 6-8. The response surface plot exhibited a directly proportional relationship of entrapment efficiency with mucilage; and inversely proportional relationship with BRF. The perturbation plot presented in Figure 7 showed that mucilage had pronounced secondary influence on entrapment efficiency that caused it to deviate which was not observed with BRF. On the other hand, slight influence of the cross-product (AB) was noticed on entrapment efficiency. The linear plot of predicted versus actual exhibited almost even distribution of points with points at higher range as exception.

Effect of concentration of BRF and mucilage on release$_{10hr}$

The "model F-value" of 119.40 implied the model was significant. In this model, B (Linear) and B^2 (Quadratic)

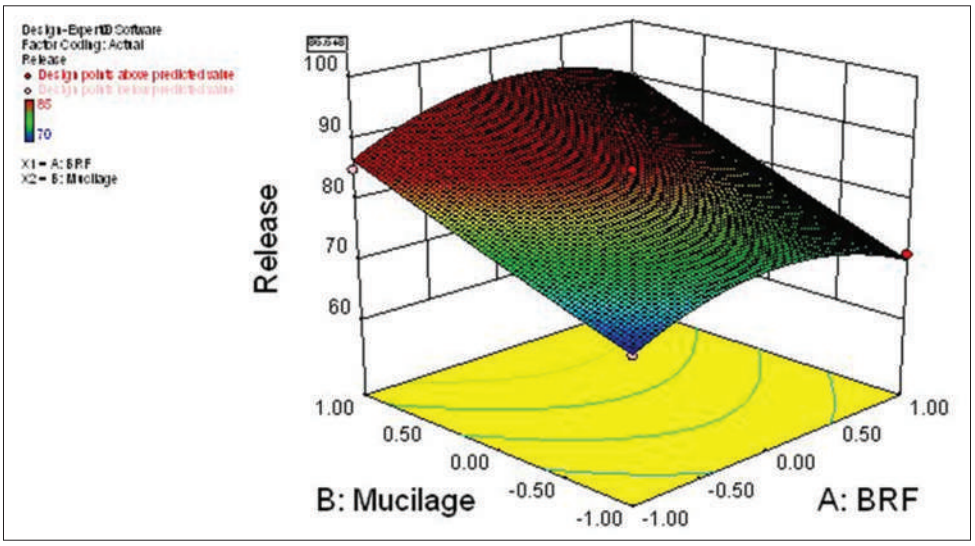

Figure 9: Three-dimensional response surface plot for release (Rel_{10hr})

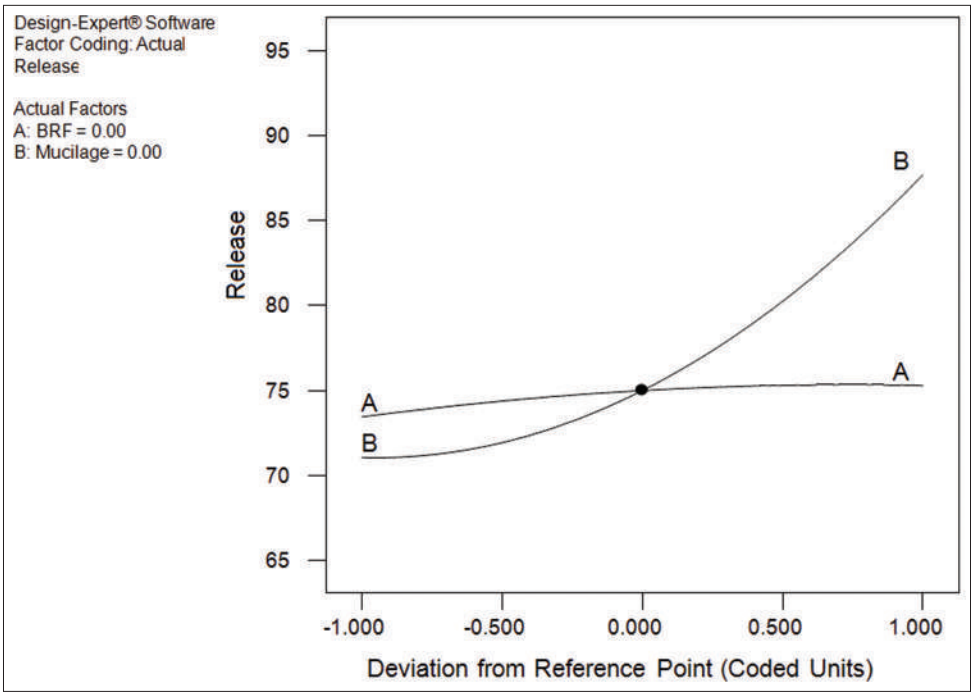

Figure 10: Perturbation plot for release (Rel_{10hr})

were significant model terms. The adequate precision ratio of 28.933 indicated an adequate signal. Hence, this model could be used to navigate the design space. The mucilage (B) would affect the release of MH from the microspheres.

The mathematical relationship generated using MLRA expressed in Equations 8 and 9 in terms of coded and actual values are presented below.

Final equation in terms of coded factors:

$$\text{Release}_{10hr} = +75.00+0.93A+8.32B+ 1.00AB- 0.62A^2+4.37B^2 \quad (8)$$

Final equation in terms of actual factors:

$$\text{Release}_{10hr} = + 75.0+0.927BRF + 8.316\ \text{Mucilage}+ 1.0BRFMucilage - 0.625BRF2 + 4.375\ \text{Mucilage2} \quad (9)$$

The positive (+ve) and negative (–ve) sign in the mathematical expression indicated the influence of BRF and mucilage on the release of MH from the formulation. The positive values indicated directly proportional relationship of A, B, AB, and B^2 with the release, whereas negative value indicated inversely proportional relationship of A^2 with release. The effect of mucilage alone on release would be more pronounced than both BRF (A) and the combination of BRF and mucilage (AB). The effect of BRF and mucilage in combination (AB) would be slightly more than that of BRF (B) alone. However, at a given set of factor levels, the final result would be the net effect of all the coefficient terms contained in the equation.

3-D response surface, 2-D perturbation, and predicted versus actual plots were constructed based on the model polynomial functions using Design-Expert software, to see the interaction effects and deviation from reference corresponding responses

Table 8: Predicted and experimental values of response variables, and percentage prediction error

Formulation code	Response variable	Mean experimental value	Predicted value	Percentage error
				(Exp. value-pred. value)/exp. value×100
OP-ES-1	Particle size	128.0	129.444	–0.8156
	DEE	78.42	69.7966	2.0701
	Release_{10hr}	82.86	70.977	1.0657
OP-ES-2	Particle size	145.0	146.367	0.2297
	DEE	84.67	82.9534	–0.3347
	Release_{10hr}	90.23	90.3959	–0.1839
OP-ES-3	Particle size	140.0	117.891	1.5064
	DEE	70.37	76.9608	–6.5238
	Release_{10hr}	84.65	69.3735	2.6893
OP-ES-4	Particle size	120.0	187.943	–14.9525
	DEE	72.75	86.7892	–5.5522
	Release_{10hr}	83.68	91.023	–5.19
OP-ES-5	Particle size	230.0	151.389	3.7439
	DEE	83.22	84.036	–0.9805
	Release_{10hr}	79.65	83.872	–4.0452
OP-ES-6	Particle size	210.0	173.823	–1.8205
	DEE	81.14	81.7867	–0.797
	Release_{10hr}	82.03	89.6265	–9.2606
OP-ES-7	Particle size	140.0	149.75	–6.9643
	DEE	76.3	74.167	2.7955
	Release_{10hr}	82.62	85.3493	–0.8827
OP-ES-8	Particle size	155.0	149.881	–1.8587
	DEE	74.28	73.9861	0.3957
	Release_{10hr}	83.21	85.044	–2.2041
OP-ES-9	Particle size	165.0	140.571	14.8055
	DEE	81.18	73.7389	–4.384
	Release_{10hr}	81.21	83.3081	–2.58354

DEE: Drug entrapment efficiency

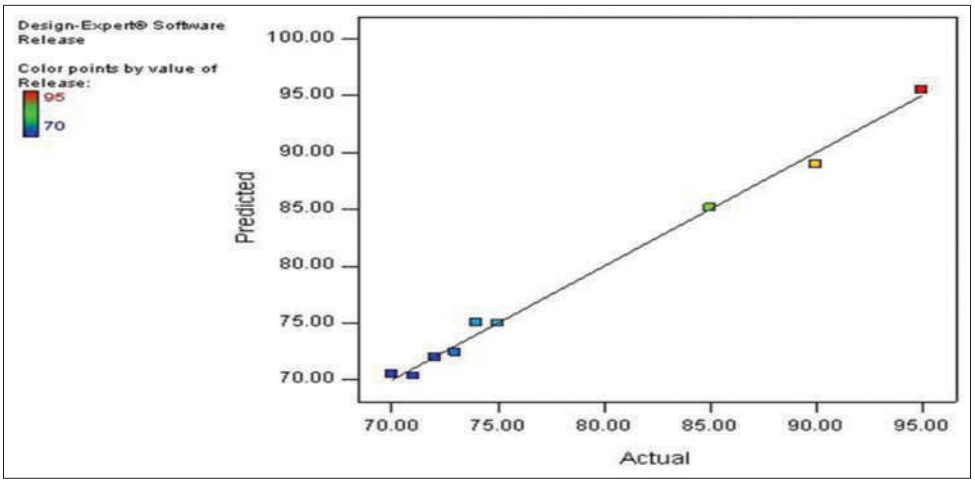

Figure 11: Predicted versus actual plot for release (Rel_{10hr})

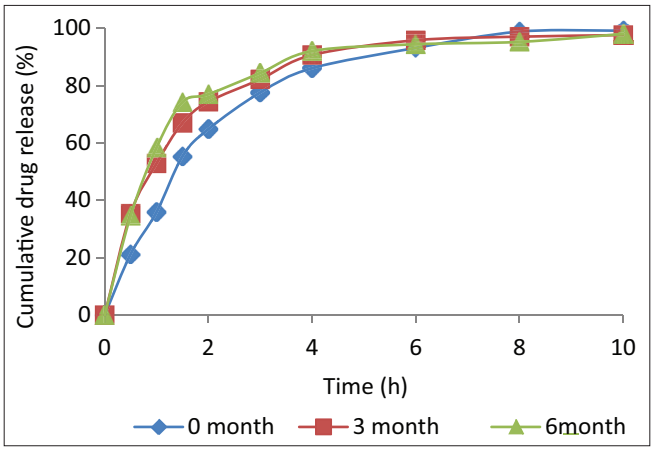

Figure 12: *In vitro* drug release profile of OP-ES-2 after 0, 3, and 6 months of stability study at 40°C ± 2°C/75% ± 5% RH

Table 9: Evaluation of OP-ES-2 after 0, 3, and 6 months of stability study at 40 °C±2 °C/75 %±5 % RH

Parameters	0 month	3 months	6 months
Similarity factor (f_2)	-	76	71
Difference factor (f_1)	-	3	3

and presented in Figures 9-11. The response surface plot exhibited a directly proportional relationship with the mucilage.

The perturbation plot presented in Figure 10 compared the effect of the factors at midpoint (coded 0 and 0) in the design space. It can be observed from the plot that the effect of B (mucilage) was linear and pronouncing. The linear plot of predicted versus actual exhibited few scattered points outside the line.

Selection and comparison of the optimized batch formulations of MH

The summary of the experimental values of response variables of corresponding formulations (OP-ES-1 to OP-ES-9) is presented in Table 4. In addition to that, the experimental values of liquid uptake capacity, *in vitro* wash-off test, *ex vivo* mucoadhesive test, release exponent (n) of Korsmeyer–Peppas model are also presented in Table 4. On comparing the results of the formulations (OP-ES-1 to OP-ES-9), it was observed that OP-ES-2 exhibited better experimental values than other formulations.

A further comparison of the predicted and experimental values of response variables were carried out, and the percentage prediction error was calculated. The results are summarized in Table 8. From the data presented in Table 8, it was observed that OP-ES-2 exhibited least error in all the three response variables. Therefore, OP-ES-2 was confirmed as the optimized formulation.

Stability study of the optimized formulation (OP-ES-2) of MH

The results of the stability study of the formulation OP-ES-2 indicated no deviation in drug release from the initial condition [Figure 12]. The resemblance of the drug release profile of pre- and post-stability study was indicated by the similarity factor (f_2) and difference factor (f_1). The similarity factor (f_2) was found to be 76 and 71 (>50) for 3 and 6 months periods, respectively, and the difference factor (f_1) in both the cases was found to be 3 [Table 9].

CONCLUSION

In the statistical optimization process, the models for the selected response variables were significant. It was observed that there was a variable influence of the concentration of the independent variables (BRF and mucilage) on the responses. Mucilage exhibited more pronounced effect on the properties of microspheres than BRF. However, the observed effect was the resultant effect of the influence of individual variables on microspheres. Therefore, there was deviation in the observed

experimental values from the predicted values, as indicated by the percentage errors. Such variation between *in silico* prediction and experimental results is not uncommon. In this study, the percentage errors signified the resemblance of both predicted and experimental values and indicated not much deviation of the experimental values from the predicted values. Hence, the process of optimization was successful. The selection of the optimized formulation on the basis of the percentage errors, drug release kinetics and other parameters as described in relevant sections, indicated OP-ES-2 as the optimized formulation. The optimized formulation was also stable under accelerated condition without much change in the release property, which was indicated by the similarity factor (f_2).

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DISCLOSURE STATEMENT

The authors report no conflicts of interest.

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