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Original Article

DEVELOPMENT AND EVALUATION OF NANOSPONGES-BASED BUCCAL TABLETS FOR DELIVERY OF QUERCETIN USING BOX-BEHNKEN DESIGN

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ABSTRACT

Objective: The goal was to develop a controlled-release formulation of quercetin utilizing the cyclodextrin-based nano-sponges as a nanocarrier.

Methods: Based on the preliminary trials a 3-factor, 3-level Box-Behnken design was employed. Five types of nano-sponges from β-cyclodextrin (NS1-NS5) were purposely designed. Quercetin was loaded into nano-sponges by freeze-drying method. The prepared nano-sponges were characterized and formulated into tablets and evaluated for weight variation, hardness, friability, disintegration studies, dissolution and stability studies.

Results: The particle sizes of quercetin-loaded nano-sponges are in between 36.45 to 135.27 nm, encapsulation efficiency of 42.37 to 88.44 % and drug release% at 6h of 53.04 to 82.64 %. *In vitro* release studies showed that more than 90 % of drugs were released from nano-sponge formulations as compared to only around 45% from free drug suspension after 24 h. The FTIR, DSC and XRPD studies confirmed the interaction of Quercetin with nano-sponges. TEM image revealed the spherical structure of drug-loaded nano-sponges. The drug loaded in the nano-sponge structure can be retained and released slowly over time. The nano-sponges were formulated into tablets and evaluated for weight variation, hardness, friability and disintegration studies and obtained satisfactory results. *In vitro* drug release from a tablet exhibited a maximum release of 99.75 percent with controlled release behaviour over 24 h, and stability studies suggested no major significant changes within 6 mo.

Conclusion: Cyclodextrin-based nano-sponges showed superior complexing ability with increased solubility of poorly soluble Quercetin tablets made for controlled drug delivery, which can reduce dosing frequency.

Keywords: Quercetin, β-Cyclodextrin, Nano-sponges, Experimental design, Buccal tablets, *In vitro* drug release

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INTRODUCTION

Quercetin (3,3',4',5,7-pentahydroxy-flavone) is the biggest flavonol subclass member among the various flavonoids identified to date. It has been proven to have anti-cancer, anti-oxidation, anti-inflammation, reducing blood cholesterol, dilating coronary arteries, anti-platelet aggregation, anti-anemia, and antianaphylaxis properties, among other biological and pharmacological actions [1]. However, due to its poor solubility, low hydrophilicity (log P value of 1.81), gastrointestinal instability, significant first-pass metabolism, and minimal absorption in the gastrointestinal system, quercetin is a difficult chemical to deliver pharmaceutically. Quercetin is classified as BCS class II [2]. It dissolves in water at 7.7 ng/ml, 5.5 lg/ml in simulated gastric fluid, and 28.9 lg/ml in simulated intestinal fluid (SIF). The drug's therapeutic application in conventional dose forms is constrained due to its low oral bioavailability, which was demonstrated to be less than 17% in rats and even less than 2% in humans [3]. It is therefore required to develop a better oral formulation of quercetin with increased bioavailability and activity. With so many different drug delivery methods available described in the literature, quercetin nanoparticulate formulation appears to be a viable option for improving solubility and stability at the same time. Hypercross-linked cyclodextrin polymers that have been nanostructured to create three-dimensional networks have recently been created as nano-sponges. They are made by cyclodextrin interacting with an appropriate cross-linking agent, such as carbonyl diimidazole or diphenyl carbonate. Natural cyclodextrins were not as good in complexing various compounds as cyclodextrin-based nano-sponges. They have been utilized to protect the labile groups, enhance the solubility of poorly soluble actives, and regulate the release [4].

Due to their unique physiological characteristics, buccal mucoadhesive dosage forms have been studied extensively over the last decade. The buccal route could be employed for both local and systemic administration. These formulations could be conveniently administered into disease sites, reducing potential side effects, improving patient compliance, and exhibiting long-term retention within the specific site of action. Using cyclodextrin nano-sponges as unique nanocarriers, the goal of the current study was to create nano-sponges incorporating buccal tablets containing quercetin.

MATERIALS AND METHODS

Materials

Quercetin was obtained as a gift sample from MSN laboratories Pvt. Ltd, β-Cyclodextrin was obtained from Gangwal Chemicals Pvt. Ltd. (Mumbai, India)., Diphenyl carbonate, HPMC K100LV, Carbopol 934P purchased from Euclid Pharmaceuticals Limited, Mumbai, Dimethyl sulfoxide and Ethanol was purchased from Qualigens, Thermo Fisher Scientific India Ltd, Mumbai.

Preparation of β-cyclodextrin nano-sponges (NS)

Cyclodextrin-based nano-sponges were prepared in our laboratory using diphenyl carbonate for the crosslinking as reported elsewhere [5]. Five types of nano-sponges were prepared using different molar ratios of reactants. The molar ratios and concentrations of both the reactants were used as shown in table 3. Briefly, in a 250 ml flask, required quantity of anhydrous β-Cyclodextrin was dissolved in dimethyl sulphoxide. Diphenyl carbonate was added to this reaction mixture and refluxed in an oil bath at 90 °C for 6 h under stirring. After completion of the reaction, the obtained product was washed with water and subsequently purified by Soxhlet extraction with ethanol up to 6 h. The white powder thus obtained was dried overnight in an oven at 60 °C and subsequently ground in a mortar. The fine powder obtained was redispersed in water. The colloidal part that remained suspended in water was recovered by lyophilization. The obtained nanosponges were termed as NS1, NS2, NS3, NS4 and NS5 (table 1).

Characterization of β-cyclodextrin nano-sponges

The particle size distribution of cyclodextrin nano-sponges were observed by the dynamic light scattering method. The

measurements were made at a fixed angle of 90° for all samples. The samples were suitably diluted with Milli Q water before measurement. The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated using cumulant analysis after averaging three measurements. Zeta potential measurements were also made using an additional electrode in the same instrument. All the experiments were conducted in triplicate at 25±2 °C [6].

Table 1: Molar ratios and concentrations of β-cyclodextrin and diphenyl carbonate

S. No.	Type of NS	Molar ratio (β-CD: DPC)	Concentration of β-cyclodextrin (g)	Concentration of diphenyl carbonate (g)
1	NS1	1:2	4.548	1.712
2	NS2	1:4	4.548	3.424
3	NS3	1:6	4.548	5.136
4	NS4	1:8	4.548	6.848
5	NS5	1:10	4.548	8.560

Fabrication of quercetin-loaded β-cyclodextrin nano-sponges

Quercetin loaded nanosponges were prepared by lyophilisation technique. 500 mg of nano-sponges were suspended in 100 ml of Milli Q water using a mechanical stirrer. To the above mixture, 500 mg of quercetin was added and the mixture was sonicated for 20 min to prevent aggregation. Then this mixture was kept under continuous stirring for specified time period. To separate the uncomplexed drug, the suspensions were centrifuged at 2000 rpm for 20 min. The colloidal supernatant was separated and freeze-dried using a lyophiliser at a temperature of-20 °C and pressure of 13.33 mbar. After lyophilisation the collected dry powder was stored in a desiccator [7].

Design of experiments

The Box-Behnken design (BBD) was used to prepare quercetin nano-sponges, which consisted of three independent variables: the molar ratio of polymer to the cross-linker (A), Stirring speed (B) and Stirring time (C). Box-Behnken design (BBD) with three factors at three levels was employed to optimize and evaluated the main, interactive and quadratic effects of influencing variables on response parameters. BBD is rotatable and requires at least three levels of each factor. BBD is appropriate to build second-order polynomial models and to describe the quadratic response surfaces [8]. The range of level of each independent variable was set according to the preliminary experiments and is listed in table 5. On the basis of the Box-Behnken design model provided by Stat-Ease Design Expert® software V8.0.1, 17 model experiments were randomly arranged (table 2 and 3).

Data analysis

The relationship between the selected factors and responses was described quadratic model based on a comparison of different statistical measures, including model p-value, multiple correlation coefficient (R2), adjusted R2 and coefficient of variation (CV) values. Quadratic model of each individual response parameter was evaluated using multiple regression analysis [8].

Table 2: BBD with list of dependent and independent variables with their respective levels and goals

Independent variables			Levels		
Variable		Units	Low	Intermediate	High
A	Molar ratio of polymer to cross-linker		0.2	0.5	0.8
B	Stirring speed	Rpm	2000	3500	5000
C	Stirring time	Min	350	450	550
Dependent variables			Goal		
Y1	Mean particle size	Nm	Minimize		
Y2	Encapsulation efficiency	%	Maximize		
Y3	Percent drug release at 6h	%	Minimize		

Table 3: Observed responses of trial experiments as per BBD

Expt	Molar ratio of polymer to cross-linker	Stirring speed (rpm)	Stirring time (min)	Mean particle size (nm)	Encapsulation efficiecny (%)	Percent drug release at 6h (%)
1	0.5	3500	450	68.25	90.82	60.28
2	0.2	5000	450	60.02	57.12	79.82
3	0.5	3500	450	69.49	90.18	60.92
4	0.5	5000	350	68.37	86.32	62.22
5	0.2	3500	550	63.14	57.88	79.12
6	0.5	2000	350	153.2	84.23	68.23
7	0.5	5000	550	52.27	93.21	60.54
8	0.5	2000	550	147.38	89.74	65.69
9	0.2	2000	450	153.42	53.44	83.11
10	0.8	2000	450	155.28	76.54	55.18
11	0.8	3500	550	81.77	79.82	54.11
12	0.5	3500	450	73.65	91.34	59.86
13	0.8	3500	350	79.21	76.56	54.45
14	0.8	5000	450	62.84	75.12	52.34
15	0.5	3500	450	69.33	90.88	59.76
16	0.5	3500	450	70.9	91.86	60.34
17	0.2	3500	350	89.68	54.13	81.05

Optimization

The nanoformulation was prepared in triplicate under optimal conditions to verify the validity optimization technique [9].

Characterization of prepared quercetin nano-sponges

Particle size, polydispersity index and zeta potential

The particle size distribution of quercetin nano-sponges were observed by dynamic light scattering method. The measurements were made at fixed angle of 90° for all samples. The samples were suitably diluted with Milli Q water before measurement. The mean hydrodynamic diameter (Dh) and polydispersity index (PI) of the particles were calculated using cumulant analysis after averaging three measurements. Zeta potential measurements were also made using an additional electrode in the same instrument. All the experiments were conducted in triplicate at 25±2 °C.

Drug payload and encapsulation efficiency

Encapsulation efficiency is the ratio of the weight of the drug entrapped into a carrier system to the total drug added. Drug

loading is the ratio of drug to the weight of total carrier system [10]. Weighed amount of quercetin-loaded nano-sponges complex was dissolved in methanol, sonicated for 10 min to break the complex, diluted suitably, and then analysed by UV spectrophotometer at 369 nm to determine the amount of quercetin present in the formulation. The "percent drug payload" and "percent drug encapsulation efficiency" were calculated using the following equation 1 and 2:

$$\%\ \text{Drug pay load} = \frac{\text{Weight of drug encapsulated in NS formulation}}{\text{Weight of the NS formulation taken for analysis}} \text{ X } 100 \ldots . (1)$$

$$\%\ \text{Drug encapsulation efficiency} = \frac{\text{Weight of drug encapsulated in NS formulation}}{\text{Initial weight of the drug fed for loading}} \text{ X } 100 \ldots . (2)$$

Transmission electron microscopy (TEM)

The morphology of the plain nano-sponges and quercetin-loaded nano-sponges was observed under transmission electron microscopy. One drop of diluted nanoparticle suspension was deposited on a film-coated copper grid and stained with one drop of 2% (w/v) aqueous solution of phosphotungstic acid and then allowed to dry for contrast enhancement. The samples were examined at a magnification of 45000× by transmission electron microscopy.

Fourier-transformed infrared (FTIR) spectroscopy

The FTIR spectra of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and Quercetin loaded nano-sponges were carried out by potassium bromide disc method using Tensor 27 FTIR Spectrophotometer in the region of 4000to 600 cm^{-1}.

Differential scanning calorimetry (DSC)

DSC of The FTIR spectra of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and Quercetin loaded nano-sponges were carried out using a Perkin Elmer DSC/7 differential scanning calorimeter (Perkin-Elmer, CT-USA) equipped with a TAC 7/DX instrument controller. The instrument was calibrated with indium for melting point and heat of fusion. A heating rate of 10 °C/min was employed in the 30-400 °C temperature range. Standard aluminum sample pans (Perkin-Elmer) were used; an empty pan was used as the reference standard. Analyses were performed in triplicate on 5 mg samples under nitrogen purge.

X-ray powder diffraction (XRPD)

X-ray powder diffraction patterns of Quercetin, plain nano-sponges and Quercetin loaded nano-sponges were recorded on X-ray diffractometer (Bruker D8 Advance) at a scan rate of 5°/min in the 2θ range from 2.5° to 60°.

Preparation of quercetin-loaded nano-sponges buccal tablets

An accurately weighed quantities of quercetin-loaded nano-sponges (250 mg) equivalent to 100 mg quercetin and the calculated Avicel PH-102, which was added to attain 300 mg tablet, were mixed for 10 min using mortar and pestle, after which the magnesium stearate (6 mg) was added and blended for another 2 min. The final mixtures were compressed using a single punch tablet machine with 8 mm, round, flat-faced single punch [11].

Evaluation of tablet formulation

Uniformity of weight

Twenty tablets were selected at random, individually weighed in a single pan electronic balance and the average weight was calculated. The uniformity of weight was determined according to the specifications of British Pharmacopoeia (BP 2013).

Hardness test

Hardness of the prepared tablets was measured using the tablet hardness tester (Monsanto). Three tablets were selected for testing and results were expressed in kg/cm^2.

Friability test

Friability test was done in a digital tablet friability tester apparatus, where the tablets were subjected to the combined effect of abrasion and shock by utilizing a plastic chamber that revolves at 25 rpm, while dropping the tablets at a distance of six inches with each revolution. Pre-weighed samples of 20 tablets were placed in the friability chamber, which was operated for 100 revolutions. At the end of the rotation, the tablets were removed from the drum, carefully brushed to free them from adhering dust and reweighed. Conventional compressed tablets lose less than 0.5–1.0% of their weight which is generally considered acceptable [13].

The percent friability (% F) is given by equation 3:

$$\%\ F = \frac{(1 - W_0)}{W} \times 100 \ldots . (3)$$

Where W_0 is the weight of the tablets before the test and W is the weight of the tablets after test.

Drug content

The Quercetin content of the prepared tablets was carried out according to the method mentioned previously. The triturated tablets were treated with ethanol for the extraction of the drug from nano-sponges. The sample was suitably diluted and drug content was estimated using UV spectrophotometer. The drug content from tablets was calculated using equation [11, 12].

Swelling test

From each batch, three tablets were individually weighed (W1) and placed separately in petri dishes with 5 ml phosphate buffer of pH 6.8. At the time interval of 1, 2, 4, and 8 h, they were taken out from the petri dish and excess water were removed by using filter paper. The swollen tablets were reweighed (W2) and the percentage of hydration was calculated for each tablet, using the Equation 4 as follows

$$\text{Swelling index} = \frac{W2 - W1}{W1} X100 \ldots . (4)$$

Measurement of adhesion force/Mucoadhesive strength

Measurement of adhesion force was determined by using bovine buccal mucosa, which was obtained from a slaughterhouse. The underlying tissues were separated and washed thoroughly with phosphate buffer solution (pH 6.8). The membrane was then tied to the bottom of the lower vial using rubber band. The vial was kept in a glass bottle, which was filled with phosphate buffer solution at 37±1 °C in such way that buffer just reaches the surface of mucosal membrane and kept it moist. The tablet to be tested was stuck on the lower side of the hanging Glass vial by using adhesive tape and the weight (2 gm) on the right pan was removed.

Ex-vivo residence time

The ex-vivo residence time was determined using a locally modified USP disintegration apparatus. The disintegration medium was composed of 900 ml (pH 6.8) of phosphate buffer maintained at 37±1 °C. The bovine buccal mucosa was tied to the surface of a wooden scale, vertically attached to the disintegration apparatus. The buccal tablet was hydrated using phosphate buffer (pH 6.8) and the hydrated surface was brought in contact with the mucosal membrane by keeping the backing membrane outside. The wooden scale allowed moving up and down, so that the tablet was completely immersed in buffer solution at the lowest point and was out at the highest point. The time taken for the complete displacement of the tablet from the mucosal surface was noted and repeated thrice.

***In vitro* release study of quercetin**

In vitro release of drug from pure quercetin drug, quercetin NS powder and quercetin NS loaded tablets was performed using the type II USP dissolution apparatus [12]. The dissolution medium was 900 ml of phosphate buffer pH 6.8 at a speed of 50 rpm and a temperature of 37±0.5 °C. The samples (5 ml) were withdrawn at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, and 24 h. Equal amount of the fresh dissolution medium, retained at the same temperature, was immediately replaced. The samples were suitably diluted and analysed using UC-spectrophotometer (Labindia UV-3000+, Labindia instruments Pvt. Ltd) at 369 nm. The dissolution experiments were conducted in triplicate.

Short-term stability studies

Stability studies of the optimized formulation was carried out according to ICH guidelines for 6 months. The stability of Quercetin buccal tablets was estimated after filling and sealing in light protective amber-colored bottles with rubber caps and aluminum covering. These were stored at three different temperatures and relative humidity (i.e., 25±2 °C, 60% RH±5%; 30±2 °C, 65% RH±5%; and 40±2 °C, 75% RH±5%) and were inspected visually and the samples were withdrawn at specified time points and were examined for appearance, hardness, disintegration time, dissolution, and drug content.

RESULTS AND DISCUSSION

Five types of nano-sponges were prepared using different molar ratios of reactants [13]. The percent practical yield, Particle size, polydispersity index and zeta potential were measured and are as presented in table 4. From the trials, the range of polymer to cross linker ratio (0.2-0.8), stirring speed (2000-5000 rpm) and stirring time (350-550 min) were identified. Based on the initial results, a Box-Behnken design was employed to optimize the influencing variables.

Mean particle size

Particle size determination is an important quality control measure to measure the ability of any nanoformulation. Size distribution is significant in terms of stability, solubility, dissolution and permeation through various tumour tissues and organs [14]. Particle size of the nanoformulation ranges from 36.45–135.27 nm. The polynomial model shown that all the variables (A, B and C) have a significant effect on the particle size of drug-loaded nano-sponges. The effect of individual variables on particle size was described by using perturbation plot (fig. 1). The variable B has main and major effect followed by C and A which have moderate effect on particle size. Three-dimensional response surface and plots and corresponding contour plots were used to describe the interactive effect of independent variables. The interactive effect of AB on particle size at a constant level of C is as shown in fig. 2a and 2b.

As the stirring speed increases the particle size was decreased. Likewise, as the cross-linking density increases the particle size reduced. Particle size was reduced as the stirring time was increased. The particle size was reduced at lower values of C and at higher values, C had an antagonist effect.

Table 4: The percent practical yield, particle size, polydispersity index and zeta potential of different nano-sponges

S. No.	Type of NS	Molar ratio (β-CD: DPC)	Practical yield (%)	Mean particle size (nm)	Polydispersity index	Zeta potential
1	NS1	1:2	77.64±2.76	111.96±3.52	0.251±0.005	-22.64±2.12
2	NS2	1:4	82.27±1.98	107.21±4.88	0.308±0.005	-25.16±1.13
3	NS3	1:6	85.82±3.12	115.67±3.42	0.262±0.005	-26.38±3.24
4	NS4	1:8	90.35±2.44	120.28±4.26	0.418±0.005	-23.02±1.74
5	NS5	1:10	92.48±1.89	99.33±2.48	0.270±0.005	-22.48±1.46

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

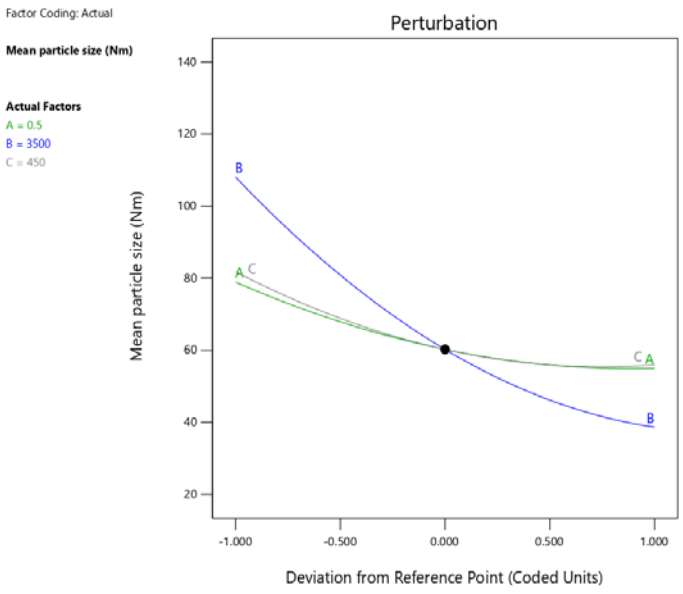
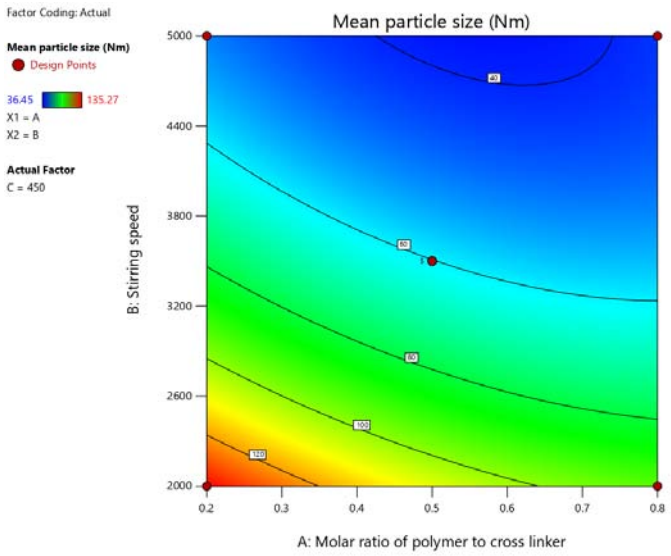


Fig. 1: Two-dimensional perturbation plot-effect of A, B and C on mean particle size

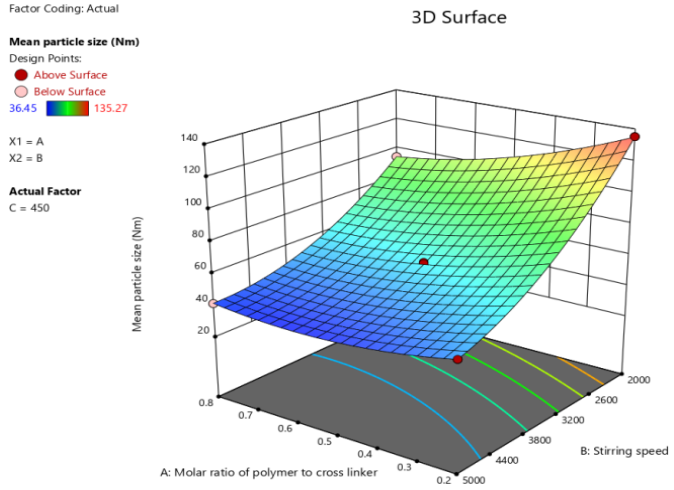
Encapsulation efficiency

Encapsulation of the drug in the nano-sponges can be important for enhancing the oral bioavailability and controlled release of the drug. Hence, the second part of the study was aimed to investigate the factors affecting drug encapsulation in the nano-sponges cavities. The encapsulation efficiency of nano-sponges was found to be in the range of 42.37 % to 88.44 % (table 2). The polynomial model shown that factors A, B and C have a significant effect on encapsulation efficiency.

The effect of individual variables on encapsulation efficiency was described by using a perturbation plot (fig. 3). Variable A has the main effect on encapsulation efficiency, followed by C and B, which have moderate and little effect, respectively. Three-dimensional response surface plot and corresponding contour plots were used to explain the interactive effect. The interactive effect of AC on encapsulation efficiency at a constant level of B is as shown in fig. 4a and 4b. The interactive effect of BC on encapsulation efficiency at a constant level of A is as shown in fig. 5a and 5b.



(a)



(b)

Fig. 2: (a). 2D-Contour plot showing the interactive effect of A and B on mean particle size at a constant level of C. (b). 3D-response surface plot showing the interactive effect of A and B on mean particle size at a constant level of C

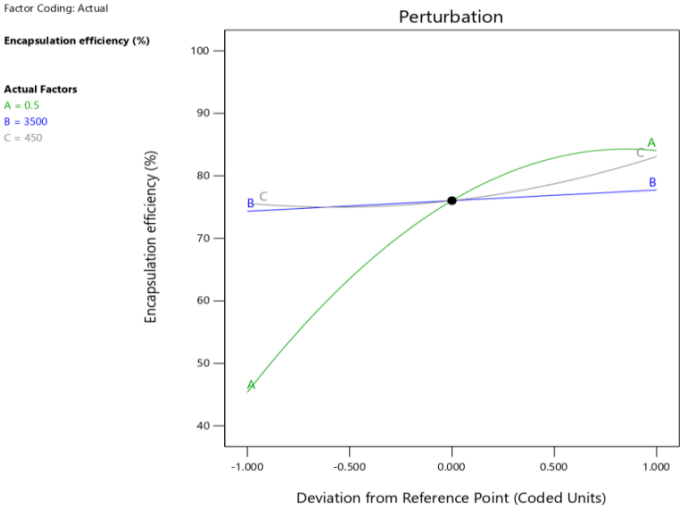
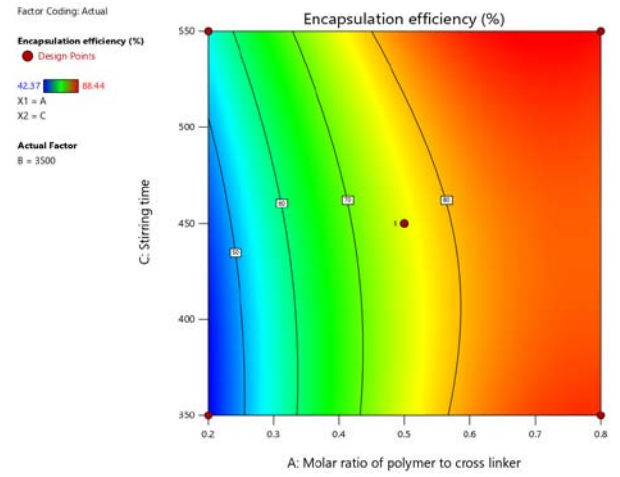
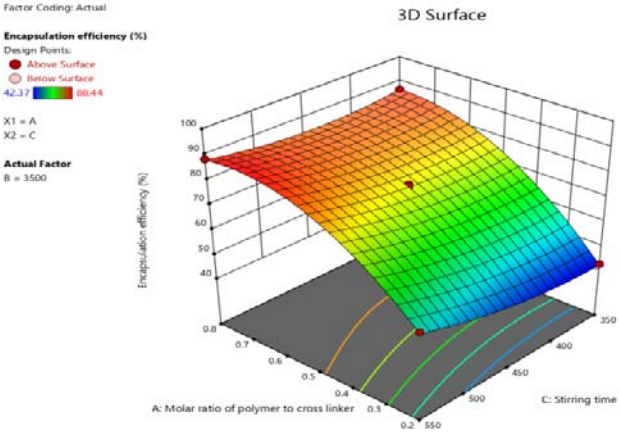


Fig. 3: Two-dimensional perturbation plot-effect of A, B and C on encapsulation efficiency

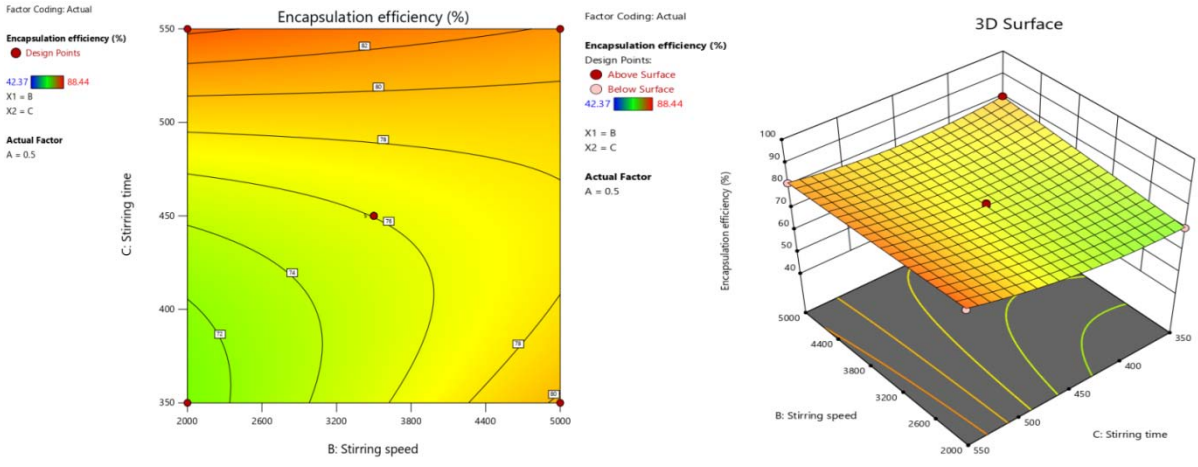


(a)



(b)

Fig. 4: (a). 3D-Contour plot showing the interactive effect of A and C on encapsulation efficiency at a constant level of B. (b). 3D-response surface plot showing the interactive effect of A and C on encapsulation efficiency at a constant level of B



(a) **(b)**

Fig. 5: (a). 3D-Contour plot showing the interactive effect of B and C on encapsulation efficiency at a constant level of A. (b). 3D-response surface plot showing the interactive effect of B and C on encapsulation efficiency at a constant level of A

Percent drug release at 6h

Percent drug release at 6h is an important measure to assess the ability of nano-sponges to control the release of the drug for a desired period of time. Percent drug release from the nano formulation ranges from 53.04-82.64 % (table 2). The polynomial model shown that only the variable A (Molar ratio) had a significant effect on the percent drug release from nano-sponges.

The effect of variable A on Y3 was described by using a perturbation plot (fig. 6).

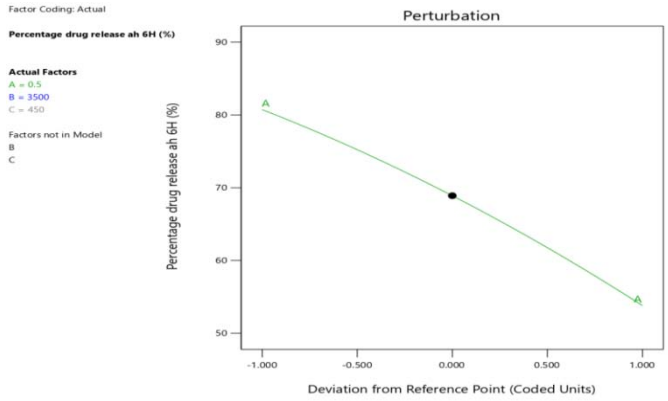


Fig. 6: Two-dimensional perturbation plot-effect of a on percent drug release at 6h

Optimization

Derringer's desirability function (D) was used to optimize the selected variables which influence the response parameters. All the responses (Mean particle size, Encapsulation efficiency and percent drug release at 6h) were transformed into a desirability scale. Y_{max} and Y_{min} were considered as the objective function (D) for each response parameter. At last, each individual desirability function was merged as a function of geometric mean by extensive grid and feasibility search over the domain to obtain the global desirability value using Design Expert® software. The extreme desirability function value obtained at A: 0.80, B: 4999.897 rpm and C: 524.844 min with the confirming D value of 0.985. To confirm the appropriateness of the model, three executive batches of cyclodextrin nano-sponges with molar ratio (0.800) were prepared and drug loading was carried out under optimal conditions. The response parameters for the prepared batches are as shown in table 5. A close agreement between predicted and experimental values demonstrates the validity of the experimental design (BBD) combined with derringer's desirability function for the optimization of quercetin nano-sponges [15].

Table 5: Optimum conditions attained by applying restrictions on response parameters

Independent variables	Optimized values	Predicted values			Actual values			
		Mean particle size (Y_1) Nm	Encapsulation efficiency (Y_2) %	Percent drug release at 6h (Y_3)	Batch	Mean particle size (Y_1) nm	Encapsulation efficiency (Y_2) %	Percent drug release at 6h (Y_3)
Molar ratio of polymer to cross linker	0.80	36.831	85.991	53.813	F1	40.62±4.62	87.06±1.67	55.50±1.28
Stirring speed	5000				F2	46.39±4.19	86.27±2.49	56.04±2.17
Stirring time	525 min				F3	48.21±2.50	87.60±1.28	56.75±1.05

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

Morphology and sizes of the quercetin-loaded nano-sponges

The particle size analysis of quercetin-loaded nano-sponges revealed that the average particle size measured by laser light scattering method is around 40-50 nm with low polydispersity index. The particle size distribution is unimodal and having a narrow range as seen in the table 6. A narrow polydispersity index means that the colloidal particles are homogenous in nature. A sufficiently high zeta potential indicates that the complexes would be stable and the tendency to agglomerate would be minuscule. The entire formulations prepared were found to be fine and free-flowing powders. The particle size of the complexes as observed under TEM was consistent with the data obtained through dynamic light scattering. The percent drug loading and encapsulation efficiency of prepared quercetin nano-sponges were determined and are presented in table 6.

Table 6: Particle size, polydispersity index and zeta potential of plain nano-sponges and drug-loaded nano-sponge formulation

Sample	Mean particle size±SD (nm)	Polydispersity index	Zeta potential (mV)	Drug payload	Encapsulation efficiency
Plain NS	108.24±3.67	0.30±0.005	-21.37±1.12	-	-
F1	41.36±4.32	0.44±0.005	-20.7±1.62	48.15	87.88±1.08
F2	46.9±3.72	0.12±0.005	-23.04±1.74	49.37	86.73±1.65
F3	48.72±4.51	0.32±0.005	-24.68±1.19	48.02	87.64±3.27

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

Characterization of cyclodextrin nano-sponges

Fig. 7 shows a comparison of FTIR spectra of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and Quercetin loaded nano-sponges. FTIR spectra of free drug had characteristic peaks at O-H stretch at 3416.05 cm^{-1}, =C-H stretch at 2928.04 cm^{-1}, conj C=O stretch at 1656.91 cm^{-1}, aromatic C=C stretch at 1500.67 cm^{-1} and 1602.90 $cm^{-1}$and aromatic C-O stretch at 1234.48 cm^{-1}. Plain nano-sponge showed a characteristic peak of carbonate bond at around 1540–1650 cm^{-1} which confirms the formation of cyclodextrin-based nano-sponges. Other characteristic peaks of nano-sponges were found at 2918 cm^{-1} due to the C–H stretching vibration and 1026 cm^{-1} due to C–O stretching vibration of primary alcohol. The FTIR spectra of physical mixtures indicated all the peaks of the drug along with some additional peaks of polymers. The Comparison of FTIR spectra of quercetin and quercetin complex showed that there is a

major change in the fingerprint region 3 i.e., 900 to 1,700 cm^{-1}. The main characteristic peaks of quercetin were disappeared in the formulations suggesting definite interactions between quercetin and nano-sponges [16].

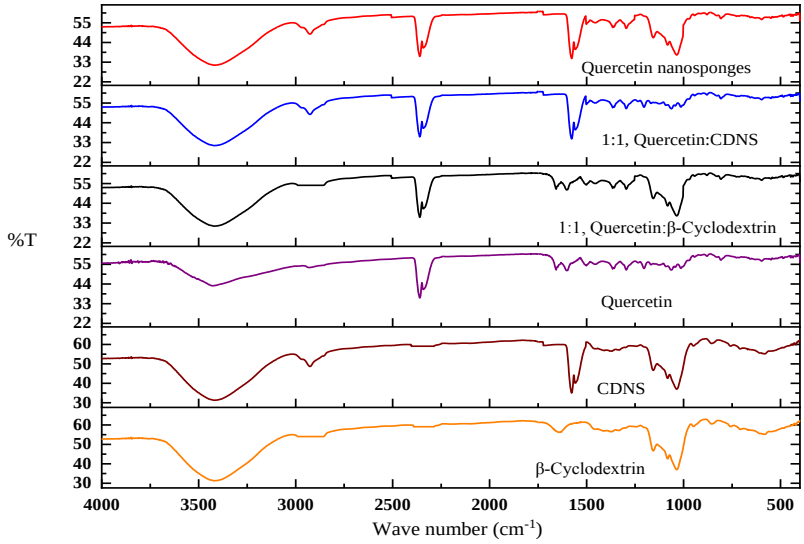


Fig. 7: FTIR spectra of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and Quercetin loaded nano-sponges

Differential scanning calorimetry curves of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and Quercetin loaded nanosponges are displayed in fig. 8. The DSC thermogram of free drug shows a sharp melting point at approximately 180.08 °C indicating the crystalline nature of the drug. The DSC thermogram of plain nano-sponges (NS2) showed exothermic peaks at around 350 °C. Quercetin nano-sponge complex also exhibited a broad exothermic peak at around at 350 °C. The complete disappearance of the quercetin endothermic peak was observed for the formulation. This phenomenon can be assumed as proof of interactions between the components of the formulation. This can be considered as indicative of drug amorphization and/or inclusion complex formation.

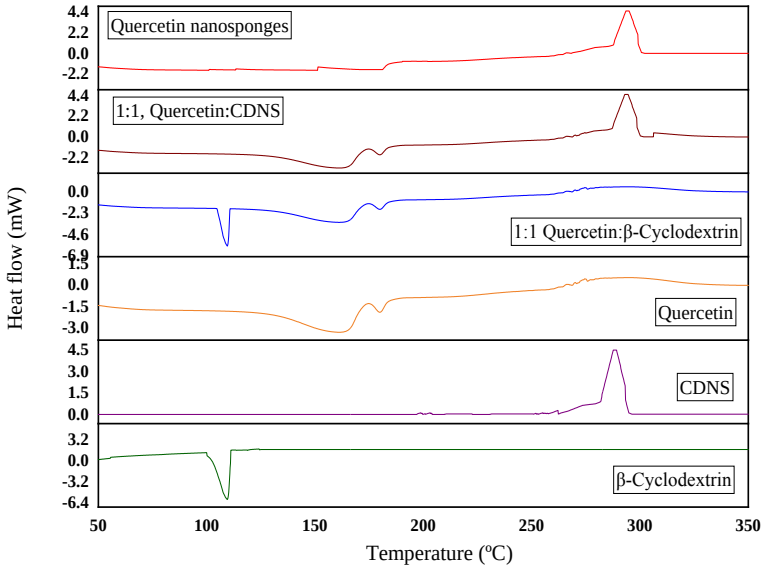


Fig. 8: DSC thermograms of β-Cyclodextrin, plain nano-sponges, Quercetin, Physical mixture and quercetin loaded nano-sponges

To study the physical nature of quercetin with in the cyclodextrin nano-sponges, XRD pattern of pure quercetin, plain nano-sponges (NS2) and quercetin-loaded nano-sponges (QNS2) were investigated. The x-ray diffractograms of plain quercetin exhibited sharp intense peaks at 2θ values of 5.417, 7.471, 10.712, 14.843, 15.232, 17.059, 22.428, 26.574 and 27.05confirming the drug's crystal form as shown in fig. 9. However, there were no characteristics peak of pure quercetin were observed in NS complexes. The absence of such crystalline peaks of quercetin in nano-sponge complex clearly indicates that the drug is encapsulated in nano-sponges. FTIR, DSC and XRD studies confirmed the formation of the inclusion complex of quercetin with nano-sponges. The freeze-drying process gives rise to fluffy mass powder showing a highly porous structure losing all its crystallinity which was confirmed by XRPD study.

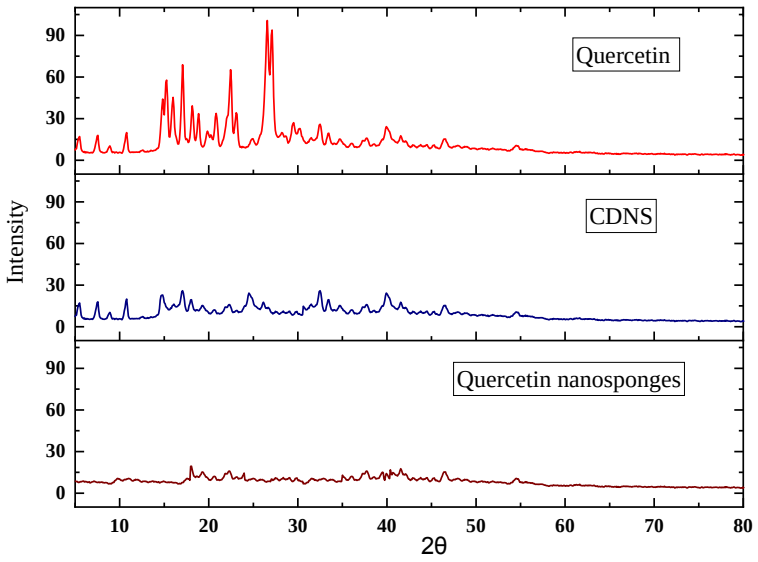


Fig. 9: XRPD pattern of quercetin, plain nano-sponges (NS2) and quercetin-loaded nano-sponge complexes (QNS)

Preparation of quercetin loaded nano-sponges buccal tablets

The mean weight ranged from 300.46 mg±2.27 to 301.97 mg±3.56. The mean thickness ranges from 4.95 mm±0.46 to 5.19 mm±0.31. The mean hardness ranges from 5.31 kg/cm²±0.38 to 5.45 kg/cm²±0.49. The mean friability values range from 0.51 %±0.24 to 0.79 %±0.18 and the average percentage drug content ranges from 98.84%±1.76 to 99.61±1.19, as shown in table 7 [17, 18].

T2 was discovered to have the greatest swelling index (table 8). The surface pH values ranged from 6.5 to 6.6, indicating that all of the formulations give an acceptable pH in the salivary pH range of 5 to 7 (table 9) [19].

Buccal pills had mucoadhesion of 19.26, 20.46, and 22.95 g, respectively (table 9). Buccal tablet residence times varied from 6.4 to 6.7 h, indicating that buccal tablets take this long to remove from the buccal mucosa.

Table 7: Evaluation parameters of quercetin tablets

Formulation	Weight (mg)	Thickness (mm)	Hardness (kg/cm²)	Friability (%)	Drug content (%)
T1	300.46±2.27	4.95±0.46	5.31±0.38	0.51±0.24	98.84±1.76
T2	301.97±3.56	5.06±0.77	5.45±0.49	0.67±0.52	99.61±1.19
T3	300.62±4.27	5.19±0.31	5.38±1.32	0.79±0.18	99.22±2.61

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

Table 8: Swelling index of quercetin nano-sponges loaded buccal tablet

Formulation no	Time (h)					
	1	2	3	4	5	6
T1	50.62	52.91	59.88	61.81	65.75	68.21
T2	65.21	68.65	71.22	74.38	78.60	84.64
T3	52.34	55.29	58.69	62.26	69.27	71.49

Table 9: Surface pH, Mucoadhesive strength and ex-vivo residence time of quercetin nano-sponges loaded buccal tablet

Formulation code	Surface pH	Mucoadhesive strength (g)	Ex-vivo residence (h)
T1	6.6±0.02	19.26±0.62	6.4±0.72
T2	6.5±0.04	20.46±0.76	6.5±0.33
T3	6.6±0.06	22.95±0.27	6.7±0.85

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

***In vitro* release study**

The dissolution profiles of pure drug suspension quercetin and from different formulations of quercetin nano-sponges powder and quercetin nano-sponges buccal tablet (fig. 10). A biphasic release pattern of quercetin from the prepared nanospongesbuccal tablets was observed. The initial burst release was ranged from 17.64 % of drug within 1 h, followed by sustained release of the drug for 24 h. The percent of quercetin released from nano-sponges buccal tablets after 24 h was 99.75 % [20].

Short-term stability studies

Stability study's results indicated that the there was no significant change in the visual appearance, hardness, disintegration time, dissolution and drug content as shown in table 10. [21-23].

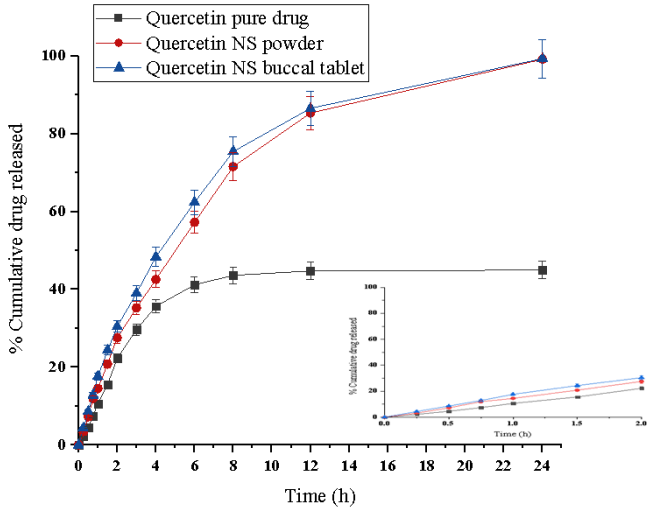


Fig. 10: *In vitro* release of quercetinpure drug, quercetin NS powder and quercetin NS buccal tablet, All determinations were performed in triplicate and values were expressed as mean±SD, n=3

Table 10: Results of stability studies of the quercetin tablets (T2)

Condition	Days	Appearance	Hardness	Percent dissolution	Drug content
25±2 °C, 60%±5 % RH	0	White	5.45±0.49	99.75±4.99	99.61±1.19
	90	White	5.22±0.67	99.66±0.88	99.55±0.74
	180	White	5.07±0.46	99.52±2.32	99.41±0.63
30±2 °C, 65%±5	0	White	5.45±0.49	99.75±4.99	99.61±1.19
	90	White	5.31±0.56	99.68±3.16	99.52±0.76
	180	White	5.06±0.34	99.59±2.98	99.41±0.69
40±2 °C, 75%±5	0	White	5.45±0.49	99.75±4.99	99.61±1.19
	90	White	4.66±0.18	99.69±0.44	99.52±0.59
	180	White	4.54±0.20	99.65±1.75	99.43±0.15

All determinations were performed in triplicate and values were expressed as mean±SD, n=3

CONCLUSION

The freeze-drying process was used to prepare quercetin-loaded nano-sponges in this investigation. The creation of a quercetin inclusion complex with nano-sponges was confirmed by FTIR, DSC, and XRD analyses. Because of the smaller drug particle size, the creation of a high-energy amorphous state, and intermolecular hydrogen bonding, the dissolution of the quercetin nano-sponges was much higher than that of the pure drug. Depending on the type of nano-sponges, the release kinetics can be slowed or sped up. The relative dissolution rate of quercetin nano-sponges buccal tablets was found to be 99 percent when compared to pure drug quercetin. As a result, cyclodextrin-based quercetin nano-sponges buccal tablets offer a viable drug delivery mechanism for oral administration. Altogether, this study showed that cyclodextrin nano-sponges can be used to increase the physicochemical characteristics, oral bioavailability, and therapeutic efficacy of the anticancer medication quercetin.

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Nil

AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICTS OF INTERESTS

Declare none

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