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QbD-based design and characterization of mucoadhesive microspheres of quetiapine fumarate with improved oral bioavailability and brain biodistribution potential

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ABSTRACT

The present work aims to discuss on Quality by Design based development and characterization of the sustained release mucoadhesive microspheres of quetiapine fumarate. The microspheres were prepared by non-aqueous solvent evaporation process. Factor screening study was carried out using fractional factorial design for identifying the influential factors. Systematic optimization of microspheres was accomplished by Box-Behnken design and characterized for particle size, entrapment efficiency, *in vitro* drug release and *ex vivo* mucoadhesion strength, which indicated that microspheres were consequence to be spherical and free flowing in nature. The microspheres exhibited high drug entrapment efficiency and *in vitro* drug release in a sustained manner, which was considered to be dependent on the concentration of rate controlling polymers. *Ex vivo* wash-off test on microspheres indicated good mucoadhesive property on excised goat intestinal mucosa. Out of all the accepted formulation, F6 was preferred as the optimized formulation. *In vivo* pharmacokinetic and brain biodistribution study revealed significant increase in the levels of drug in blood plasma and brain homogenates from the optimized formulation vis-à-vis the pure drug suspension. Overall, current study corroborated significant improvement in the biopharmaceutical attributes of quetiapine fumarate from mucoadhesive microspheres, which can be effectively used for management of depression and schizophrenia.

1. Introduction

Mucoadhesive microspheres provides improvement of oral drug absorption characteristics due to intimate contact with mucus layer, prolonged retention in the GI tract or sometimes particular targeting of the drug to the gastric absorption site [1,2]. If the bioadhesive take place basically towards the mucous membrane containing mucous layer, then the observable fact is better known as “Bioadhesion or mucoadhesion” [2,3]. Mucoadhesion can be acquired by means of; either non-specific or specific interaction with ligands of the surface at a surface of mucosa. More than the previous two decades, there has been substantial attention in mucoadhesive drug delivery systems for its competence to optimize localized drug release by retaining a dosage form at the site of action, or systemic delivery, by retaining a formulation in closer in contact with the absorption site in order to enhance the oral drug bioavailability [4,5]. Several literature reports have demonstrated that utility of mucoadhesive microspheres or microparticles can providing controlled release action and augmenting the drug absorption for extended time interval to afford better patient compliance [6–10].

Quetiapine fumarate (Seroquel®, ICI204, 636) is a psychotropic substance accepted as a medication to cure schizophrenia, acute mania, and acute bipolar depression in adult patients. Chemically, quetiapine is a dibenzothiazepine derivative that is {2-(2-(4-dibenzo [b, f] [1,4]-thiazepine-11-yl-1-piperazinyl) ethoxy) ethanol} [11]. It is an antipsychotic agent showing serotonin/dopamine binding ratio, dopamine D2-receptor and 5-HT2-receptor blocking effects and resulting minimal extrapyramidal side effects. Poor oral bioavailability (9%) of quetiapine

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fumarate due to extensive hepatic first-pass metabolism and low plasma elimination half-life (6–7 h) [12]. Therefore, it reduces the therapeutic efficacy of drug treatment through oral route of administration.

Quality by Design (QbD) regulatory initiative represents a highly systematic approach implementing the Design of Experiments (DoE) for finding the optimal product and process characteristics [13,14]. DoE, affords the remarkable and even more quantity of instructions as of the slightest number of experimental runs by methodical distinction of the factors and simultaneous evaluation of the effects of multiple variables [15]. Quality assurance (QA) has altered from the demand to elucidate that the ultimate product gets the predefined requirements and specifications to a novel circumstance where it needs to be confirmed that the product is controlled within a significant and organized design space [10]. The design space can be stated as a renowned technique enclosing multidimensional series of input variables (e.g., formulation factors) and process parameters which are detonated in order to insist typical quality assurance [16].

Administration of quetiapine fumarate in sustain release dosage mucoadhesive microspheres as once daily dose would be further enviable since this formulation is proposed to be given to the patients for the management of depression and schizophrenia. Therefore, the present investigation to performed a formulation design and characterization of sustained release mucoadhesive microspheres of quetiapine fumarate using optimized polymer blends containing ethyl cellulose (EC) along with mucoadhesive polymers such as HPMC (K4M, K15M, and K100M), chitosan and their combinations. The formulation having sustained release was employed quite a few years ago and its crucial goal is to liberate the drug slowly over an extensive period of time for improvement of therapeutic efficacy and patient compliance.

2. Materials and methods

2.1. Materials

Quetiapine fumarate was obtained as a gift sample from M/s Ranbaxy Laboratories Ltd., Gurgaon, New Delhi, India. Ethyl cellulose (EC), Hydoxypropyl methylcellulose HPMC (K4M, K15M & K100M) and chitosan were obtained from M/s S.D Fine Chemicals, Mumbai, India. Dichloromethane and Polyvinyl alcohol were obtained from M/s Loba Chem, Mumbai, India. All supplementary chemicals were utilized in the study of analytical grades and used as obtained.

2.2. Methods

2.2.1. Preformulation studies

For effective evaluation of physicochemical properties of the drug component, usually preformulation study was conducted. Melting point was determined by using minute quantity of the drug substance in a capillary tube closed at one end and was positioned in Thiele's melting point apparatus. The particular temperature at which drug melted was noted as the melting point. The solubility of the drug was evaluated by shake flask technique reported in literature in distilled water, 0.1 N HCl and pH 6.8 phosphate buffer. Also, micromeritic properties of the drug were analyzed due to its unfavorable impact on formulation properties. Flowability of the drug was investigated by determining the angle of repose, bulk density, Carr's index and Hausner's ratio as per the standard procedure.

2.2.2. Preparation of mucoadhesive microspheres

Mucoadhesive microspheres containing the drug were prepared by a non-aqueous solvent evaporation method. Initially prepared 1%w/v polyvinyl alcohol (PVA) solution by taking 2 g of PVA in a beaker containing 200 ml distilled water and stirred by a mechanical stirrer for 2 h at a speed of 100 rpm. After that the solution was filtered through Whatman filter paper and filtrate was collected in a beaker. In another 100 ml beaker, 25 ml of dichloromethane was taken and ethylcellulose (1000 mg) was dissolved slowly by stirring through a glass rod. After that drug (500 mg) was added slowly until a drug-polymer dispersion is formed. To this dispersion, selected mucoadhesive polymers (150 and 300 mg of HPMC K4M, K15M and K100M) were added (Table 1). The drug-polymer dispersion was then steadily added bit by bit to the previously prepared PVA solution. Simultaneously, the mixture was stirred with a mechanical stirrer at 900 rpm under 40 °C for 1 h. After 1 h, heating was stopped and only stirring was continued till volatile organic phase get evaporated to leave the microspheres in the remaining aqueous medium. The prepared microspheres were collected by decanting the solution on a Whatman filter paper and dried in hot air oven at 40 °C for 8 h. Table 1 enlists the configuration of preliminary formulations investigated for screening of polymers for preparation of mucoadhesive microspheres. The microspheres which equipped successfully were subjected to evaluation by calculating the percentage yield, % entrapment efficiency and % drug release in 24 h (Q_{24h}).

Table 1
. Preliminary trial mucoadhesive microsphere formulations of quetiapine fumarate.

Ingredients (mg)	Formulation composition						
	F1	F2	F3	F4	F5	F6	F7
Quetiapine fumarate	500	500	500	500	500	500	500
Ethyl cellulose	1000	1000	1000	1000	1000	1000	1000
HPMC K4M	–	150	300	–	–	–	–
HPMC K15M	–	–	–	150	300	–	–
HPMC K100M	–	–	–	–	–	150	300
Chitosan	150	150	150	150	150	150	150
Polyvinyl alcohol (gm)	2	2	2	2	2	2	2
Dichloromethane (ml)	25	25	25	25	25	25	25
Distilled water (ml)	200	200	200	200	200	200	200

2.2.3. Factor screening study

The screening of factors influencing the mucoadhesive microspheres formulation was performed using a fractional factorial design (FFD). A 2^{5-2} FFD (Resolution III) was used for performing factor screening, where a total of five factors (both formulation and process parameters) related to mucoadhesive microspheres were selected. These factors include the concentration of ethyl cellulose (EC), type of hydoxypropyl methylcellulose (HPMC), concentration of HPMC, concentration of chitosan and stirring speed. An entire of eight trial formulation was prepared (Table 2). All the mentioned factors were studied at each having two levels, and the prepared microspheres were evaluated for responses such as particle size (μm), drug release in 24 h (Q_{24h}) and % mucoadhesion behavior. The obtained data were subjected to analysis with the help of statistical modeling using linear polynomial model. The factors are actually influencing the formulation responses were identified by means of half-normal plots as well as Pareto charts.

2.2.4. Factor optimization study

The systematic optimization of mucoadhesive microspheres was performed by means of a response surface design on the influential factors identified from factor screening study. A three-factor and three-levels containing Box-Behnken design (BBD) was selected for optimization study. A total of 15 trials formulation were arranged and characterized for particle size (μm), entrapment efficiency, % drug release in 24 h (Q_{24h}) and % mucoadhesion behavior (Table 3). The entire characterization tests were carried out in triplicate for accuracy of the observations. Further, optimization data assessment was performed by evaluating the response variables. Subsequently, mathematical modeling and fitting of data were performed by multiple linear regression analysis (MLRA). The appropriateness of the model was evaluated using parameters like model p-value, coefficient of correlation (R) and lack of fit analysis. The response surface mapping was conducted by means of 3D and 2D-plots for critical sympathetic of factor-response correlation.

Table 2
. Design matrix indicating trial formulations suggested as per the 2^{5-2} fractional factorial design.

Trials	Factors					Responses		
	EC Conc. (mg)	HPMC Conc. (mg)	HPMC Type	Chitosan Conc. (mg)	Stirring Speed (rpm)	Particle Size (micron)	Drug Release Q_{24h} (%)	Mucoadhesion Strength (%)
1	500	300	K4M	50	3000	1022.13	44.89	74
2	1000	300	K15M	100	3000	1067.28	77.86	78
3	500	300	K15M	50	1000	1028.12	51.88	59
4	500	150	K15M	100	1000	1005.43	78.35	67
5	1000	150	K4M	50	1000	1176.43	46.61	62
6	500	150	K4M	100	3000	1173.59	48.43	79
7	1000	150	K15M	50	3000	1059.87	61.31	68
8	1000	300	K4M	100	1000	1012.42	49.84	85

At the end, the optimum formulation was recognized through a numerical optimization and desirability function, where target values of the responses were made to meet the desired objectives. Moreover, the graphical optimization was also performed for locating the optimum formulation within the design space. Design method validation study was executed by choosing six confirmatory check-point formulation, where the experiential and forecast values of the responses were compared with the help of linear correlation plots.

2.3. Characterization of mucoadhesive microspheres

2.3.1. Micromeritic properties

Flowability of microspheres was evaluated by determining the angle of repose, Carr's index and Hausner's ratio. Angle of repose was determined by fixed funnel method, where microspheres were poured from a funnel vertically to achieve a maximum cone height (h) and radius of the pile of microspheres (r). The angle of repose was calculated by the formula, $\theta = \tan^{-1}$ (h/r). Bulk and tapped density parameters were estimated by using standard procedures. Further, Carr's index was determined from the obtained bulk and tapped density values. Also, Hausner's ratio was determined to evaluate the flowability of granular material.

2.3.2. Particle size distribution

The mucoadhesive microspheres and its explicit distribution of particle size were found out by the help of an optical microscope (Olympus, japan) which is fixed with an ocular micrometer. Stage micrometer is the decisive instrument significantly beneficial for proper calibration of ocular micrometer. The mean diameter outlined was observed from a total of more than 50 microspheres.

2.3.3. Entrapment efficiency

Accurately weighed microspheres equivalent to 10 mg of quetiapine fumarate were crushed and dispersed in 100 ml phosphate buffer (pH 6.8) for determining the drug entrapment efficiency. The resultant dispersion was kept 24 h for extraction of drug and permitted to pass through Whatman filter paper. The net content of drug was assayed by a UV spectrophotometer after suitable dilution at λ_{max} 290 nm. The entrapment efficiency of microspheres was quantified by using the subsequent formula contained in Eq. (1).

$$\text{Entrapment efficiency} = \frac{\text{practical drug content}}{\text{theoretical drug content}} \times 100 \quad (1)$$

2.3.4. Percent yield

The percent yield of prepared microspheres was calculated using the finally dried out microspheres weight with respect to the first total quantity of the drug as well as the polymer used for the preparation. Percent yield was estimated as per the following formula mentioned in eq. (2).

$$\text{Percentage yield} = \frac{\text{practical mass(microspheres)}}{\text{Theoretical mass(polymer + drug)}} \times 100 \quad (2)$$

2.3.5. In vitro drug release

The drug release from mucoadhesive microspheres was evaluated by using USP type II dissolution apparatus (Electrolab, Mumbai, India). The microspheres equivalent to 25 mg of the drug were taken and filled in to a hard gelatin capsules. The capsules were placed in to the dissolution flask containing 900 ml of 0.1 N HCl (pH 1.2) and study was conducted for initial 2 h and then, phosphate buffer (pH 6.8) for additional 22 h maintaining temperature 37 ± 0.5 °C and paddle speed

Table 3
. Design matrix with trial formulations suggested as per the Box-Behnken design.

Runs	Factors			Responses			
	HPMC Conc. (mg)	Chitosan Conc. (mg)	Stirring Speed (rpm)	Particle Size (μm)	Entrapment Efficiency (%)	Drug Release, Q24h (%)	Mucoadhesion Strength (%)
1	225	75	2000	1272.13	69.24	77.05	68
2	150	50	2000	1067.28	57.59	82.26	78
3	300	75	1000	1428.12	78.45	61.88	59
4	150	100	2000	1005.43	58.51	88.35	67
5	150	75	1000	1176.43	59.14	86.61	62
6	300	75	3000	1473.59	78.44	68.43	79
7	225	50	1000	1359.87	67.67	71.31	68
8	225	100	1000	1399.15	69.56	75.54	73
9	150	75	3000	1012.42	78.58	85.34	85
10	225	50	3000	1014.22	64.22	47.56	85
11	300	50	2000	1462.36	77.23	67.23	73
12	300	100	2000	1454.65	72.48	69.84	75
13	225	75	2000	1282.13	68.52	72.11	69
14	225	100	3000	1158.58	65.35	78.69	78
15	225	75	2000	1222.13	62.63	72.36	68

rate of 100 rpm. Aliquot samples (5 ml) from time to time removed out, followed by replacement by way of an equivalent quantity of a fresh dissolution medium. The samples were analyzed by spectrophotometer at λ_{max} 290 nm using the previously validated analytical method of the drug on UV-Vis spectrophotometer (Shimadzu, Tokyo, Japan).

2.3.6. Swelling index

The swelling characteristics of microspheres were resolved correctly in the phosphate buffer (pH 6.8). Microspheres of known weight (50 mg) from different batches were placed in the dissolution medium (phosphate buffer pH 6.8) for 24 h and swollen microspheres were accumulated in a centrifuge. The swollen microsphere weight or mass was found out by first blotting the microsphere with filter paper to eradicate water which is absorbed on surface and after that undergone taking weight instantly in an electric balance. The percentage of swelling of microspheres in the dissolution media was then calculated by the formula contained in eq. (3).

$$\text{Swelling Index(Sw)} = \frac{\text{Wt}-\text{W0}}{\text{W0}} \times 100 \quad (3)$$

where, S_w = Percentage of swelling microsphere, W_t = Weight of microsphere at time 't' and W_o = Initial weight of the microspheres

2.3.7. Drug-excipients compatibility study

2.3.7.1. Fourier transform infrared (FT-IR) spectra. FT-IR spectroscopic analysis was performed on a Shimadzu IR Affinity-I instrument (Shimadzu, Tokyo, Japan) and measurements of spectra were performed using KBr pellets. Interaction studies between drug and various mucoadhesive polymers were analyzed by comparing the FT-IR spectra.

2.3.7.2. Differential scanning calorimetry (DSC). DSC analysis was performed using a Shimadzu DSC-60 instrument (Shimadzu, Tokyo, Japan). Calibration of instrument was performed with indium and temperature was calibrated with lead for measurement of enthalpy. The samples weighting 6–10 mg were placed into the DSC under aluminum plate and heated from 0 to 270 °C at a scanning rate 10 °c/min. Each investigation was made in duplicated experiments. For comparison, the same procedure was followed for the raw materials and physical mixtures.

2.3.7.3. Scanning electron microscope (SEM). The outline or shape and exterior morphology of organized chitosan based mucoadhesive microspheres were ascertained by SEM (Joel Scanning Microscope JSM-5800, Japan). The SEM analysis was performed by means of an accelerating voltage of 20 kV after they were gold sputtered.

2.3.8. In vitro mucoadhesion test

The microspheres and its mucoadhesive characteristics were undergone for evaluation by an *in vitro* adhesion testing technique well-known as wash-off technique. In this study, freshly excised pieces of intestinal mucosa (5.5 × 1.5 cm) from goat were mounted onto glass slides (5.5 × 1.5 cm) with cotton thread. Glass slides were connected with a suitable support. Around 50 microspheres were rolled out onto each wetted specimen of tissue and soon thereafter the support was set aside to the arm of a disintegrating test machine with a USP tablet. When the disintegrating test appliance was set off or in working mode, then the specimen of tissue was stated in a moderate use up and down motion in the test fluid at 37°C contained in a 1L vessel of the machine. Reading was taken at the end of the 30 min, during last part of 1 h and at hourly intervals up to 6 h. Then the apparatus was closed and the amount of microspheres yet sticking at the tissue were counted at each reading interval up to 6 h. The test was performed at intestinal pH (phosphate buffer pH 6.8). Percentage of mucoadhesion was determined from the formula in eq. (4).

$$\% \text{ Mucoadhesion} = \frac{\text{Number of microspheres}}{\text{Initial microspheres}} \times 100 \quad (4)$$

2.3.9. In vivo pharmacokinetic study

A single dose and analogous design pharmacokinetics review were intended in male Wistar rats (n = 6) under fasting conditions. The optimized microsphere formulation (F6) and pure drug suspension were administered with the help of feeding cannula in the oral dose of 10 mg/kg body weight. All experimental procedures were reviewed and permitted by the institutional animal ethical committee of Roland Institute of Pharmaceutical Sciences (Odisha, India). The samples of blood were taken away by means of the *retro*-orbital venous plexus puncture at 0, 1, 3, 6, 12, 24 and 48 h after dose. About 0.5 ml of blood samples were drawn out in eppendorf tubes and undergone centrifugation at 3000 rpm for 30 min. The plasma was relocated to a different new eppendorf tube and kept aside at −20 °C till analysis with the help of reported HPLC method of quetiapine fumarate [17]. Pharmacokinetic data analysis was performed by the way of noncompartmental modeling. Various parameters such as peak plasma concentration (C_{max}) and corresponding time (T_{max}), and area under curve (AUC) were noted for the treatment formulations and statistically compared using ANOVA followed by post-hoc *t*-test at 5% level of significance.

2.3.10. In vivo brain biodistribution study

The *in vivo* brain biodistribution phenomenon was executed by using male Wistar rats (n = 6) under fasting conditions. The animals were injected with optimized formulation (F6) and pure drug suspension with the help of feeding cannula in the oral dose of 10 mg/kg body weight. At prearranged time intervals (0, 1, 3, 6, 12 and 24 h), rats were immolated by cervical and brain was collected from the animals. Brain homogenates were prepared by suspending the brain slices in phosphate buffer solution (pH 6.8) and subjected to filtration using a 0.45 μm membrane filter. The clear filtrates were subjected to HPLC analysis using the previously reported method after suitable modification for estimation of drug content in the brain homogenate samples [17].

2.3.11. Accelerated stability study

Stability study was executed for the optimized formulation by storage at 40 °C/75% RH for 6 months. At defined time intervals (1, 2, 3 and 6 months), the samples were drawn out and subjected to evaluation of physical appearance, size, shape, surface morphology, drug content and *in vitro* drug release for recognizing any fundamental major distinction with the initial value.

3. Results and discussion

3.1. Preformulation studies

The preformulation study indicated that melting point of drug at 173°c, which was observed to be in consonance with the literature. The solubility of drug was determined in distilled water, 0.1 N HCl and phosphate buffer (pH 6.8), where maximal solubility was achieved with phosphate buffer solution. Also, micromeritic properties of the drug analyzed by measuring the angle of repose, bulk density, Carr's index and Hausner's ratio indicated superior flow property and compaction behavior.

3.2. Preliminary screening of polymers

In the present work, the preliminary trial for the formulations bearing mucoadhesive microspheres were developed and evaluated as per the methods provided in experimental section. Table 1 provides the list of trial formulations prepared for identifying the best suitable polymer combinations for preparing mucoadhesive microspheres of

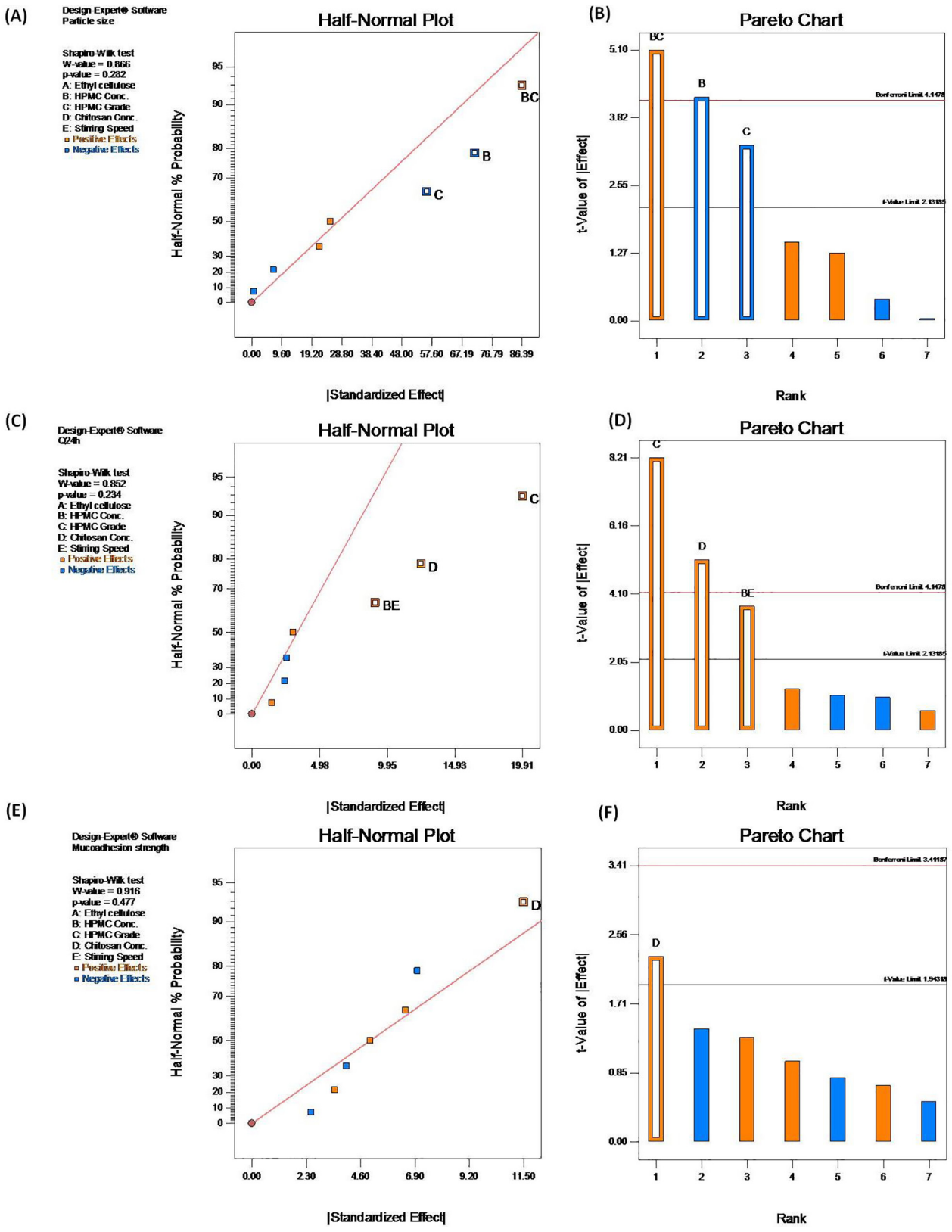

Fig. 1. . Half-normal plots and Pareto charts indicating factor influence on response variables of mucoadhesive microspheres, (A-B) particle size, (C-D) Q24h, (E-F) mucoadhesive strength.

quetiapine fumarate. Among the prepared trial formulations, the spherical shape microspheres with smooth textures was formed in batches F2 and F3, while no microspheres were observed in the remaining formulation batches. Thus, aforementioned batches containing HPMC K4M were preferred for furthermore optimization study.

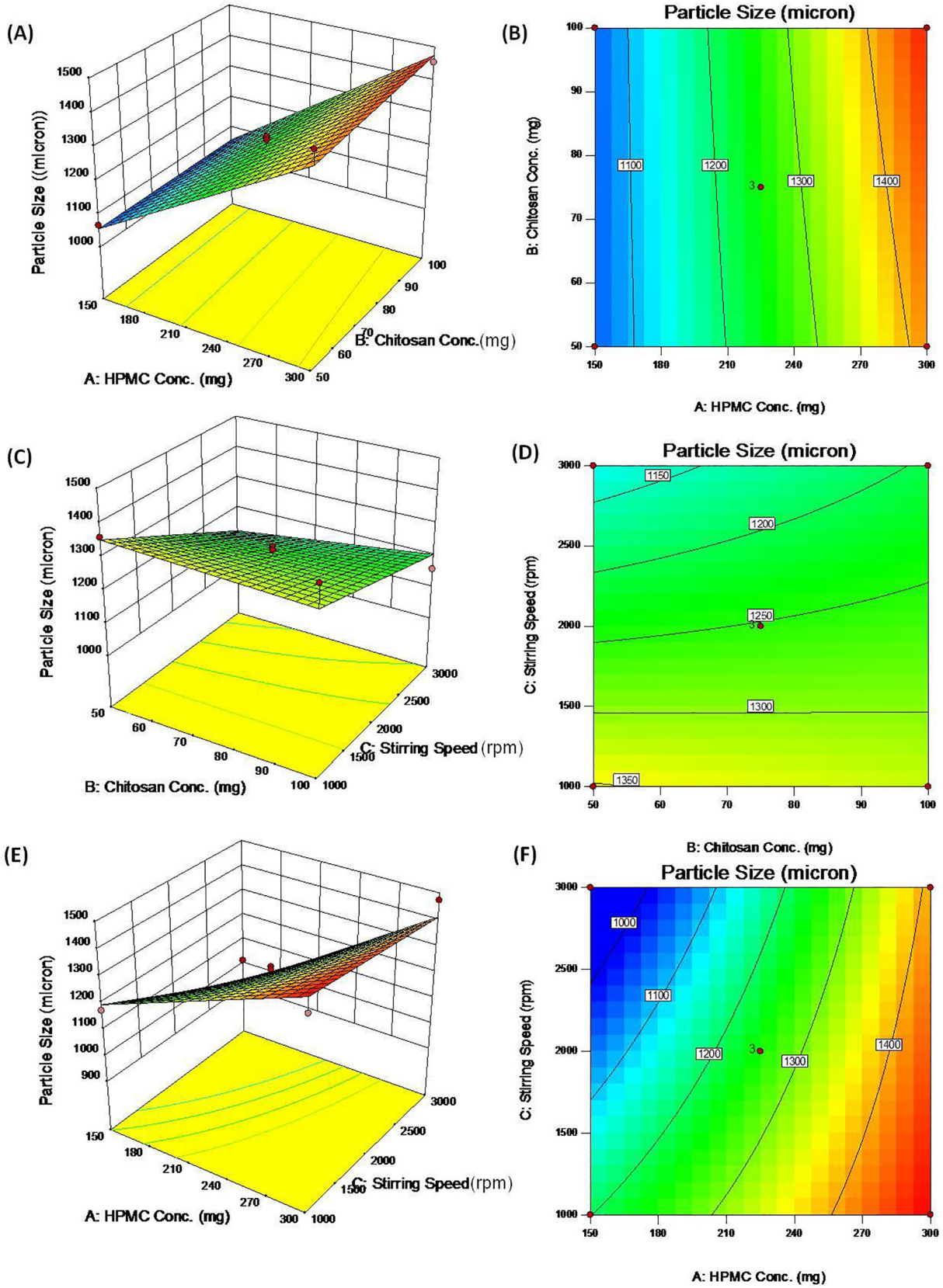

Fig. 2. . 3D-Repsonse surface plots and 2D-contour plots showing the relationship among the factors such A (HPMC conc.), B (Chitosan conc.) and C (stirring speed) on particle size as the response variable.

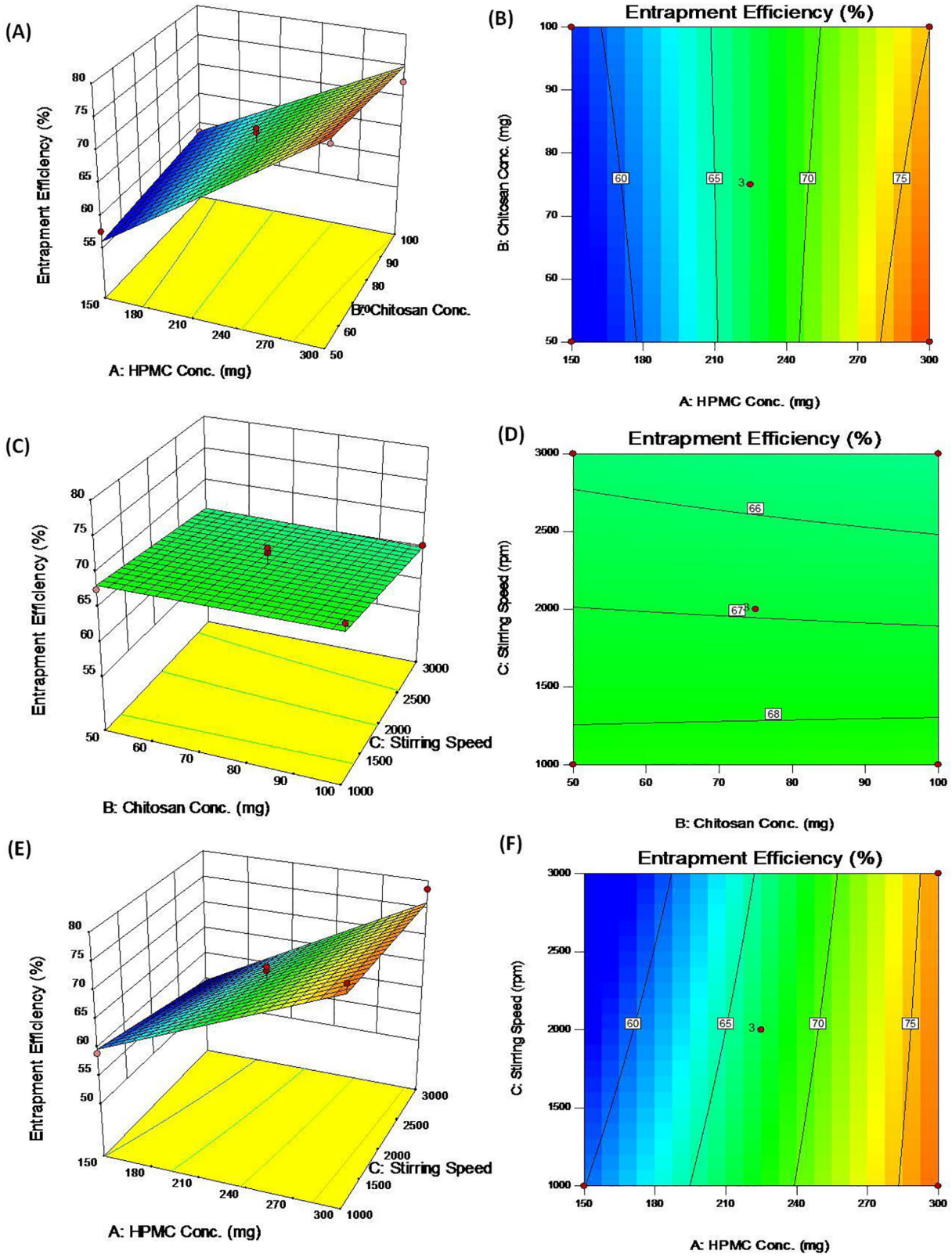

Fig. 3. . 3D-Repsonse surface plots and 2D-contour plots showing the relationship among the factors such A (HPMC conc.), B (Chitosan conc.) and C (stirring speed) on entrapment efficiency as the response variable.

3.3. Preparation and optimization of mucoadhesive microspheres

3.3.1. Factor screening study

Table 2 enlists the trials suggested as per the fractional factorial design and the obtained responses. The mathematical modeling of data obtained for the prepared formulations using linear polynomial model equation (5) revealed a good fitness to detect the main effects. The interactions are holding a two-factors, however, observed were ignored due to the chances of aliasing.

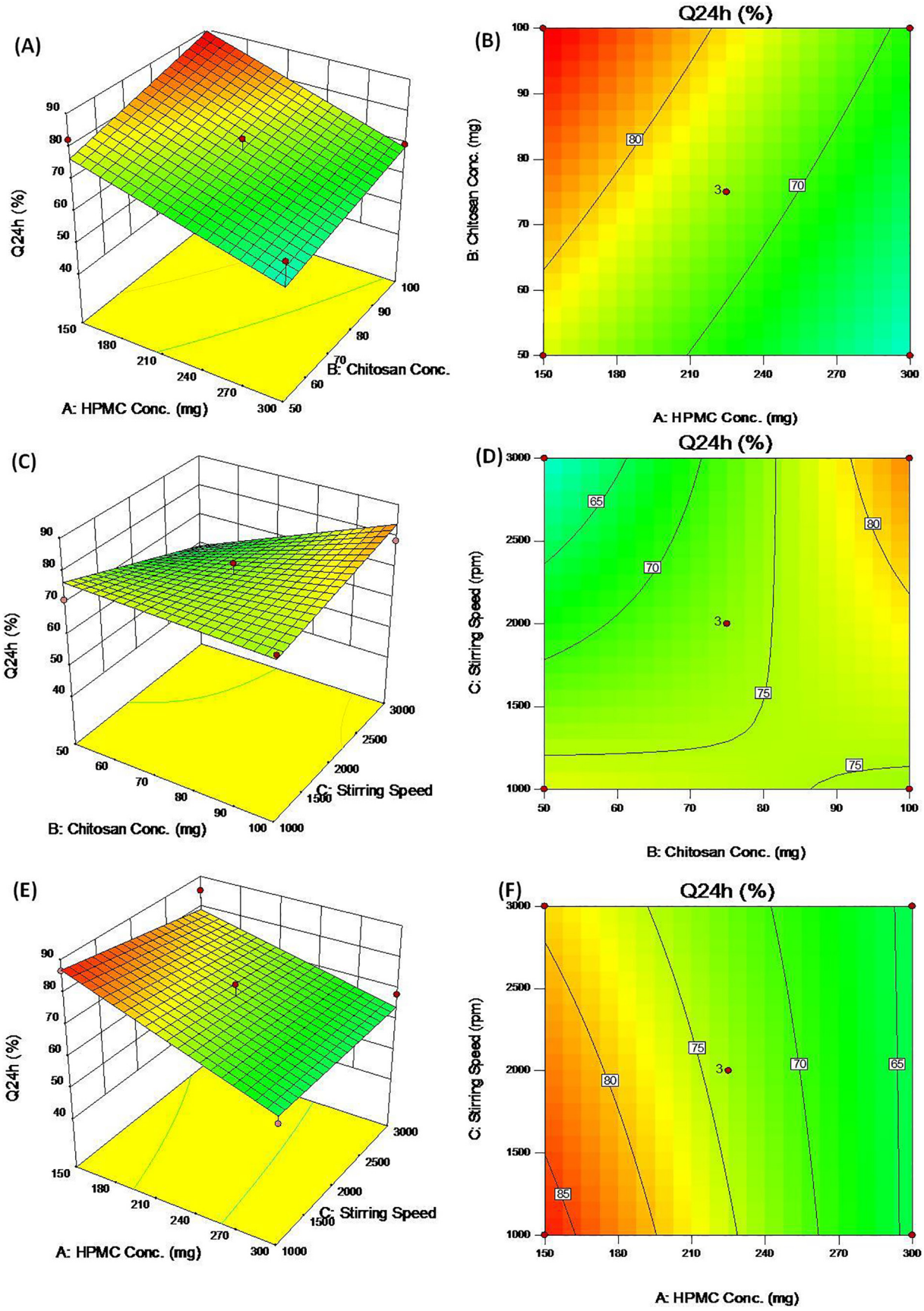

Fig. 4. . 3D-Repsonse surface plots and 2D-contour plots showing the relationship among the factors such A (HPMC conc.), B (Chitosan conc.) and C (stirring speed) on Q24h as the response variable.

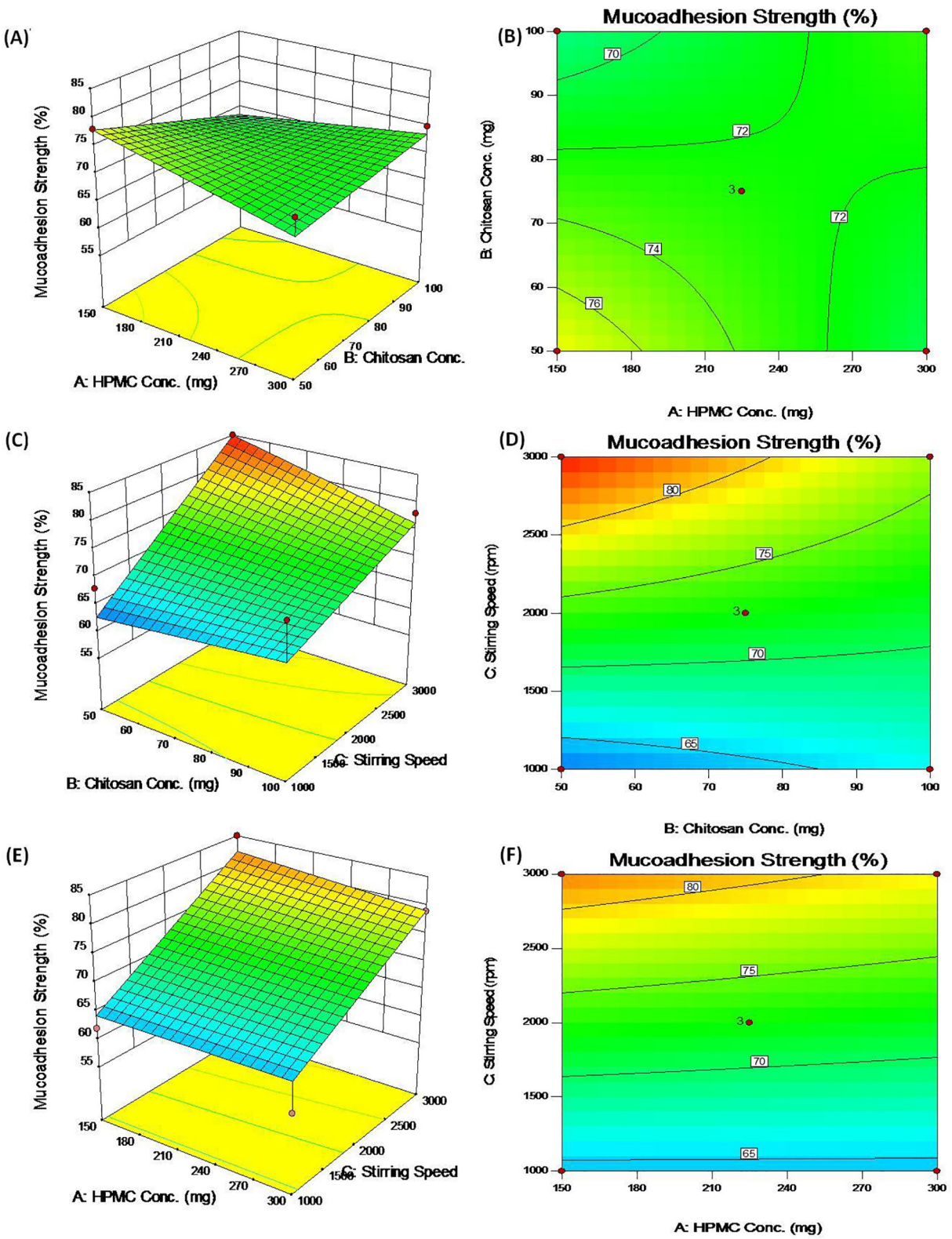

Fig. 5. . 3D-Repsonse surface plots and 2D-contour plots showing the relationship among the factors such A (HPMC conc.), B (Chitosan conc.) and C (stirring speed) on mucoadhesion strength as the response variable.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_5 X_5 + \beta_n X_n \quad (5)$$

where, Y = response variables, β_0 = intercept, β_1 to β_5 = coefficients of linear model terms

Factors with statistically significant main effects on the response variables were identified through half-normal plots and Pareto charts (Fig. 1). On the response variable, particle size, factor B (HPMC conc.) and C (HPMC grade) were observed to be highly influential, as the bars for both the factors above t-value limit. For response variable, Q_{24h}, the factor C and D (Chitosan conc.) exhibited statistically significant impact, as indicated by the bar graph in the Pareto chart above t-value limit and Bonferroni limit. Likewise, in case of %mucoadhesion as the

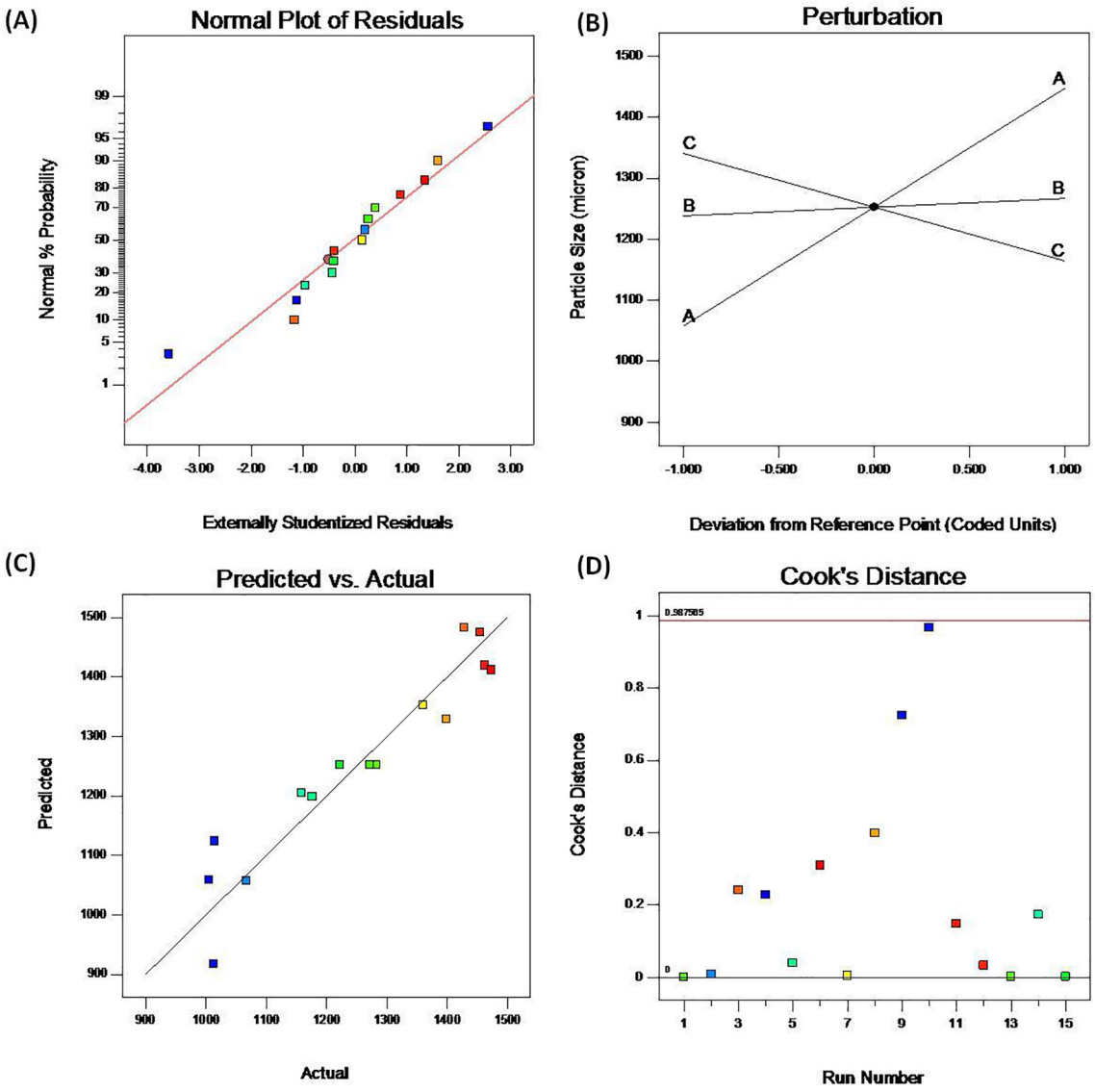

Fig. 6. . Model diagnostic plots indicating experimental data fitting for particle size as the response variable, (A) Normal plot of residual, (B) Perturbation plot, (C) Predicted vs. Actual plot, (D) Cook's distance.

response variable, only factor D showed influential effect with characteristic bar above the t-value limit. Overall, the factor screening study revealed that factors B, C and D were highly significant on the studied response variables. Applying these factors, the response surface optimization study was performed and optimum microsphere formulation was identified.

3.3.2. *Factor optimization study*

Table 3 enlists the trials suggested as per the Box-Behnken design along with obtained responses of the trial formulations. The obtained data were subjected to mathematical modeling, which indicated suitability with quadratic polynomial model equation (6). Further, ANOVA was applied for analysing the coefficients of polynomial equation for each of the studied response variables. Supplementary data Tables S1–S4 indicate the details on the ANOVA parameters, which indicated statistically significant model terms ($p < 0.05$), insignificant lack of fit and low values of predicted residual sum of squares.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1^2 + \beta_5 X_2^2 + \beta_6 X_3^2 + \beta_7 X_1 X_2 + \beta_8 X_2 X_3 + \beta_9 X_1 X_3 \quad (6)$$

By implementation of 3D-response surface plots and 2D-contour plots, the response surface investigation was performed for understanding the concept of factor-response correlation. Figs. 2–5 identifies the response surface plots for the studied response variables. As depicted in Fig. 2, the 3D-response surface plot and 2D-contour plots for particle size as the response variable indicated relationship among HPMC conc., chitosan conc. and stirring speed. In Fig. 2 (A-B), both the effect of HPMC and chitosan conc. indicated a sharp linear increase in the values of particle size between low to high levels of the factors. On the other hand, Fig. 2 (C-D) portraying the 3D and 2D-plots indicated insignificant influence of chitosan conc. and a negative impact of stirring motion or speed on particle size. Moreover, Fig. 2 (E-F) illustrating the plots indicated analogous relationship between HPMC conc. and stirring speed upon the size of particle. In general, the curve shape manifestation of the response surface plots confirmed presence of interaction effect among the factors.

The concept related to entrapment efficiency with respect to 3D-response surface plot and 2D-contour plots are illustrated in Fig. 3 indicated the relationship among HPMC conc., chitosan conc. and stirring speed. The plots for HPMC and chitosan conc. shown in Fig. 3 (A-B) indicate a positive impact of HPMC conc. on entrapment efficiency, where as chitosan conc. did not exhibit any influence on the

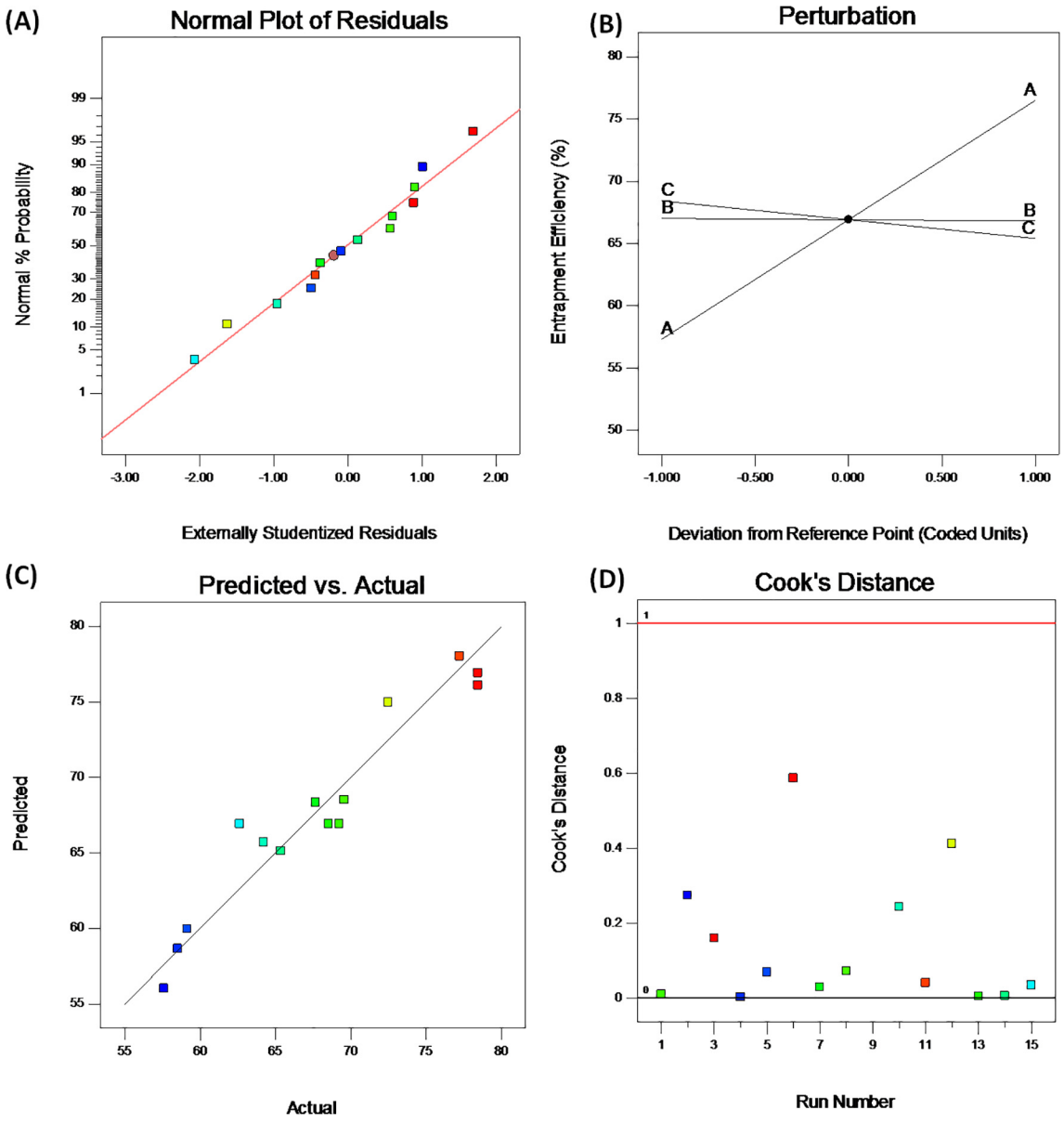

Fig. 7. . Model diagnostic plots indicating experimental data fitting for entrapment efficiency, (A) Normal plot of residual, (B) Perturbation plot, (C) Predicted vs. Actual plot, (D) Cook's distance.

entrapment. In Fig. 3 (C-D), no significant influence was observed for effect of chitosan conc. and stirring speed on entrapment efficiency. On the contrary, the plot between HPMC conc. and stirring speed shown in Fig. 3 (E-F) revealed that only the effect of an increase in HPMC conc. was observed to be highly pronounced.

Fig. 4 portrays the 3D-response surface and 2D-contour plots for Q_{24h} indicating the relationship among the factors, HPMC conc., chitosan conc. and stirring speed. Fig. 4 (A-B) indicating the relationship between HPMC and chitosan concentration showed that increase in HPMC decreases the amount of drug release, while increase in chitosan conc. showed a positive influence on the drug release. In another way, Fig. 4 (C-D) presenting the correlation, among chitosan conc. and stirring speed showed that the earlier one exhibit declining influence on drug release while later one showed positive influence on drug release profile. Moreover, Fig. 4 (E-F) illustrating the influence of HPMC conc. and stirring speed indicated that earlier one exhibit a sharp declining trend on drug release, while the later one showed no significant influence on drug release profile.

The 3D-response surface plots and 2D-contour plots depicted in Fig. 5 for % mucoadhesion efficiency indicate the relationship among the factors, HPMC conc., chitosan conc. and stirring speed. Fig. 5 (A-B) indicating two factor relationships reveal that increase in HPMC conc. showed a declining influence on % mucoadhesion, while effect of chitosan concentration on % mucoadhesion showed a positive response. On the other hand, Fig. 5 (C-D) depicting the relationship between chitosan concentration and stirring speed showed that the later one exhibited a sharp linear influence on % mucoadhesion. An analogous response was also observed in Fig. 5 (E-F), indicating the relationship between HPMC conc. and stirring speed, where impact of stirring speed exhibited a sharp inclining trend in % mucoadhesion behaviour.

Apart from the response surface analysis, the model diagnostic plots were analyzed for evaluating the fitting of data with the developed mathematical model. Figs. 6–9 illustrates the normal plot of residuals, perturbation charts, predicted vs. actual plots and Cook's distance plots. All the plots indicated excellent model fitting, closeness between the predicted and observed data, followed by an absence of any outliers.

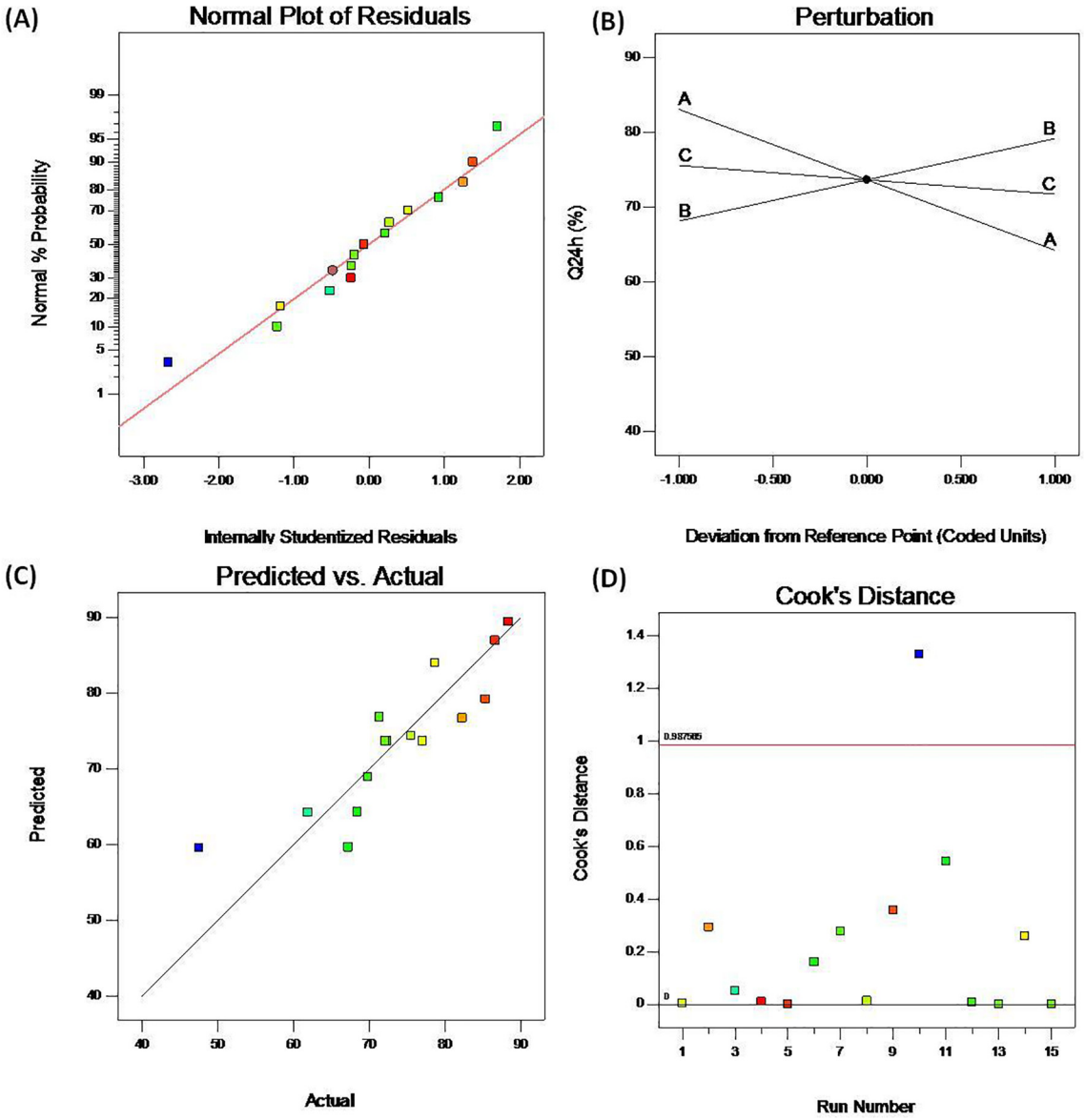

Fig. 8. . Model diagnostic plots indicating experimental data fitting for Q_{24h}, (A) Normal plot of residual, (B) Perturbation plot, (C) Predicted vs. Actual plot, (D) Cook's distance.

3.4. Characterization of the mucoadhesive microspheres

3.4.1. Micromeritic properties

Microspheres showed desired flowability due to optimal presence of moisture, diminished cohesiveness and spherical shape. The flow properties of microspheres such as angle of repose, Carr's index and Hausner's ratio are summarised in Table 4. The angle of repose of all the selected formulations ranged between 28.2 and 35.1, Carr's index was observed to be in the limit of 19.4 and 24.6, while Hausner's ratio was ranged from 1.18 to 1.34. The above results indicated excellent flow property of the prepared mucoadhesive microspheres.

3.4.2. Percentage yield

The manufacture yield or outcome of microspheres prepared by the solvent evaporation method was observed to be ranging between 90.2% and 98.5% as shown in Table 4. The yield of production was not uniform for all formulation. The frequent possible cause for its low percentage (%) yield was due to wastage of formulation ingredients during the preparation process.

3.4.3. Entrapment efficiency

The entrapment efficiency of drug of all formulation is summarized in Table 4, which was found to be ranging between 50.8 and 67.4. The dealing out processing medium volume (polyvinyl alcohol) significantly improved the entrapment efficiency of microspheres. Higher drug extraction into the processing medium could be cause of lower entrapment efficiency.

3.4.4. Particle size analysis

The distribution of dimensions especially, the shape and size in virtue of microspheres average diameter was found out by an optical microscope system. A compound microscope fixed with an ocular micrometer which has been calibrated in association with a stage micrometer was employed to count up at least 100 microspheres. Mean of the particles were taken into consideration. The microspheres were uniform in size in each formulation and the mean size ranged from 1108 to 1465 μm.

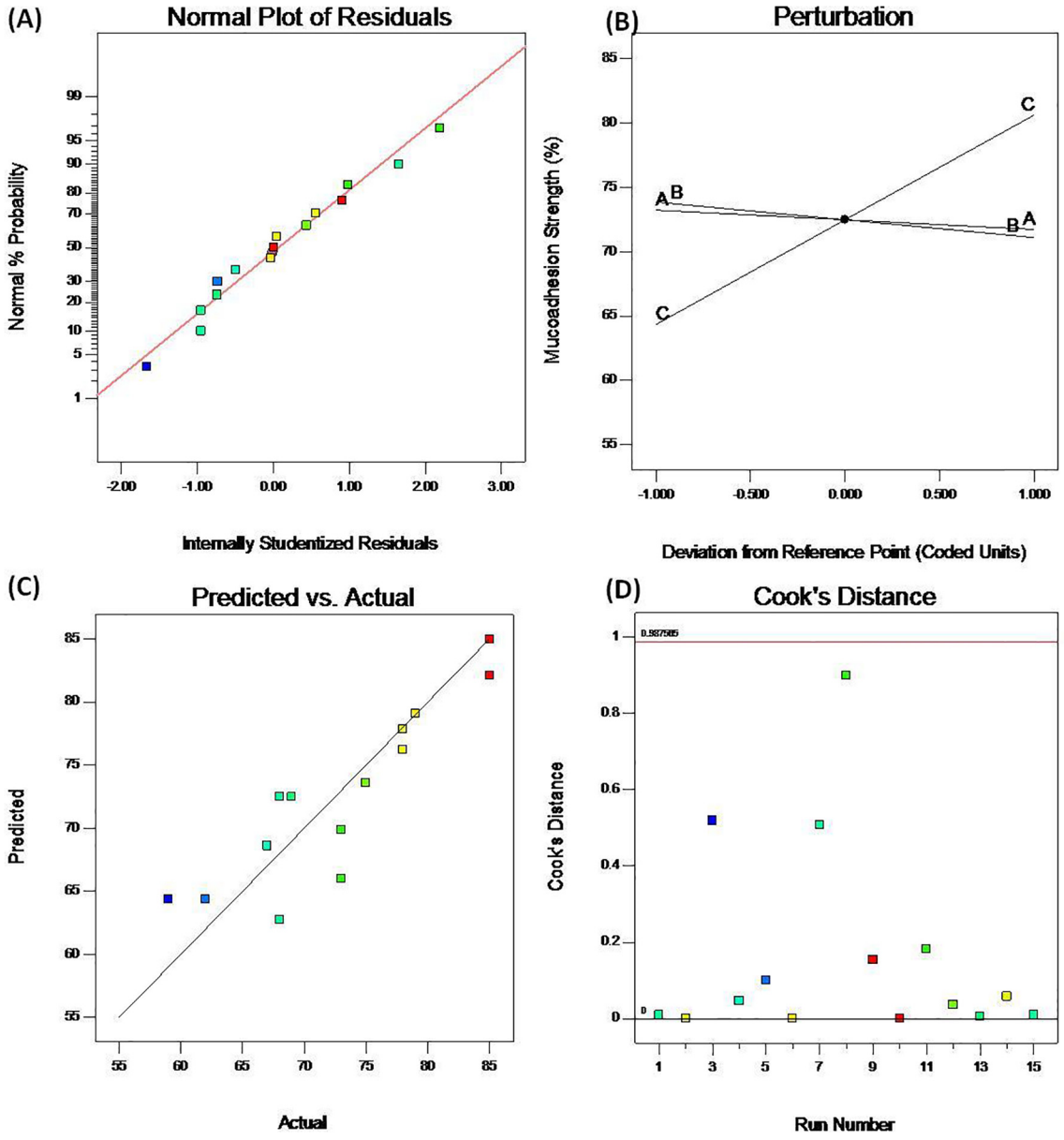

Fig. 9. . Model diagnostic plots indicating experimental data fitting for %mucoadhesion efficiency, (A) Normal plot of residual, (B) Perturbation plot, (C) Predicted vs. Actual plot, (D) Cook's distance.

Table 4
. Characterization data of mucoadhesive microspheres prepared as per the Box-Behnken design.

Formulation code	Angle of repose	Carr's index	Hausner's ratio	Percent Yield	Entrapment efficiency (%)	Swelling index (%)
*F1	28.2	22.7	1.19	94.1	50.8	65
F2	29.4	19.4	1.23	92.3	55.7	74
F3	24.8	21.2	1.21	98.5	62.0	78
F4	30.3	26.3	1.27	91.8	56.0	77
F5	34.6	20.2	1.14	98.3	59.8	69
F6	31.5	21.5	1.13	96.5	61.1	83
F7	33.7	22.4	1.26	97.5	65.8	67
F8	30.8	19.8	1.21	90.2	66.2	68
F9	32.6	24.6	1.31	97.3	67.4	75
F10	34.8	18.4	1.25	97.9	65.2	72
F11	35.1	22.5	1.34	98.2	63.1	69
F14	33.3	23.8	1.18	93.4	62.9	74

*Indicate F1, F12 and F13 are center points and values are average of 3 center points; Data presented as Mean ± S.D. (n = 3).

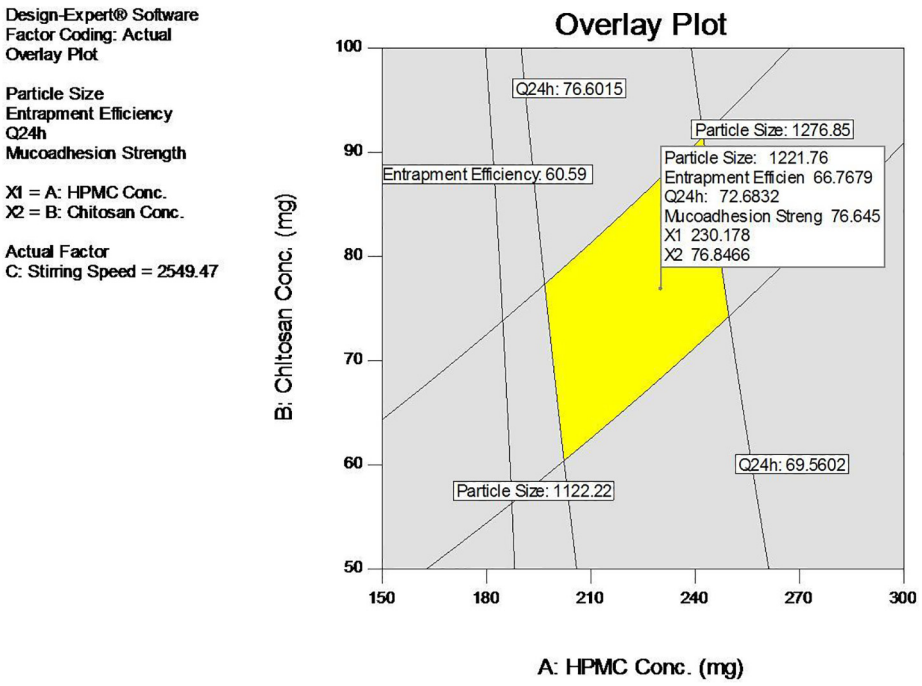

Fig. 10. . Overlay plot with yellow color region as the design space and flagged point with composition of optimized formulation along with predicted values of the responses.

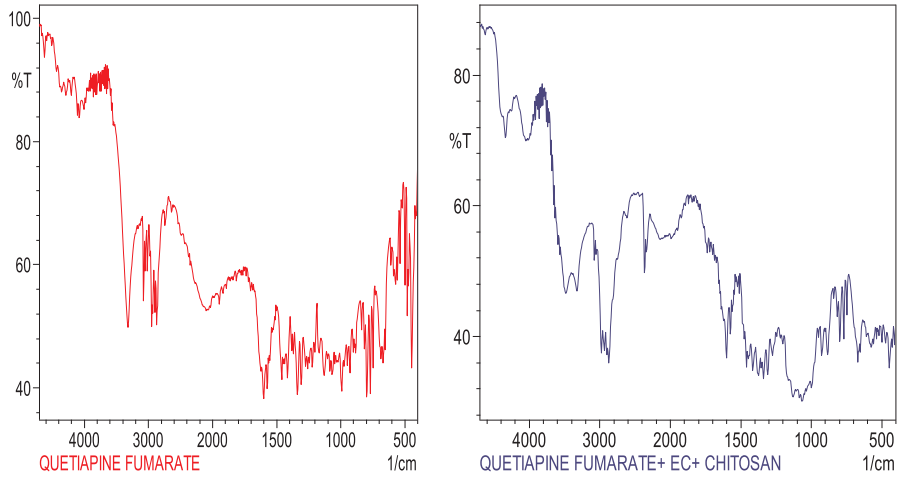

Fig. 11. . FT-IR spectra of Quetiapine fumarate (A) and optimized formulation (B).

3.5. Search for the optimum formulation and validation studies

The optimized mucoadhesive microspheres formulation was identified by numerical optimization and desirability function by "trading off" of various response variables for attaining the desired goals, minimization of particle size, maximization of entrapment efficiency, amount of drug release (Q_{24h}) and % mucoadhesion. The design space with yellow colour region was demarcated in grey colour overlay plot region, as showed in Fig. 10. The optimum formulation, comprising of HPMC (230 mg), chitosan (77 mg) and stirring speed (2550 mg), which exhibited particle size of 1221 μm, entrapment efficiency of 66.6%, Q_{24h} of 73% and mucoadhesion efficiency of 77%. Validation of the predicted values of responses was performed by comparing with the observed data, which indicated high degree closeness between the predicted and observed values of the responses (data not shown) and confirmed excellent prognostic ability of the employed mathematical model.

3.6. Characterization of the optimized mucoadhesive microspheres

3.6.1. Drug-polymer interaction studies

3.6.1.1. Fourier transform infrared (FT-IR) spectroscopy. The FT-IR spectrophotometer was used to identify as well as determine the possibilities of any interaction between the formulation components at the optimized composition. As showed in the Fig. 11, there was no substantial differentiation in the FT-IR spectra of the drug when compared to the spectra of the physical mixture of drug and polymers. The FT-IR spectra of the drug and polymer showed that there was no shift in the major peaks. This further revealed that there was no variation in the properties of the drug and polymers in the formulation. Hence, the drug and polymers were compatible with each other.

3.6.1.2. Differential scanning calorimeter (DSC). The thermal analysis was carried out upon pure drug, physical mixture and optimized

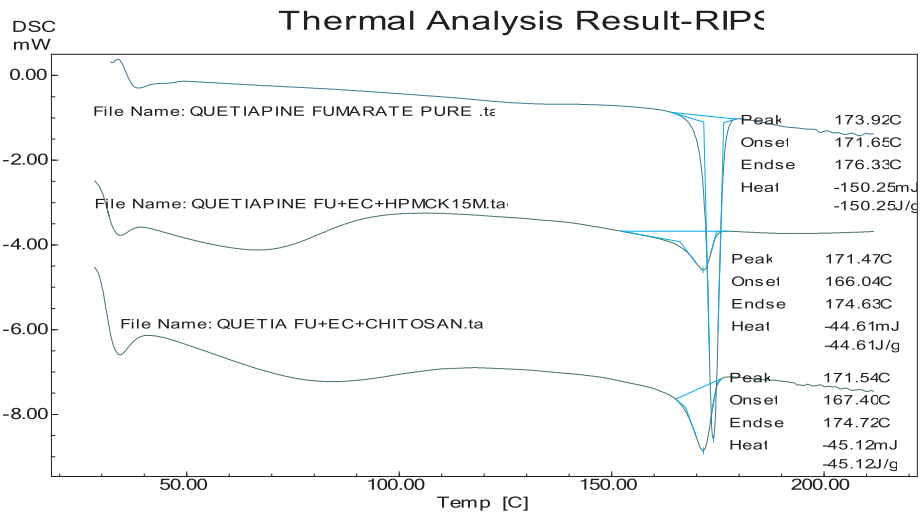

Fig. 12. . DSC thermograph of pure drug, physical mixtures of drug and selected polymers and optimized formulation.

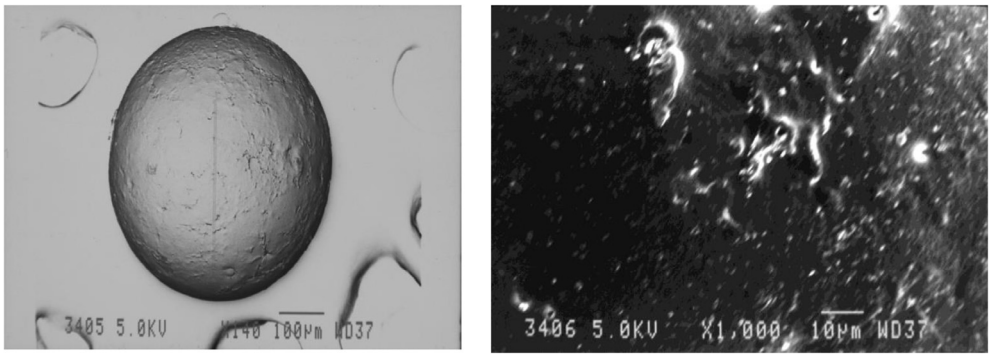

Fig. 13. . SEM images of optimized formulation at low resolution (a) and at high resolution (b).

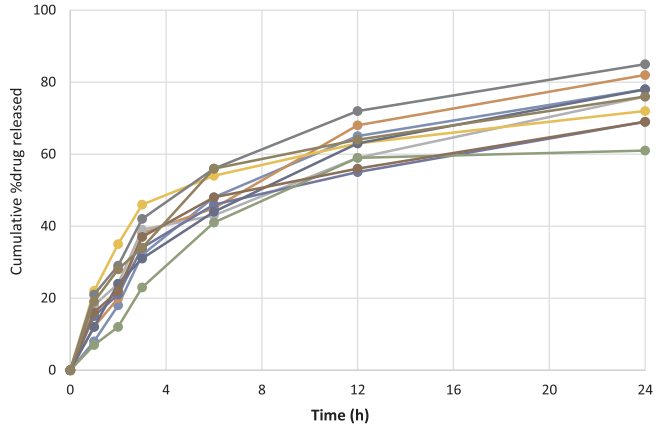

Fig. 14. . *In vitro* drug release profile of quetiapine from mucoadhesive microspheres.

Table 5
. *In vitro* drug release kinetics of mucoadhesive microsphere formulations.

Formulation code	Correlation coefficient (R2)		
	Zero order	First order	Higuchi plot
*F1	0.973	0.869	0.960
F2	0.963	0.754	0.929
F3	0.978	0.844	0.950
F4	0.973	0.833	0.947
F5	0.983	0.809	0.937
F6	0.980	0.724	0.953
F7	0.988	0.829	0.987
F8	0.976	0.887	0.924
F9	0.968	0.879	0.981
F10	0.971	0.778	0.975
F11	0.988	0.878	0.954
F14	0.974	0.785	0.943

*Indicate F1, F12 and F13 are center points and values are average of 3 center points.

mucoadhesive microsphere formulation and thermograms are depicted in Fig. 12. The result of DSC thermograms of pure drug, physical mixture and optimized s polymers showed an endothermic peak at 175 °C. In addition to this, additional decline in sharpness of endothermic peak in loaded with drug microspheres formulation was notified. This might be since the modification of microspheres crystalline state to the subsequent amorphous geometry.

3.6.1.3. Scanning electron microscopy (SEM). The microspheres were communicated to be distinct as well as non aggregated, free-flowing. These also lead to being a category of monolithic matrix. Fig. 13 depicts the SEM images indicating that the microspheres are spherical in shape with a smooth appearance of the surface.

3.6.2. Swelling index

Swelling depends on polymer concentration, ionic strength as well as the presence of water. Table 4 shows the % swelling index of different preliminary formulations at diverse time duration. The results reveal that all the formulations became swell enormously when allowed to dip in to phosphate buffer pH 6.8. It is outlined that swelling

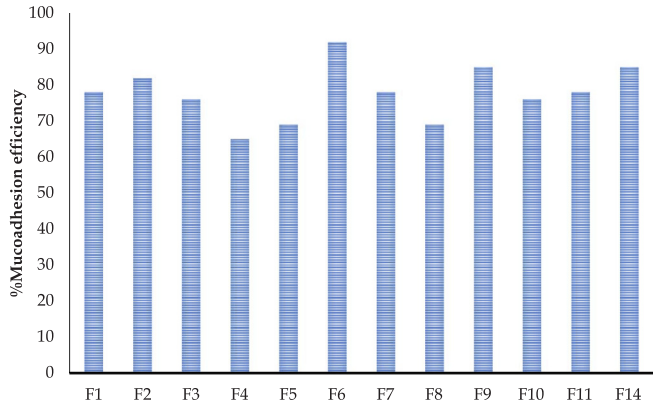

Fig. 15. . Bar chart showing *ex vivo* mucoadhesion of the prepared microspheres.

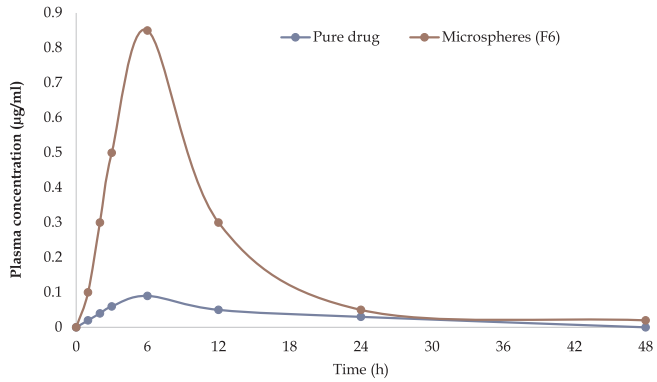

Fig. 16. . *In vivo* pharmacokinetic study of pure drug and optimized mucoadhesive microspheres of quetiapine fumarate in Wistar rats (n = 6).

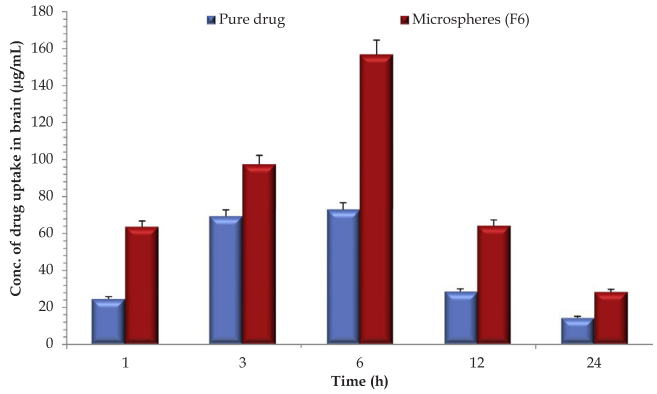

Fig. 17. . *In vivo* brain biodistribution of quetiapine fumarate from pure drug suspension and optimized mucoadhesive microspheres (F6); Data expressed as Mean + 1SD (n = 3).

behaviour is the prime most factor which commonly has significant impact upon the adhesive characteristics and cohesiveness of mucoadhesive polymers. Mucoadhesive microspheres are expected to start on with water from beneath the layer of mucosal tissue by swelling, absorbing, and capillary effects results in to substantially stronger adhesion. Higher swelling was observed for formulation F6 containing chitosan as mucoadhesive polymer might be caused by its high ionization at pH 6.8 which is competent of absorbing a high amount of water.

3.6.3. In vitro drug release study

The *in vitro* drug release research profile of quetiapine fumarate were initially carried out in 0.1 N HCl for 2 h and followed by phosphate buffer pH 6.8 for 22 h. The microspheres exhibited sustained drug release action throughout the study period. No drug was released in the acidic medium during 2 h, while controlled release was seen after 2 h in phosphate buffer pH 6.8. Fig. 14 depicts the *in vitro* drug release status from the mucoadhesive microsphere formulations of quetiapine fumarate. Every one of the formulations revealed a superior retardation of the drug release upto 16 h. This could be due to a combination of mucoadhesive polymer particularly HPMC with EC, which is widely used for sustained release matrix polymer having hydrophobic property. Among all the formulations, F6 showed maximum sustained drug release profile up to 24 h ostensibly owing to the higher proportion of HPMC K15M and EC content. As HPMC is commonly used in a hydrophilic matrix type delivery system, it produces a viscous gel in touch with aqueous media that might be helpful in controlled delivery of highly water soluble drugs. The release of the drug was prolonged with the increase in viscosity of HPMC. Effective and rapid liberation of the drug from the hydrophilic matrices were possibly in virtue of effective and quicker dissolution of the aqueous soluble drugs from the core and is spreading out of the spheres producing the pores for access of solvent molecules [8–10].

3.6.4. Drug release kinetics evaluation

Table 5 enlists the modelling rely on release of drug data using various mathematical equations (zero, first and Higuchi), which revealed that drug release was quite in accordance with zero order kinetics, but Higuchi model also holds good for regulating the drug release from all the prepared microsphere formulations.

3.6.5. Ex vivo wash-off test

Fig. 15 summarized the results of % mucoadhesion of overall formulations with goat intestine. The results indicated that the polymer viscosity was strongly associated with the adhesion. Viscosity and molecular weight were directly proportional to the mucoadhesion property. The microspheres consisting of ethyl cellulose in combination with mucoadhesive polymers (as mentioned earlier) revealed superior mucoadhesive properties observed in the in-vitro wash-off experiment when compared to non mucoadhesive polymer (i.e. ethyl cellulose). The evaluated value was obtained from 94 to 87% for F6 to F2 formulations. The wash-off was slow in case of microspheres consisting of mucoadhesive polymers when compared to that of ethyl cellulose alone. The result of the wash-off test designated that the microspheres exhibited good mucoadhesive property, which is required for prolonged residence time at the absorption site for enhancing the oral bioavailability [5].

3.6.6. In vivo pharmacokinetic study

Fig. 16 illustrates the plasma concentration-time profile of quetiapine fumarate from optimized mucoadhesive microsphere (F6) and pure drug. A drastic augmentation in pharmacokinetic profile was clearly observed from the microspheres. The mean peak plasma concentration (C_{max}) of quetiapine fumarate from microspheres was found to be 0.85 µg/mL, while during the condition of pure drug, the peak plasma concentration is 0.09 µg/mL. Besides, the area under curve (AUC) was also found to be significantly increased in case of microspheres vis-à-vis the pure drug. However, time at peak plasma concentration (T_{max}) was found to be unchanged. The results indicated a significant improvement ($p < 0.001$) in the oral bioavailability of drug, as clearly evident from the values of C_{max} and AUC of optimized microspheres vis-à-vis pure drug suspension.

3.6.7. In vivo brain biodistribution study

Fig. 17 portrays the *in vivo* brain biodistribution data of quetiapine fumarate from optimized mucoadhesive microspheres formulation and pure drug suspension. Higher brain levels of the drug were observed from microspheres (153.36 µg/mL) within 6 h time period, while pure

drug suspension showed brain drug levels (73.65 μg/mL) quite less. A statistically significant variation ($p < 0.001$) was observed from the microspheres vis-à-vis the pure drug. This could be attributed to the higher systemic levels of the drug, ostensibly by virtue of the rise in drug absorption potential.

3.6.8. Accelerated stability study

The accelerated stability profile was performed in the laboratory at room temperature for 6 months for the best formulation (F6). The drug content, drug release, and other physical properties were tested. There was not any significant change; hence the formulation is said to stable.

4. Conclusions

The foremost endeavor of this research stated that an acid labile drug quetiapine fumarate was chosen and mucoadhesive microspheres were formulated by means of solvent evaporation method in order to improve its biopharmaceutical attributes. The microspheres were efficiently optimized using experimental designs, which furnished good understanding on the factor-response relationship. Moreover, the microspheres were subjected to characterization, where the obtained results corroborated micromeritic properties, sustained drug release profile and mucoadhesion within the desired limits.

5. Conflicts of interest

The authors confirm that this article content has no conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bfopcu.2018.09.002.

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