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Original Article

OPTIMIZATION AND CHARACTERIZATION OF MICROSPHERES OF BERBERINE HYDROCHLORIDE USING BOX-BEHNKEN DESIGN

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ABSTRACT

Objective: The current work sought to optimize Berberine hydrochloride (BBH)-loaded microspheres by examining the link between design parameters and experimental results.

Methods: BBH-loaded microspheres were prepared by using the water-in-oil emulsion cross-linking process and optimized with a three-factor, three-level Box-Behnken design (BBD). Grafted gum polyvinyl alcohol (PVA) ratio (w/w) (A), Revolutions per minute (RPM) (B), and Span 20 (%) (C) were independent variables. The dependent variables were Percent Entrapment Efficiency (% EE) (R1), Percent Drug Loading (% DL) (R2), and Particle Size (µm) (R3). The generated polynomial equations and response surface plots were used to relate the dependent and independent variables. Microscopic examination, %EE, and % DL were determined to evaluate the optimized formulation. Fourier transforms infrared (FT-IR) spectroscopy studies and stability studies of optimized formulation were also carried out.

Results: The optimized formulation (FMS6) had a polymer content of 2% w/v [Grafted gum (36.96): PVA (63.04)], a span 20 (0.78 %), and a prepared at the speed of 1225.92 rpm. The observed responses were close to the improved formulation's predicted values. The particle size, % EE, and % DL were found to be 1.10 µm, 82.79% and 16.48%, respectively. FT-IR spectroscopy study indicated that the drug was entrapped in microspheres.

Conclusion: BBD provides a systematic approach to optimize the BBH microsphere preparation process. Additionally, the stability study results confirmed that FMS6 is not only the ideal formulation but also stable, ensuring its suitability for practical applications.

Keywords: Box-behnken design, Water in oil emulsion cross-linking method, Berberine HCl, Microspheres

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INTRODUCTION

Many clinically significant medicinal plants contain the yellow isoquinoline alkaloid berberine HCl (BBH), which has been the subject of numerous sudies in recent years and has shown a variety of health benefits. These plants include Hydrastis Canadensis (goldenseal), Berberis aquifolim (Oregon grape), *Berberis aristata* (tree turmeric), and *Berberis vulgaris* (barberry). In doses of up to 100 mg three to four times per day, BBH is safe and well tolerated. The poor bioavailability of BBH has long limited its medicinal potential. BBH has limited bioavailability, solubility in water, and absorption, all of which contribute to subtherapeutic plasma levels. Berberine HCl's solubility and bioavailability must be increased using an oral medicine administration route [1]. BBH has been shown to have a wide range of pharmacological actions, including anti-inflammatory, anti-tumor, and anti-diabetic activity [2]. The objective of this study was to create a sustained-release multi-particulate BBH system for the treatment of ulcerative colitis. In comparison to the single unit system, the multi-particulate system has several advantages, such as faster transit through the colon, a larger surface area for localized action, less inter-subject variability, homogenous drug absorption, homogeneous drug distribution, and less local irritation. The seeds of the drought-tolerant Leguminosae plant *Cyamopsis tetragonoloba* are used to make guar gum [3]. This naturally occurring polymer is cheap, plentiful, and possesses exceptional emulsifying and surface-active properties. Chemical grafting is one of the most effective methods for changing the composition and properties of biopolymers. Natural polysaccharides can be given better functional properties by graft copolymerization, a crucial process for creating novel materials. Gamma rays, an electron beam, a microwave, or the conventional redox grafting method are frequently used to produce grafted polymers [4]. Another form of multi-particulate carrier that is frequently utilized to improve medicine targeting and absorption in the colon is microspheres [5]. These uniform, monolithic particles, whose sizes range from 1 to 1000 µm, are frequently employed as medication carriers for controlled release. Microspheres smaller than 125 µm are utilized for the injection method of distribution. These solid, almost spherical particles of the drug are released either as a solution or in microcrystalline form. Utilizing microspheres enables controlled drug release in the colon, enabling targeted treatment for conditions like colorectal cancer or inflammatory bowel disease [6]. These carriers offer Controlled drug release, extending the duration of the drugs' stay in the colon. Improving therapeutic effectiveness. Additionally, scattering multi-particulate systems in the GIT can result in more consistent medication absorption [7]. Drug entrapment during manufacturing can be achieved through coacervation, phase separation, polymerization, gelation, or encapsulation techniques, as well as covalent and ionic attachment. Either the medication is uniformly dispersed throughout the microsphere or it is encapsulated inside a special capsule wall. Polymer breakdown, drug solubility, and diffusion through the microsphere matrix, the wall of the microcapsule, or both and control drug release. Microspheres have been extensively researched as prospective drug delivery systems.

The response surface methodology (RSM), a combination of statistical and mathematical methodologies based on the fit of empirical models to the experimental data acquired in connection to experimental design, is a useful tool for process optimization [8, 9]. RSM has successfully been used to improve the circumstances of food and pharmaceutical research [10]. In a 3-factor experimental design, the BBD, an RSM design, was mostly employed since it needed fewer runs than all other RSM designs and avoided extreme treatment combinations. The best microsphere formulation may be achieved by using BBD to evaluate the liner and interactive impacts of various parameters on reaction [11, 12]. The goal of this research was to improve the BBH-loaded microspheres utilizing the BBD while also

examining the primary and interaction effects of compositional change. Due to its excellent drug entrapment effectiveness, light liquid paraffin (LLP) was identified in this study as a lipid material. The emulsifiers used were Span 20. Glutaraldehyde was used to cross-link this emulsion-free mixture. BBH-loaded microspheres were created using the emulsion evaporation technique. In-depth research was done on the physicochemical characteristics, such as surface shape, particle size, drug loading, and FT-IR investigation.

MATERIALS AND METHODS

Materials

BBH was bought from PRS Infotech and Engineers, Herbal Division in Ballabhgarh, Faridabad, while PVA, span 20, acrylamide, and guar gum were bought from India Scientific Corporation at Guru Tegbhadur Market, Sirsa. The process of making fresh water using the miliQ system has just ended. Laboratory-grade solvents were used throughout the whole investigation.

Characterization of drug

The chemical and physical characteristics of BBH were identified using HPLC and FT-IR.

HPLC analysis of BBH

Procedure

A reverse-phase HPLC-based analytical method for measurement and quantification of BBH was developed and used for the HPLC analysis of BBH. Using an HPLC (Shimadzu Prominence I LC2030 Plus) equipped with a PDA detector and a C-18 column (250x4.6 mm, i.d., 5 mm particle size), BBH content was ascertained. Acetronitrile and phosphate buffer are distributed equally in the mobile phase at a flow rate of 1 ml/min and the injection volume was 5 ml. The 346 nm detecting wavelength was chosen.

The assay was linear (r^2=0.9985) in the concentration range of 1 to 10 µg/ml, and the test's lowest detection limit was 0.018786 µg/ml. All samples were filtered via 0.22 µm millipore membrane filters before analysis [13].

FTIR spectroscopy

The purpose of the FTIR drug characterization is to identify any impurities in the drug being studied. The infrared spectra of BBH were ascertained using the potassium bromide dispersion technique. The spectrum displays the functional groups present in medication candidates. After obtaining the drug's infrared spectrum, its peaks were identified by contrasting it with reference spectra. The scanning ranged from 400 to 4000 cm^{-1} [14, 15].

Methods

Microwave-assisted guar gum grafting trials using acrylamide

In 50 ml of water, the desired amount of 0.5-2g gum solution was formed. Separately, various acrylamide (monomer) solution concentrations of 5–10 g in 50 ml distilled water were created for experiments. A different amount of initiator was then added after adding the monomer solution drop by drop to the gum solution while stirring continuously for two hours. This solution was heated in a microwave oven at 50–100 MW for 2–4 min, or until it began to boil. The addition of acetone precipitated this heated solution after it had been rapidly cooled in ice-cold water. To get rid of any homopolymers, the filtered precipitates were washed with plenty of acetone. The dried precipitates were eventually ground and sieved. Optimized formulation was selected for further use.

Preparation of microsphere

Preparation of microspheres BBH microspheres were synthesized utilizing a grafted copolymer by employing the water-in-oil (w/o) emulsion cross-linking process. PVA and grafted gum at various concentrations were dissolved in double-distilled deionized water to make a 20-ml polymer solution with the strength of 2% (w/v) [16]. The aforementioned polymer mix solution, which contained precisely calibrated amounts of BBH, was gradually emulsified into light liquid paraffin (100 g, w/w), incorporating varying volumes of different surfactants, for approximately 15 min at various stirring speeds. This w/o emulsion was cross-linked with glutaraldehyde in a variety of concentrations, each containing 0.5 ml of HCl, and the liquid was stirred for three hours**.** Filtering, followed by benzene and water washing, removed the unreacted glutaraldehyde and associated surfactants from the solid microspheres [17]. Before usage, the solid microspheres were dried at 40 °C for 24 h and stored in desiccators. The formulations with varying components are shown below [18].

Experimental design

The chosen independent variables comprised a 3-factor, 3-level Box-Behnken design that was optimized for microspheres using Design-Expert version 7. To assess the impact of independent factors throughout the formulation development, the BBD was utilized. The ratio of Grafted gum with PVA(w/w) (A), RPM (B), and Span 20 (C) concentrations were independent variables and were divided into three levels, with the high, medium, and low values being denoted by the codes+1, 0, and-1, respectively. The three independent variables, % EE (R1), % DL (R2), and Particle Size (µm) (R3), were given in table 1 and were treated as dependent variables. The following equation was used to code the variables:

$$Xi=Xi-X/\Delta X \quad \text{..... Eq. 1}$$

Where Xi (i=1, 2, 3) is the variable's coded value; Xi is the variable's real value; X_0 is the variable's actual value at the center; and X is the step change [19].

The 3-factor, 3-level BBD was used in this investigation. Using Design-Expert software, the design was used to explore the quadratic response surface and create a second-order polynomial model.

The second-order equation may be used to analyze the relationship between the variables and the answer as follows:

$$Y=\beta 0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{11}X^2_1+\beta_{22}X_2^2+\beta_{33}X_3^2 \quad \text{..... Eq. 2}$$

Where Y is the response variable; β_0 is a constant; β_1, $\beta 2$, $\beta 3$ are linear coefficients; $\beta 12$, $\beta 13$, $\beta 23$ are interaction coefficients between the three factors; $\beta 11$, $\beta 22$, $\beta 33$ are quadratic coefficients. According to research, 3D response surface plots can grasp the primary impacts and their interactions of two parameters while keeping the levels of all other components constant. According to the regression model, the three-dimensional response surface plots for entrapment effectiveness (R1), drug loading rate (R2), and particle size (R3) were created by keeping one variable at the center level. To analyze the experimental data, Design-Expert 7 was used [20].

Table 1: Independent and dependent variables used in box-behnken design for the preparation and optimization of microspheres

Variables	Unit	Levels	
		-1 Level	+1 Level
Independent variables (factors)			
A grafted Gum ratio with PVA	Ratio	30	40
B=RPM	%	600	1400
		0.5	1
C= Span 20			
Dependent variables (response)			
R1= Entrapment Efficiency	%		
R2 = Drug Loading	%		
R3 = Particle Size	µm		

Characterization of optimized formulation

Microscopic examination and surface appearance

Process: The particle size and surface characteristics of microspheres were measured using an optical microscope. The particle size was estimated using 10-20 particles on a glass slide and normal polarized light. The produced microspheres were examined using a 10X optical microscope. The microscopic images are given in the findings section [21].

Percent entrapment efficiency (%EE)

50 mg of microspheres were dissolved in 10 ml of methanol, and the solution was filtered through 0.45 µm membrane filter paper. The filtrate was then diluted and the drug concentration was determined using a UV spectrophotometer set at 350 nm. The following formulae were used to compute the entrapment efficiency:

$$\% \text{ EE} = \frac{\text{Drug loaded in MS}}{\text{Added drug}} \times 100 \text{ ..... Eq. 3}$$

Percent drug loading (%DL)

50 mg of microspheres were crushed in a mortar and pestle, and the fluid was filtered through 0.45 µm membrane filter paper. The filtrate was then diluted with 10 ml of methanol and examined with a UV spectrophotometer at 350 nm. The drug loading capacity was calculated using the following formula:

$$\% \text{ DL} = \frac{\text{Drug loaded in microspheres}}{\text{Total Weight of Microspheres}} \times 100 \text{ ..... Eq. 4}$$

FTIR spectroscopy of optimized formulation FMS6

The chemical structure and complex formation of FMS6 were analyzed by an FT-IR; the samples used for the FT-IR spectroscopic characteristics were prepared by grinding the dry specimens with KBr and pressing them to form disks. These analyses were performed within the range of 400–4000 cm^{-1}.

Stability studies

For pharmaceutical products, stability studies are conducted to evaluate storage conditions and expiration dates. According to ICH guidelines, a stability analysis of the FMS6 was conducted. The optimized batch (FMS6) was stored in a polypropylene container and tested for accelerated stability for 180 d and long-term stability conditions for 360 d. Then, the optimized batch was kept in a humidity chamber with a temperature and humidity condition of 40±2 °C/75±5% RH for 6 mo and 30±2 °C/65±5% RH for 12 mo. Every month, samples were taken out and examined for changes to their physical characteristics, flow properties, entrapment efficiency, and drug loading capacity. HPLC was used to determine how storage affected things [22].

RESULTS AND DISCUSSION

Characterization of drug

HPLC analysis method was used to characterize the drug sample. It was discovered that the retention period for BBH was 2.978 min (fig. 1).

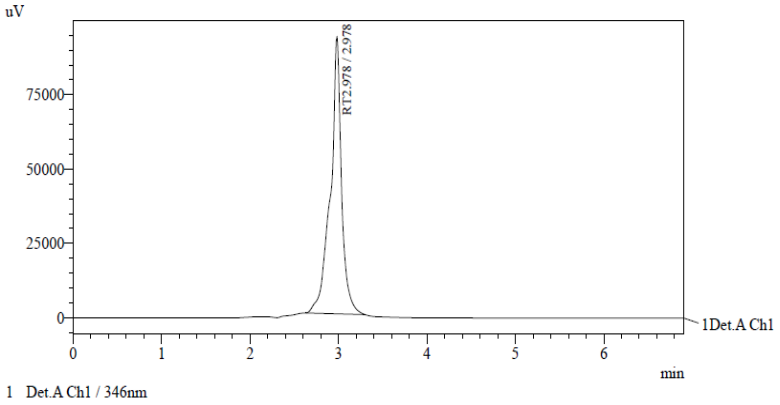

Fig. 1: Chromatogram of BBH in potassium dihydrogen orthophosphate buffer (50 mmol) with a flow rate of 1.0 ml/min

FT-IR spectra study

The BBH spectra as shown in fig. 2(b) contain all of the distinctive peaks of BBH, including those at 2834 cm^{-1} (C-H stretch structure), 1504.8 cm^{-1} (C=C stretch, C=N stretch), 1362.13 cm^{-1} (C-H deformation), and 1034.44 cm^{-1} (C-O stretch). Consequently, the drug sample was free of contamination. The absence of any additional peaks in the BBH spectra indicates that there are no impurities present in the drug sample. This confirms its purity and quality, aligning with previous research findings, which are given in fig. 2(a) [23].

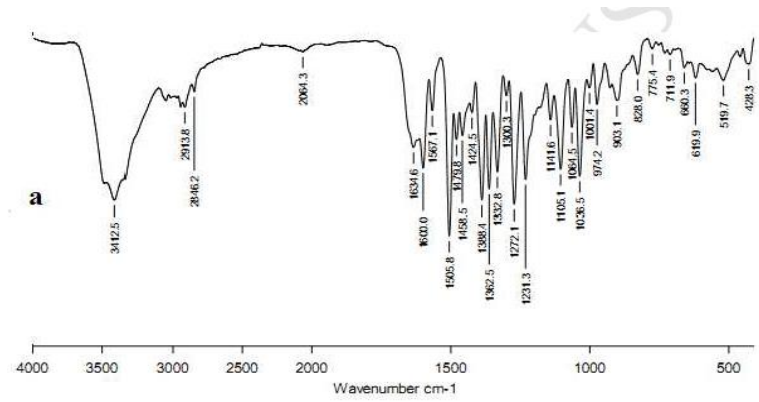

Fig. 2 (a): FT-IR spectra of BBH (Reference) [23]

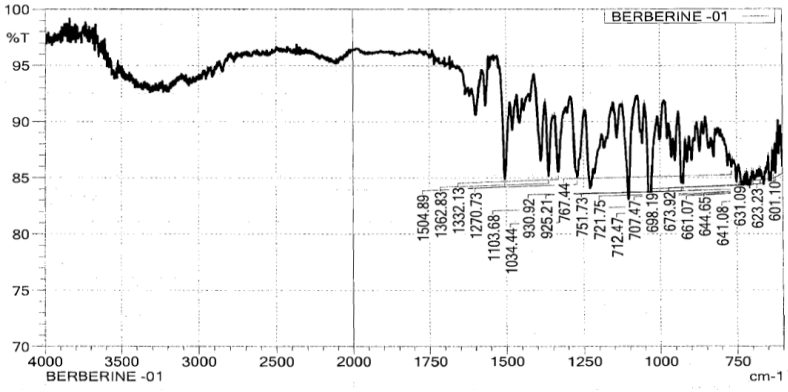

Fig. 2(b): FT-IR spectra of BBH

Box-behnken design for preparation and optimization of microspheres

Statistical analysis of experimental data by design-expert software

Microspheres of BBH were prepared by the water-in-oil (w/o) emulsion cross-linking method as stated above. The optimization of microspheres was done using the 3-factor, 3-level BBD (Design Expert software) [11]. The experiment conditions and the observed responses for the 17 formulations were analyzed using Design-Expert software and are shown in table 2. The number of experiments included the mid-point of each edge and the replicated center points. The selected independent variables including the concentration of grafted gum: PVA ratio (w/w) (A), RPM (B), and Span 20 (C) and Entrapment Efficiency (%) (R1) Drug Loading (%) (R2) and Particle Size (µm) (R3) were regarded as dependent variables. From the prepared microsphere trials and their evaluation, the size of microspheres, values of drug loading, and entrapment efficiency of different batches were determined. The amount of grafted gum: PVA ratio (w/w), RPM, and the concentration of span 20 significantly influenced the observed responses for % EE, % DL, and particle size, which are presented in table 3. Polynomial equations were generated which explained the individual main effects and interaction effects of independent factors on each dependent variable by the Design-Expert software [12]. The positive coefficients before the independent variables of the quadratic model indicate a favorable effect on the % EE, while the negative coefficients indicate an unfavorable effect on the % EE. The three-dimensional response surface plots and the contour plots for % EE (R_1), % DL (R_2), and particle size (R_3) were obtained, as seen in fig. 3(a-f). All of the observed response surfaces formed hillsides with large curvatures, which confirms that they were typically influenced by the interaction effect of concentrations of dependent factors.

Table 2: Design matrix of BBD taking into account three responses

Experimental	Factor			Response		
trial no.	A: grafted gum: PVA (w/w)	B: RPM	C: Amount of span 20 (%)	% EE±SD*	% DL±SD*	Particle size±SD* (µm)
FM 1	35:65	1000	0.75	77.88±0.010	15.45±0.022	1.17±0.002
FM 2	30:70	1000	0.5	65.74±0.025	10.77±0.001	1.84±0.032
FM 3	35:65	600	1	72.12±0.002	11.16±0.032	1.13±0.026
FM 4	35:65	1400	0.5	75.63±0.023	10.76±0.024	1.08±0.014
FM 5	35:65	1400	1	75.84±0.015	11.35±0.034	1.09±0.010
FM 6	35:65	600	0.5	67.68±0.043	09.53±0.025	1.18±0.068
FM 7	35:65	1000	0.75	78.85±0.021	15.48±0.014	1.17±0.009
FM 8	35:65	1000	0.75	78.90±0.062	15.50±0.035	1.17±0.021
FM 9	30:70	600	0.75	72.48±0.011	12.66±0.017	1.81±0.037
FM 10	35:65	1000	0.75	77.89±0.001	15.49±0.001	1.18±0.001
FM 11	30:70	1400	0.75	69.01±0.032	11.37±0.003	1.79±0.022
FM 12	35:65	1000	0.75	78.75±0.014	15.47±0.024	1.15±0.023
FM 13	40:60	1000	0.5	87.94±0.002	17.30±0.016	0.88±0.008
FM 14	40:60	600	0.75	79.08±0.015	16.49±0.013	0.88±0.012
FM 15	30:70	1000	1	73.65±0.024	12.59±0.021	1.86±0.005
FM 16	40:60	1000	1	84.68±0.032	17.71±0.012	0.85±0.032
FM 17	40:60	1400	0.75	94.93±0.035	19.20±0.012	0.75±0.095

*N=3 (mean±SD)(SD-Standard deviation)

Box Behnken design was applied using Design Expert software. All the 17 possible combinations were performed in experimental trials. The % EE, % DL, and particle size were calculated. The % EE for 17 formulations was found in between 65.74 % to 94.93 %. The % DL for 17 formulations of grafted mastic gum was found in between 9.53 % to 19.2%. The particle size for 17 formulations of microsphere was found in between 0.75 µm to 1.86 µm. ANOVA was applied to detect insignificant factors. The fit of the model was dependent upon the lower p-value, high F value, and high level of adjusted R^2 and predicted R^2.

Optimized concentration of dependent variables

Data were run again and calculated for the minimum standard deviation between theoretical and experimental response values.

Response surface (3D) and contour plot analysis

Fig. 3(a-f) illustrate 3D plots and contour plots that show the influence of independent variables on response. All of the observed response surfaces formed hillsides with large curvatures, indicating that they were typically impacted by the interaction effect of dependent variables.

Table 3: Evaluation of design parameters for optimized numerical solutions

Number of solutions	Polymer 2% (w/v)	RPM	Span 20 (%)	Design parameters (DP)			Observed parameters (OP)			±SD between DP and OP		
	PVA: Gum (w/w)			% EE	% DL	Size (µm)	% EE	% DL	Size (µm)	% EE	% DL	Size (µm)
FMS1	38.11:61.89	1206.08	0.61	86.75	16.96	0.897	81.94	16.6	1.10	3.42	0.26	0.13
FMS2	32.70:67.30	1163.36	0.59	72.51	12.62	1.413	68.50	15.8	1.88	2.83	2.25	0.32
FMS3	35.12:64.88	705.04	0.84	75.31	14.01	1.151	77.05	16.84	1.84	1.23	1.99	0.48
FMS4	34.74:65.26	901.84	0.78	77.35	15.16	1.198	79.59	14.28	1.63	1.58	0.62	0.30
FMS5	36.16:63.84	1067.92	0.55	78.69	14.22	1.061	74.08	12.4	1.31	3.26	1.29	0.17
FMS6	36.96:63.04	1225.92	0.78	83.99	16.58	0.962	82.79	16.48	1.10	0.85	0.07	0.10
FMS7	36.21:63.79	975.44	0.52	77.17	13.69	1.072	74.62	12.6	0.89	1.80	0.77	0.12
FMS8	30.26:69.74	674.08	0.95	74.54	12.48	1.777	71.66	15.44	1.34	2.03	2.08	0.30
FMS9	30.74:69.26	794.8	0.69	72.36	13.53	1.718	75.57	16.6	0.66	2.24	2.16	0.74
FMS10	37.78:61.89	1206.08	0.61	86.75	16.96	0.897	80.36	14.64	1.24	3.87	2.08	0.22

(SD-Standard deviation)

Table 4: Statistical analysis results of entrapment efficiency, drug loading, and particle size

Source	(% Entrapment efficiency) R1		% Drug loading R2		Particle size R3	
	Sum of squares	p-value Prob>F	Sum of squares	p-value prob>F	Sum of squares	p-value prob>F
Model	850.8545	<0.0001	135.5121	<0.0001	2.112207	<0.0001
A-grafted gum	540.3828	<0.0001	67.91951	<0.0001	1.94045	<0.0001
B-rpm	72.30031	<0.0001	1.0082	<0.0001	0.010513	<0.0001
C-span	10.81125	<0.0001	2.475313	<0.0001	0.000312	0.0990
AB	93.3156	<0.0001	4	<0.0001	0.003025	0.0006
AC	31.19223	<0.0001	0.497025	<0.0001	0.000625	0.0311
BC	4.473225	0.0014	0.2704	<0.0001	0.0009	0.0145
A^2	33.0813	<0.0001	11.7744	<0.0001	0.149609	<0.0001
B^2	23.89021	<0.0001	20.75583	<0.0001	0.010109	<0.0001
C^2	44.59693	<0.0001	27.54562	<0.0001	4.21E-06	0.8316
Residual	1.216945		0.001505		0.000605	
Lack of Fit	0.126025	0.9219	2.5E-05	0.9947	0.000125	0.7943
Pure Error	1.09092		0.00148		0.00048	
Cor Total	852.0715		135.5136		2.112812	

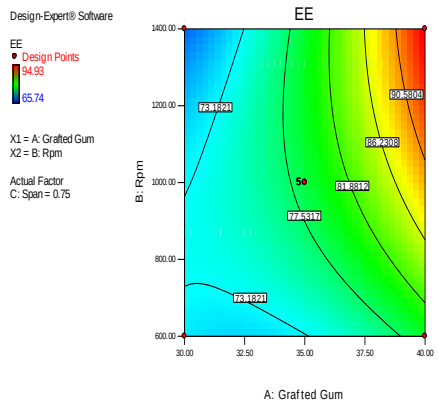

Fig. 3 (a): Contour plot of % entrapment efficiency

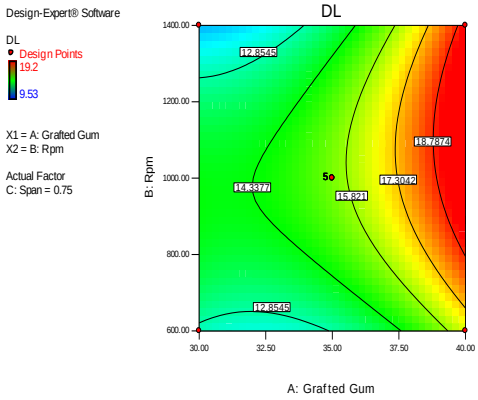

Fig. 3 (c): Contour plot of % drug loading

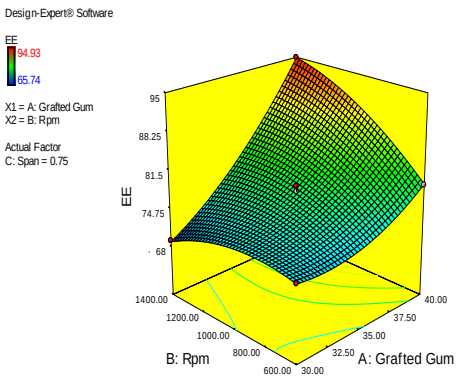

Fig. 3 (b): 3D Contour plot of % entrapment efficiency

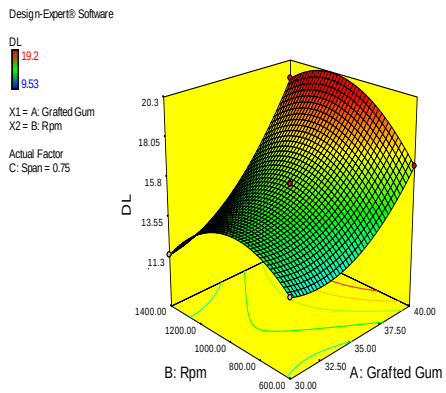

Fig. 3 (d): 3D contour plot of % drug loading

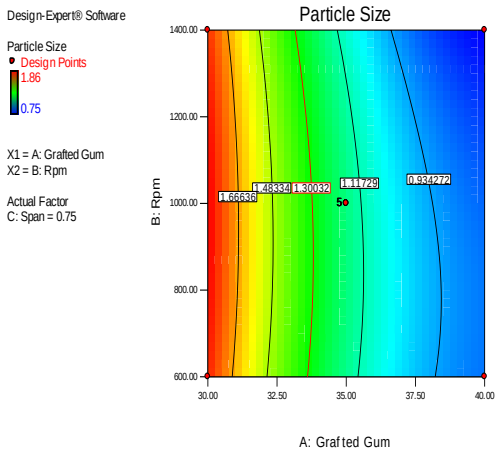

Fig. 3(e): Contour plot of particle size

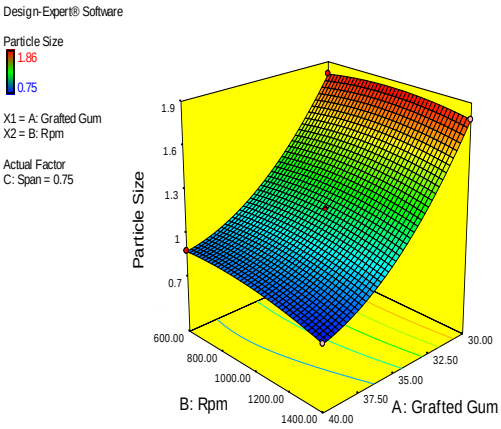

Fig. 3(f): 3D contour plot of particle size

Effects on entrapment efficiency (R_1)

According to multiple regression analysis on the experimental data, the relationship of the variables on entrapment efficiency (*R1*) was illustrated by the following equation:

$$\% EE = 78.45+8.22 * A+3.01 * B+1.16 * C+4.83 * A * B-2.79* A * C-1.06 * B * C+2.80 * A^2-2.38 * B^2-3.25 * C^2 \ldots\ldots \text{Eq. 5}$$

The summary of analysis results for the observed response is shown in Table 4. The effect of each factor was tested using an ANOVA test with a corresponding *p*-value. Probability> *F* less than 0.0001 suggests that the model is significant, while greater than 0.0001 suggests that the model is not significant. The Model F-value of 543.80 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 "Probability>F" less than 0.0500 indicate model terms are significant. In this case, A, B, C, AB, AC, BC, A^2, B^2, and C^2 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). The "Lack of Fit F-value" of 0.15 implies the Lack of Fit is not significant relative to the pure error. There is a 92.19% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good. We want the model to fit.

The "Pred R-squared" of 0.9956 is in reasonable agreement with the "Adjusted R-squared" of 0.9967."Adequate Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 91.002 indicates an adequate signal. From Table 3 and fig. 3(a) we found that the Grafted Gum PVA ratio (A), RPM (B), and interactive influence of them (AB) were the main factors that affected the % EE. Thus, we can find that the high amount of grafted gum and the high value of RPM are favorable to formulation. The negative value before the concentration of span 20 (C) in the regression equation suggested that the response R1 decreased as the concentration of span 20 increased. In fig. 3 (a and b), % EE increased by decreasing the concentration of span 20 (C). Because at high concentrations, the emulsifier might be gathered at an organic solvent/water interface to reduce the interface tension, leading to a significant increase in the net shear stress during emulsification [24, 25].

Effects on drug loading (R_2)

The following equation can explain the effect of factor levels on DL%:

$$\% DL= 15.48+2.91 * A+0.36 * B+0.56* C+1.00* A * B-0.35* A * C-0.26 * B * C+1.67 * A^2-2.22 * B^2-2.56 * C^2 \ldots\ldots\ldots \text{Eq. 6}$$

The Model F-value of 70032.08 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, AB, AC, BC, A2, B2, and C2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.02 implies the Lack of Fit is not significant relative to the pure error. There is a 99.47% chance that a "Lack of Fit F-value" this large could occur due to noise. The "Pred R-Squared" of 1.0000 is in reasonable agreement with the "Adj R-Squared" of 1.0000. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 859.868 indicates an adequate signal.

The models were significant while the lack of fit was not significant, as shown in Table 4. It can be seen from Table 4 that the model, liner coefficients (AB) were significant model terms, while others were not, which indicated that the two factors mainly affected the response (*R2*). We can see from fig. 3(c and d), showing that % DL increased rapidly with the amount of grafted gum increasing. This is because when the amount of grafted gum and RPM increased, the content of the drug entrapped in the microsphere improved.

Effects on particle size (R_3)

The obtained following equation explains the influence of different factors on response (*R3*) which was generated:

$$\text{Particle size} = 1.17-0.49* A-0.036* B-6.250E-003* C-0.028* A* B-0.013* A* C+0.015* B* C+0.19* A^2-0049* B^2+1.000E-003* C^2 \ldots. \text{Eq. 7}$$

The Model F-value of 2715.42 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, AB, AC, BC, A2, and B2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The "Lack of Fit F-value" of 0.35 implies the Lack of Fit is not significant relative to the pure error. There is a 79.43% chance that a "Lack of Fit F-value" this large could occur due to noise. Non-significant lack of fit is good--we want the model to fit. The "Pred R-Squared" of 0.9987 is in reasonable agreement with the "Adj R-Squared" of 0.9993. "Adeq Precision" measures the signal-to-noise ratio. A ratio greater than 4 is desirable. A ratio of 154.974 indicates an adequate signal. This model can be used to navigate the design space. The optimum microsphere was formulated with grafted gum PVA ratio (36.96:63.04) (w/w), RPM (1225.92), and Span 20 (0.78 %). The optimum microsphere formulation was demonstrated by practical experiments. As listed in Table 3, the predictive values, practical values, and predicted error of %EE, %DL, and particle size were compared. The practical values of %EE and %DL were similar to the predicted values; however, the measured value of particle size had a significant difference between the predicted values. Although the particle size model could not be predicted, the measured particle size was small enough and metal requirements.

Fitting the model to data

All formulations' response data were fitted to a quadratic model. According to Design Expert software, the best-fitted model for

response R1, R2, and R3 (% EE, % DL, and Particle Size) was quadratic. All responses were fitted to the model to create the full model polynomial equation.

Checkpoint analysis

These experimental values of % EE, % DL, and Size (in µm) by the optimized grafting solutions (table 5) were found to agree with the predicted values of % EE, % DL, and Size generated by design expert software, indicating that the optimized formulation was rational and reliable.

Optimized numerical solution

Based on the above values, FMS6 was selected for further evaluation (table 5). The size of microspheres, EE, and % drug loading values of optimized formulation was 1.10 µm, 82.79%, and 16.48%, respectively.

Table 5: Optimized numerical solution

Formulation code	Polymer 2% (w/v)	RPM	Span 20(%)	Design parameters (DP)			Observed parameters (OP)			±SD between DP and OP		
	Grafted Gum: PVA (w/w)			% EE	% DL	Size (µm)	%EE	%DL	Size (µm)	%EE	%DL	Size (µm)
FMS6	36.96:63.04	1225.92	0.78	83.99	16.58	0.962	82.79	16.48	1.10	0.85	0.07	0.10

(SD-Standard deviation)

FT-IR spectra of optimized formulation (FMS6)

However, the peak of BBH significantly disappeared in FT-IR spectra of FMS6 (fig. 4) and two new characteristic peaks at 3423.44 cm^{-1} and 2922.56 cm^{-1} appeared. The results indicate that BBH has been entrapped in the microsphere, and BBH-loaded microsphere formulations were formed. These characteristic peaks suggest that the BBH molecules have been successfully incorporated into the microsphere structure. This finding is consistent with the results reported in a previous study [26], further supporting the formation of BBH-loaded microspheres.

Stability studies

The results of stability studies at 40±2 °C/75±5% RH for 0-180 d and 30±2 °C/65±5% RH for 0-360 d of formulation were determined. No significant amounts of change were observed in the different parameters after 180 d of storage at accelerated stability conditions or after 360 d of storage at long-term stability conditions. Based on the results, it was concluded that the optimized BBH microsphere formulation (FMS6) was found stable even after 6 mo and 1 y of storage at accelerated stability conditions and long-term stability conditions, respectively.

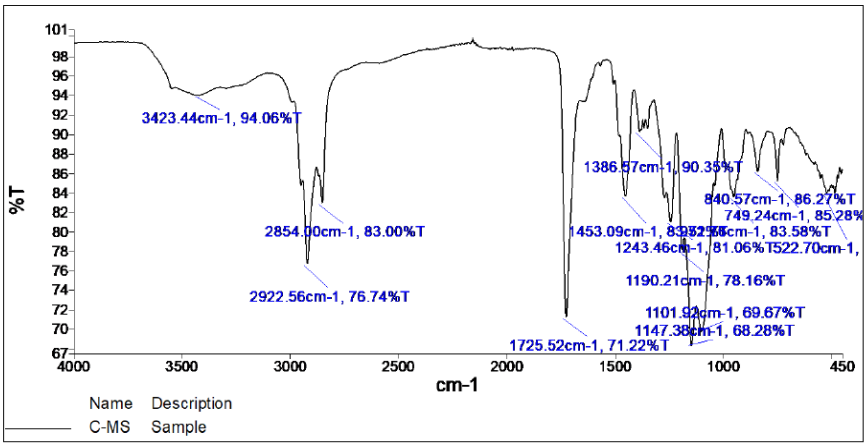

Fig. 4: FTIR spectra of FMS6

CONCLUSION

The modified w/o emulsion cross-linking approach was effective in formulating the BBH-loaded microspheres. To assess the interaction and quadratic impacts of the three primary influencing variables on the % EE,% DL, and particle size as well as to optimize the formulation parameters, a three-factor, three-level BBD was applied. The ideal microsphere formulation was found to be polymer concentration 2% w/v [Grafted gum (36.96): PVA (63.04)] and span 20 (0.78 %) with speed 1225.92 rpm based on experimental results and mathematical analysis of the restrictions. In addition, the use of a three-factor, three-level BBD allows for efficient optimization of the BBH microsphere preparation process. This approach ensures that all key factors are considered and adjusted to achieve the desired outcome. Additionally, the BBD methodology provides a systematic and reliable framework for conducting experiments and analyzing the results, leading to accurate optimization of the microsphere preparation.

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AUTHORS CONTRIBUTIONS

Conceptualization Gautam Kumar, Narendra Kumar Pandey, Surajpal Verma; review and editing, GK, NKP, and Sachin Kumar Singh, Vijay Mishra; resources, GK, NKP, Bimlesh Kumar, SKS, Jitender Singh, SV; Design, GK, NKP, Kalvatala Sudhakar, Saurabh Singh; data collection and/or Processing GK, NKP, BK, SS, KV; Analysis and Interpretation, NKP, SV, VM, Dileep Singh Baghel; writing original draft, GK, DSB, NKPJS; supervision, NKP, SKS, BK

CONFLICT OF INTERESTS

No conflict of interest was declared by the authors.

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