OPTIMIZATION OF QUERCETIN LOADED FAST DISSOLVING FILMS BY EMPLOYING QBD AS A DESIGNING TOOL FOR IMPROVED BIOAVAILABILITY

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Abstract:
Quercetin was found to be BCS class-4 molecule with desired cancer preventive activity but characterized by lower solubility and permeability. To overcome the issues associated with Quercetin, the current research was designed for the development of cyclodextrin based inclusion complexes loaded orally fast dissolving films (ICQF). For the preparation of ICQFs, the film former, plasticizer and the super disintegrant was selected as methyl cellulose, PEG 400, either croscarmellose sodium/ starch citrate respectively. To develop the formulation with desired properties, box behnken design of quality by design (QbD) was employed and the same was analyzed to find the optimized combination of different variables. The ICQFs were evaluated for their physical characterization, drug content and disintegration time of the ICQFs were observed to be in the range of 0.13-0.27 mm, 447-663, 6.6-10.4 MPa, 20.4-30.7%, 96.3-102.4% and 32-312 sec. respectively. Along with the physico chemical characterization, the ICQFs were evaluated for its dissolution and pharmacokinetic behavior. The effect of different factors were studies on the desired responses like disintegration time and dissolution after 10 minutes (D_{10}). From the graphical optimization, a fresh formulation was prepared with 500 mg of film former, 1.16% v/v of plasticizer and 7.5% of super disintegrant. The DT and D_{10} for the optimized formulation was found to be 32.9 sec. and 82.4% respectively. Along with the *in-vitro* performance, the *in-vivo* behavior was also evaluated by performing the pharmacokinetic study using male Sprague–Dawley (SD) rats and the bioavailability of the formulation found to be improved in comparison to the plain drug suspension. The investigation was found to be innovative with successful attainment of the hypothesis.

Keywords: Quercetin, BCS class, inclusion complex, Quality by Design (QbD), Disintegration time, Dissolution, Box- Behnken design, bioavailability.

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INTRODUCTION:

Oral administration is the most desirable and convenient route for the patients by having its own advantages like non-invasive, ease of single time as well as repeated drug administration, and cost-effective. Effective delivery of medicine through oral route may depends on many factors like gastric emptying rate, gastrointestinal transit time, drug release from formulation and site of absorption of drug etc. Along with the advantages oral route is also having the disadvantages like first pass metabolism, unacceptable taste and poor bioavailability due to its limited solubility and permeability by majority of the drugs [1]. So, as to overcome these disadvantages it is required to design the novel dosage forms with improved bioavailability and patient compliance. In the current work we have engrossed on the development of orally fast dissolving films by taking Quercetin as a model drug.

Quercetin is a flavonoid with chemical name 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, having rich anti-oxidant properties which helps in preventing the formation of cancer cells. Quercetin is also having the anti-inflammatory and anti-allergic effect along with the anti-aging and prevention of heart diseases [2]. Especially Quercetin gain attention of researchers for its rich therapeutic activities but having poor bioavailability due to its low solubility and permeability. Till date many formulations were developed based on different solubility enhancement techniques such as co-crystallization, co-solvency, solid dispersion, cyclodextrin complexes, nano-suspensions etc. to enhance their bioavailability [3].

Among the various solubility enhancement techniques cyclodextrin complexes are attaining the attraction due to their unique advantages like high solubility, tendency to encapsulate the lipophilic drugs by having lipophilic core [4]. There are different types of cyclodextrins available based on the presence of number of rings. Among all those the ß-form with 7 ringed structure was the most suitable for pharmaceutical dosage form development by having the ease of availability, lesser cost and ability to encapsulate both hydrophobic and lipophilic drugs. Along with the functional advantages, cyclodextrins can also enhance the organoleptic properties of the drug such as taste masking, moisture protection, improving the elegant appearance of finished product etc.

The current research work focused on the development of cyclodextrin inclusion complex based orally fast dissolving films to improve the bioavailability of Quercetin which belongs to the BCS class-4. Quality by design was used a quality tool to develop the formulation with desired responses and quality [5]. During this development process we had chosen our desired outcomes of the formulation as quality target product profiling (QTPP) followed by the selection of factors which can have the impact on the QTPP. These factors were termed as Critical quality attributes (CQA) which will have a direct impact on the QTPP and the changes in CQA will have direct impact on the desired response i.e. QTPP. The process or formulation variables were termed as critical process parameters (CPPs) which will have the capability to alter the CQA there by the QTPP of the formulation. To optimize the CPP and CQA to have the best formulation with desired QTPP, Box-behnken design was selected and the same was graphically optimized to find its suitability of the selected model [6]. The formulated films were quality tested for their physicochemical properties and *in-vivo* behavior.

Materials and Methods:

Quercetin was sample gifted from Hetero labs, methyl cellulose, PEG 400, Hydroxy propyl ß-cyclodextrin was purchased from Merck, croscarmellose sodium was obtained as a gift sample from BASF and starch citrate was purchased from vertex chemicals. All the chemicals used in the process are of analytical grade.

Development of Quercetin cyclodextrin complex based fast dissolving films (ICQF):

The ICQFs were developed using Box-Behnken design (BBD) [7] of StatEase design expert software. The quality elements of the formulation were selected based on the prior experience and the CQAs and CPPs were selected and their impact on the quality of the product were studied.

Quality Target Product Profiling (QTPP)

The formulated ICQFs should disintegrate and dissolve immediately to facilitate the faster absorption in the oral cavity.

Critical Quality Attributes (CQA)

The CQA will have the ability to impact the QTPP directly. As the performance of the film depends on the disintegration time and dissolution, in the current research disintegration time (DT) and

dissolution after 10 minutes (D_{10}) of the film were selected as CQA.

Critical Process/Formulation Parameters (CPPs)

Upon thorough literature study, the amount of the film former (Methyl Cellulose A4CP grade), concentration of the plasticizer (PEG 400), concentration of super-disintegrant (SDis.) and the weight of solids were selected as CPPs.

Experimental Design

BBD was selected as the suitable model to develop the ICQFs and the same was optimized by desirability functions approach to identify the design space (combination of the optimum levels of the factors to get desired CQA values). The blends of the selected factors and their levels as different formulations according to this BBD are presented in the Table 1.

Preparation of the ICQFs

The ICQFs were developed using the optimized cyclodextrin complexes of Quercetin. Briefly the combination 60% w/w HP-β-CD and 5% w/w tween 80 by solvent evaporation method and the same were incorporated into fast dissolving films by taking 100 mg of Quercetin equivalent cyclodextrin complexes. The films were formulated with the solvent casting method [8] using the petri plates. Briefly the film former methyl cellulose followed by super-disintegrants, plasticizer and quercetin loaded cyclodextrins were added to the solvent mixture prepared with water and methanol under continuous stirring until the formation of homogeneous mixture. The formed mixture was poured into the petri plate and dried.

Table 1: Blends of the factors and levels for different formulations of fast dissolving oral films of Quercetin according to the BBD.

Std. Order	Run Order	Formulation code	Factor A: Film former (mg)	Factor B: Plasticizer (% v/v)	Factor C: Super disintegrant conc. (% w/w)	Factor D: Type of Super disintegrant
1	17	QFC1	400.00	0.50	5.00	CCS
5	10	QFC2	400.00	1.00	2.50	CCS
7	25	QFC3	400.00	1.00	7.50	CCS
3	5	QFC4	400.00	1.50	5.00	CCS
9	12	QFC5	600.00	0.50	2.50	CCS
11	8	QFC6	600.00	0.50	7.50	CCS
13	13	QFC7	600.00	1.00	5.00	CCS
10	26	QFC8	600.00	1.50	2.50	CCS
12	9	QFC9	600.00	1.50	7.50	CCS
2	22	QFC10	800.00	0.50	5.00	CCS
6	1	QFC11	800.00	1.00	2.50	CCS
8	2	QFC12	800.00	1.00	7.50	CCS
4	4	QFC13	800.00	1.50	5.00	CCS
14	3	QFS1	400.00	0.50	5.00	SC
18	6	QFS2	400.00	1.00	2.50	SC
20	11	QFS3	400.00	1.00	7.50	SC
16	20	QFS4	400.00	1.50	5.00	SC
22	18	QFS5	600.00	0.50	2.50	SC
24	7	QFS6	600.00	0.50	7.50	SC
26	14	QFS7	600.00	1.00	5.00	SC
23	23	QFS8	600.00	1.50	2.50	SC
25	21	QFS9	600.00	1.50	7.50	SC
15	19	QFS10	800.00	0.50	5.00	SC
19	16	QFS11	800.00	1.00	2.50	SC
21	15	QFS12	800.00	1.00	7.50	SC
17	24	QFS13	800.00	1.50	5.00	SC

Different formulations were made as suggested by the BBD. To develop the ICQFs, four variables i.e. quantity of film former, concentration of plasticizer, concentration of super disintegrants and type of super disintegrants were selected and different levels of the same factors were evaluated and the same formulation was characterized for their physical and chemical parameters.

Physicochemical evaluation of ICQF

Thickness

It was measured by screw gauge at five different positions of the films preferably at the four corners and center. The thickness of the film was measured only at the position without any breaks and air bubbles to ensure the correctness of the uniformity of film thickness.

Surface morphology

It was examined using the scanning electron microscopy (SEM) [9]. The ICQF samples were fixed with double sided carbon tape and gold coated sputter on to aluminum stubs and the samples were analyzed at 5kV excitation voltage.

Folding Endurance

The brittleness of the film can be determined using folding endurance. Briefly the folding endurance of the film can be determined using the repeated folding of the 2*2cm film until a crack or break observed. The ability of film to retain its integrity while folding can be termed as its folding endurance [10].

Tensile strength and % Elongation

These parameters were estimated using texture analyzer [11]. The tensile strength was obtained by dividing the maximum load at which the film breaks by the initial cross sectional area of the film and hence it was expressed as the force per unit area (MPa). The distance between the tensile grips before (D1) and after (D2) fracture of the film was used to calculate the percentage elongation with below mentioned formula.

$$\% \text{ Elongation} = (D2 - D1)/D2*100$$

Assay/ Drug Content

A piece of ICQF with an area of 15.2 cm^2 was dissolved in phosphate buffer pH 6.8 in a beaker and filtered. Later the volume of the filtrate was made up to 100 mL in a volumetric flask with the buffer. Then the solution was filtered, diluted suitably and quantified for Quercetin content using UV-Visible spectrophotometry [12].

Disintegration

Petri dish method was used to estimate the disintegration time. Phosphate buffer pH 6.8 of volume 6 mL was transferred into the petri dish and the cut films of 15.2 cm^2 were positioned in it. The films were observed for complete dispersion and the time was noted when it happened. This time was taken as disintegration time [13].

***In vitro* Dissolution**

Dissolution studies were carried out in USP-II apparatus, paddle method. 300 ml of phosphate buffer or acidic buffer (pH 6.8) was placed in the vessel and maintained at 37±0.5°C. the paddle was set to rotate at 50 rpm. A piece of ICQF with area 15.2 cm^2 (2.2 cm radius) was added into the vessel. Dissolution sample volume of 5 mL were withdrawn at regular time intervals and the same volume was replaced with fresh buffer [14]. The taken samples were quantified for the amount of Quercetin dissolved using UV-Visible spectrophotometry. Then the obtained data analyzed for dissolution kinetic of the ICQFs.

Optimization and Validation of the selected model:

The suitability of the selected model to understand the impact of the factors on the responses and to identify whether the factors have significant effect on the responses was studied by ANOVA test [15]. Upon successful validation, optimization was performed using desirability functions approach. The desirability criteria were set as minimizing the DT and maximizing the D10 so that the disintegration and dissolution rate of QUE would be improved towards achieving high bioavailability [16]. Optimization was performed and the best formation of the factors having maximum desirability was identified. All this validation and optimization was done using the Design Expert software.

***In vivo* Pharmacokinetic Studies**

In-vivo pharmacokinetic study was conducted for the ICQFs using male Sprague–Dawley (SD) rats, weighing around 250 – 300 g. All the animals except one Group will be administered with required amount of the dose (50mg/kg) in the form of aqueous dispersion through oral route. After administration, the blood samples will be withdrawn from the lateral saphenous vein of the rats at designated time points and transferred into heparinized containers. During the study, the animals are supplied with water to maintain the fluid balance. After 12 hours, the animals will be injected with sufficient fluids and subjected to rehabilitation and revival [17].

The blood samples were analyzed to determine the plasma Quercetin concentration. The obtained data were subjected to non-compartmental analysis[21] for the estimation of pharmacokinetic parameters of Quercetin using PK Solver software.

RESULTS AND DISCUSSION

The ICQFs were prepared using the solvent casting method using the BBD as a quality tool. The manufacturing formulae suggested by the model is shown in table 1.

Physicochemical characterization of ICQFs:

The physico chemical evaluation tests like thickness, folding endurance, tensile strength, % elongation, assay and disintegration time was performed for the manufactured ICQFs and the results are presented in Table 2.

Table 2: Physico-chemical characterization results of ICQFs

Formulation code	Thickness (mm)	Folding endurance	Tensile strength (MPa)	Elongation (%)	Drug content (%)	DT (sec.)
QFC1	0.13 ± 0.02	462 ± 29	6.8 ± 0.2	21.3 ± 1.6	97.5 ± 2.3	86 ± 9
QFC2	0.15 ± 0.01	514 ± 36	7.8 ± 0.5	24.2 ± 2.4	99.2 ± 0.8	219 ± 11
QFC3	0.16 ± 0.03	497 ± 17	7.5 ± 0.3	23.8 ± 2.1	96.8 ± 1.3	58 ± 4
QFC4	0.18 ± 0.04	572 ± 24	8.4 ± 0.2	25.3 ± 2.5	98.5 ± 2.4	187 ± 12
QFC5	0.15 ± 0.02	504 ± 33	7.6 ± 0.1	23.7 ± 0.9	102.4 ± 1.1	124 ± 8
QFC6	0.16 ± 0.05	523 ± 16	7.8 ± 0.4	24.4 ± 1.3	97.9 ± 3.2	45 ± 3
QFC7	0.18 ± 0.03	571 ± 28	8.9 ± 0.6	26.1 ± 1.9	96.4 ± 1.5	137 ± 12
QFC8	0.19 ± 0.02	614 ± 41	9.7 ± 0.2	28.3 ± 2.1	99.7 ± 0.6	231 ± 15
QFC9	0.19 ± 0.02	632 ± 38	10.1 ± 0.7	29.6 ± 0.7	101.2 ± 1.4	119 ± 7
QFC10	0.18 ± 0.04	531 ± 24	7.9 ± 0.4	24.3 ± 1.2	98.8 ± 1.7	134 ± 10
QFC11	0.19 ± 0.01	582 ± 22	8.6 ± 0.5	25.7 ± 2.5	100.5 ± 0.8	296 ± 18
QFC12	0.20 ± 0.02	569 ± 37	8.3 ± 0.1	25.1 ± 1.7	98.1 ± 1.2	165 ± 9
QFC13	0.22 ± 0.04	647 ± 43	10.2 ± 0.3	29.9 ± 3.1	99.4 ± 0.9	312 ± 21
QFS1	0.14 ± 0.01	447 ± 22	6.6 ± 0.4	20.4 ± 2.6	96.7 ± 1.3	51 ± 6
QFS2	0.17 ± 0.02	521 ± 16	7.9 ± 0.8	24.1 ± 2.2	100.2 ± 0.5	103 ± 7
QFS3	0.18 ± 0.02	469 ± 27	7.2 ± 0.6	21.7 ± 0.5	101.6 ± 1.2	37 ± 3
QFS4	0.21 ± 0.03	591 ± 15	8.7 ± 0.3	25.6 ± 2.4	97.9 ± 2.4	94 ± 4
QFS5	0.18 ± 0.01	482 ± 19	7.2 ± 0.5	23.2 ± 1.9	96.8 ± 1.5	76 ± 6
QFS6	0.18 ± 0.02	463 ± 25	6.7 ± 0.1	20.8 ± 2.3	102.1 ± 0.6	32 ± 3
QFS7	0.20 ± 0.03	519 ± 13	8.0 ± 0.2	24.7 ± 3.0	100.7 ± 1.1	82 ± 10
QFS8	0.23 ± 0.01	552 ± 24	8.2 ± 0.2	25.3 ± 1.5	96.3 ± 2.4	119 ± 12
QFS9	0.24 ± 0.05	574 ± 34	8.3 ± 0.4	25.6 ± 2.1	97.5 ± 1.3	61 ± 8
QFS10	0.19 ± 0.04	526 ± 38	7.9 ± 0.3	24.9 ± 0.6	99.3 ± 1.5	78 ± 5
QFS11	0.21 ± 0.02	609 ± 14	9.1 ± 0.6	28.7 ± 3.2	98.1 ± 2.0	133 ± 9
QFS12	0.23 ± 0.03	622 ± 31	9.6 ± 0.4	29.2 ± 1.8	96.6 ± 2.6	85 ± 7
QFS13	0.27 ± 0.01	663 ± 27	10.4 ± 0.5	30.7 ± 1.5	98.9 ± 1.3	142 ± 11

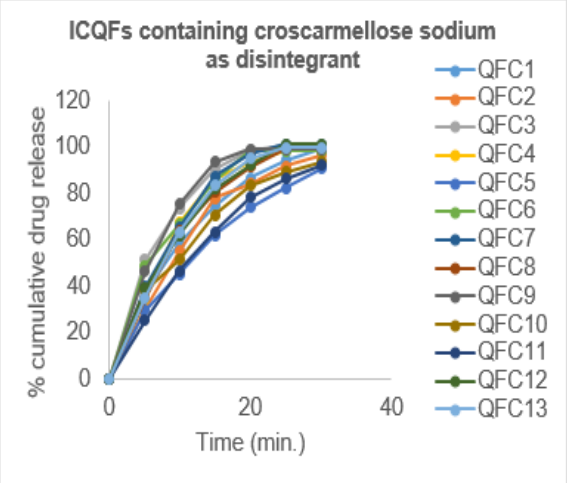
The ICQFs were inspected visually using SEM and were found to be uniform, clear, smooth and transparent in nature. Thickness of the film was evaluated and the results were found to be in the range of 0.13 – 0.27 mm. The impact of the film former and plasticizer were also studied on the thickness of the film and it was found that, the thickness of the film found to be increased upon increasing the film former which might be due to the formation of high viscous casting solution with increment in film forming agent. It was also found that the thickness was increased upon increment in plasticizer concentration which might be due to the intercalation of the plasticizer into the polymer network [18].

Tensile strength, % elongation and the folding endurance will help in understanding the flexibility and elasticity of the film which will hinder the breakage of the film while handling. The tensile strength of the film was found to be in the range of 6.8 – 10.4 MPa, and it was also observed that it is depending on the concentration of plasticizer and film former. With increment in plasticizer i.e. PEG400, the tensile strength, % elongation was found to be improved whereas the brittleness was found to be reduced, which might be due to interaction of plasticizer with the methyl cellulose polymer by breaking its original interactions [19].

The ICQFs were evaluated chemically for its total drug content and disintegration to understand its behavior at in-vivo conditions. The total drug content of the film was estimated for each formulation at various locations of the film and the mean values were observed to be in the range of 96.3- 102.3%, which is indicating the uniform distribution of the drug throughout the film. As the disintegration time will have direct impact on the dissolution and absorption of the film, it was also evaluated at very initial stage to pick up the best film for further tests [20]. The disintegration time was found to be in the range of 32 – 312 sec. To understand the impact of different variables on the disintegration time of the film, it was selected as one of the CQA. This uniform drug content and disintegration results, excellent physical properties of the film indicating that the selected solvent casting method was found to be effective and the same process can be utilized to produce uniform, clear and smooth films for further evaluation tests.

Dissolution studies of ICQFs:

Time versus % Quercetin dissolved data are displayed as dissolution profiles in Figure 1.



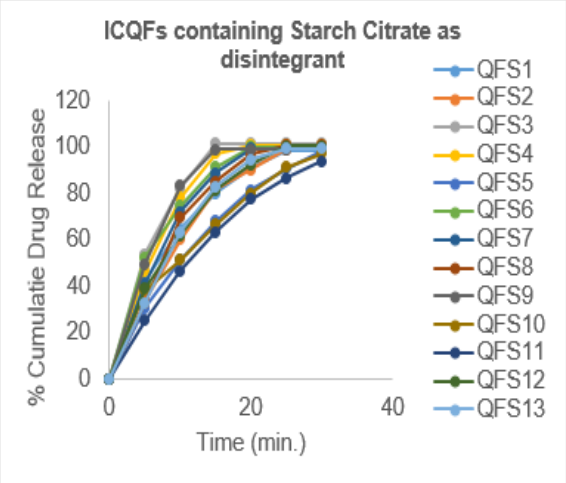


Fig 1: (a) Dissolution profile of ICQFs containing disintegrants as croscarmellose sodium

(b) Dissolution profile of ICQFs containing disintegrants as starch citrate

Dissolution studies were performed for the formulated ICQFs and it was observed that almost all the formulations were able to release the total drug content within 30 minutes. As the dissolution of the film directly impact the behavior of the film, it was also considered as one of the CQA and decided to evaluate the impact of different variables on it.

Analysis of QbD model:

The box behnken model was analyzed using sequential model square of analysis [21] on desired responses.

DoE analysis of response factor-1 (R1): Disintegration time:

As the disintegration time has a major role in the performance of the formulated ICQF, it was selected as one of the response factor and the same was analyzed with sequential sum of square analysis followed by ANNOVA and the results were displayed in table 3 and figure 2

Table 3: ANOVA test for response surface quadratic model for the DT (R1)

Source	**SS[a]**	**Df[b]**	**MSS[c]**	**F value**	**p-Value**	**Inference[d]**
Model	$1.332x10^5$	13	10244.74	24.59	< 0.0001	**Significant**
A: FF	16256.25	1	16256.25	39.02	< 0.0001	**Significant**
B: Plasticizer Conc.	25520.06	1	25520.06	61.25	< 0.0001	**Significant**
C: SDis Conc.	30537.56	1	30537.56	73.29	< 0.0001	**Significant**
D: Type of SDis.	40015.38	1	40015.38	96.04	< 0.0001	**Significant**
AB	1200.50	1	1200.50	2.88	0.1154	
AC	288.00	1	288.00	0.69	0.4220	
AD	2601.00	1	2601.00	6.24	0.0280	**Significant**
BC	276.13	1	276.13	0.66	0.4315	
BD	4935.06	1	4935.06	11.84	0.0049	**Significant**
CD	4455.56	1	4455.56	10.69	0.0067	**Significant**
A^2	4410.88	1	4410.88	10.59	0.0069	**Significant**
B^2	117.16	1	117.16	0.28	0.6056	
C^2	58.02	1	58.02	0.14	0.7155	
Residual	4999.87	12	416.66			
Cor Total	$1.382x10^5$	25				
Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-Mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant						

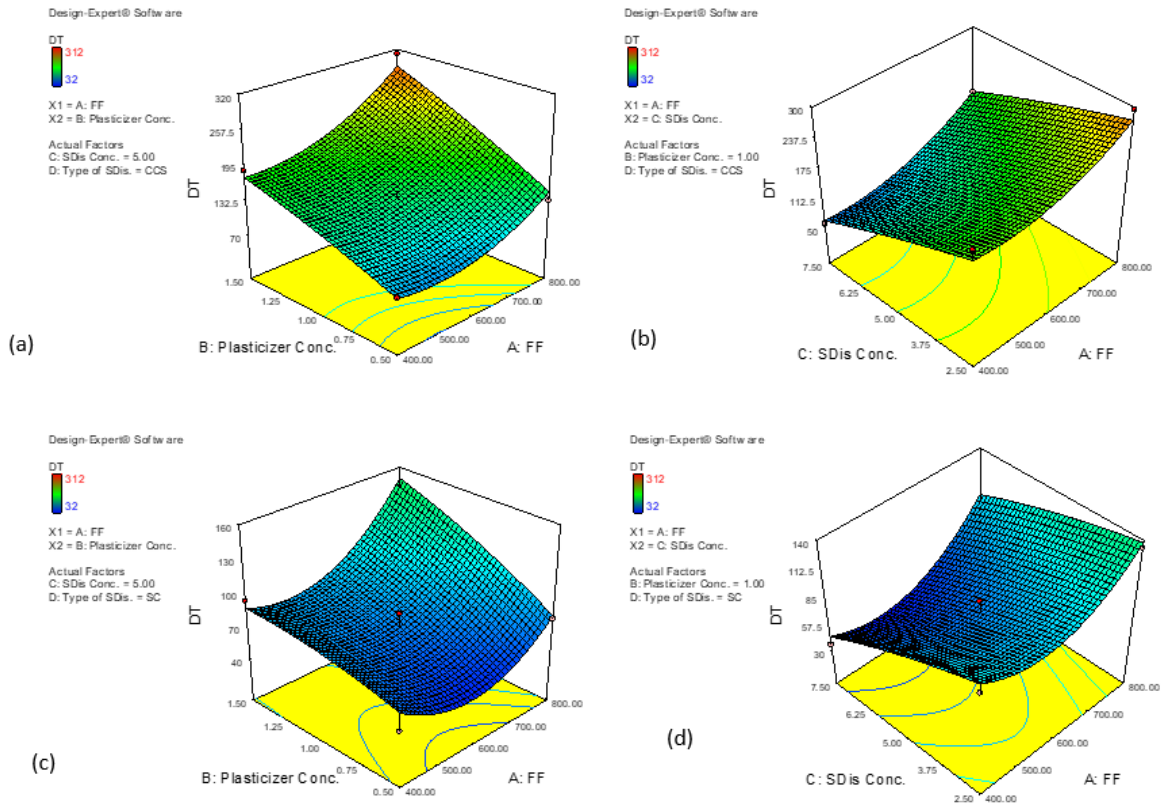


Fig 2: 3D-surface plots illustrating the influence of (a) the factors A and B on DT in case of CCS (b) the factors A and C on DT in case of CCS (c) the factors A and B on DT in case of SC (d) the factors A and C on DT in case of SC.

Sequential sum of squares analysis was applied to find the best regression model to identify the impact of different factors on DT. The analysis results were indicated that the quadratic model was the best suitable model to evaluate the impact of selected factors on DT. To confirm the suitability of the model, further ANNOVA also applied to the quadratic model and the results indicated that the selected model is this model can be navigated to develop design space.

From the 3D surface plots, it was observed that with increase in the film former concentration (factor-A), the disintegration time was found to be decreased which might be due to the higher thickness of the film thereby increases the diffusion path length for water to penetrate through it [22]. Whereas with increment in plasticizer concentration (Factor-B), the disintegration time was found to be increased which might be due to intercalation of the plasticizer molecules with the film forming polymer, thereby reducing the swelling index of the film which will ultimately facilitates the faster water absorption through it and leads to rapid disintegration. With increment in super disintegrant concentration (Factor-C), the disintegration time was found to be increased, this is obvious, which might be due to the absorption of more water thereby facilitating the rapid disintegration. Along with the concentration of different factors, the type of disintegrant (Factor-D) was also studied between two disintegrants i.e. stach citrate (SC) and croscarmellose sodium (CCS). It was observed that the ICQFs made with SC were found to have the faster disintegration than CCS, this could be due to the extensive swelling index of of SC than CCS. As the higher swelling index leads to the increase in volume upon water absorption and hence facilitate the rapid disintegration [23].

DoE analysis of response factor-2(R2): Dissolution after 10 minutes (D10)

The dissolution after 10 minutes is the desired criteria to know the performance of the ICQFs. To support the selected criteria and to know the impact of various factors on the selected response sequential sum of square analysis followed by ANNOVA was applied and the results were shown in Table 4 and Figure 3.

Table 4: ANOVA test for response surface quadratic model for the D10 (R2)

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
Model	2785.94	13	214.30	138.27	< 0.0001	**Significant**
A: FF	289.00	1	289.00	186.47	< 0.0001	**Significant**
B: Plasticizer Conc.	673.40	1	673.40	434.48	< 0.0001	**Significant**
C: SDis Conc.	1354.24	1	1354.24	873.77	< 0.0001	**Significant**
D: Type of SDis.	314.31	1	314.31	202.80	< 0.0001	**Significant**
AB	1.20	1	1.20	0.78	0.3959	
AC	3.51	1	3.51	2.27	0.1581	
AD	2.89	1	2.89	1.86	0.1971	
BC	47.53	1	47.53	30.67	0.0001	**Significant**
BD	2.25	1	2.25	1.45	0.2515	
CD	9.92	1	9.92	6.40	0.0264	**Significant**
A^2	82.33	1	82.33	53.12	< 0.0001	**Significant**
B^2	3.25	1	3.25	2.10	0.1729	
C^2	9.86	1	9.86	6.36	0.0268	**Significant**
Residual	18.60	12	1.55			
Cor Total	2804.53	25				

Note: [a]-Sum of Squares; [b]-Degrees of Freedom; [c]-Mean Sum of Squares; [d]-p-Value less than 0.05 indicates model terms are significant

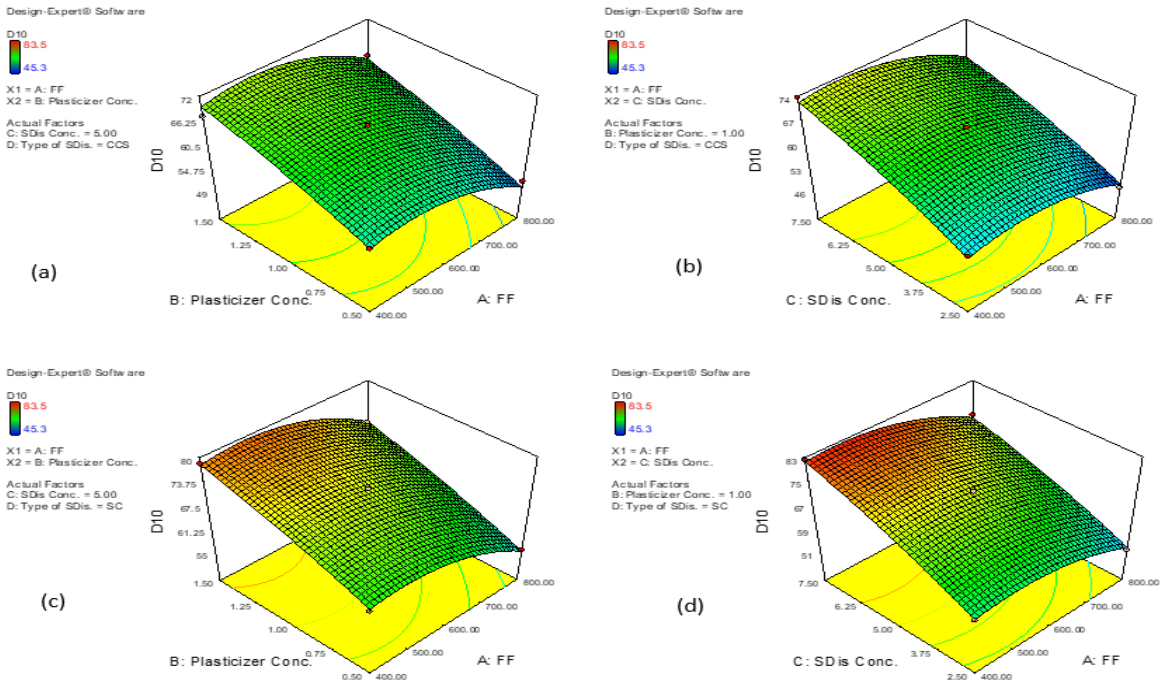


Fig 3: 3D-surface plots illustrating the influence of (a) the factors A and B on D10 in case of CCS (b) the factors A and C on D10 in case of CCS (c) the factors A and B on D10 in case of SC (d) the factors A and C on D10 in case of SC

From the 3D surface plots, it was observed that with increase in the film former concentration (factor-A), the D_{10} was found to be decreased which might be due to increased thickness of the film thereby inhibiting the faster penetration of media into the film. Whereas with increment in plasticizer concentration (Factor-B), the D_{10} was found to be increased which might be due to improved hydrophilicity of the film, as well as the co-solvent effect by the Plasticizer PEG 400. With increment in super disintegrant concentration (Factor-C), the disintegration time was found to be increased, which will ultimately facilitate the faster dissolution [24]. Along with the concentration of different factors, the type of disintegrant (Factor-D) was also studied between two disintegrants i.e. starch citrate (SC) and croscarmellose sodium (CCS) to understand their impact on the selected response D10. It was found that the ICQFs made with SC were found to have the faster dissolution than CCS, this could be due to the extensive swelling index of SC than CCS. As the higher swelling index leads to the increase in volume upon water absorption and hence facilitate the rapid disintegration followed by faster dissolution [25].

Optimization and Validation of the selected model:

To find the best possible composition of the selected factors to formulate the ICQF with minimal disintegration time and maximum D_{10}, the graphical optimization was performed and the results were shown in Figure 4.

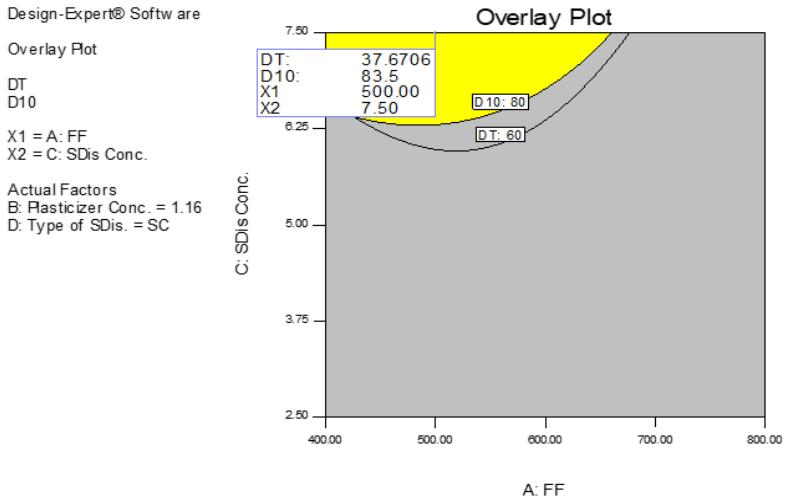


Fig 4: Overlay plot showing the design space for the set desirability criteria

From the yellow region of the graph, it is understood that any combination of factors A, B and C would lead to the formulation of best ICQF with desired responses. A fresh formulation was prepared with the suggested combination and evaluated for desired responses and the predicted and actual responses were displayed in Table 5.

Table 5: Predicted and observed responses of the the optimized ICQF

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: FF (500 mg) B: Plasticizer Conc. (1.16 %v/v) C: SDis. Conc. (7.5% w/w) D: Type of SDis. (SC)	R1: Disintegration time (sec.)	37.7	10.11	65.23	32.9
	R2: % Drug dissolved after 10 min.	83.5	81.82	85.18	82.4

The observed values of the desired responses were found to be aligned between the ranges predicted by the graphical optimization, hence the formulation was considered as optimized and the same was moved forward for in-vivo pharmacokinetic studies.

***In vivo* Pharmacokinetic Studies**

The optimized formulation was studied for its behavior at *in-vivo* conditions in comparison with control (Quercetin suspension), the blood plasmas samples were analyzed with the HPLC and from the data various parameters like plasma concentration data of Quercetin, various pharmacokinetic parameters like C_{max}, T_{max} and AUC [26] were also calculated and the results are displayed in Table 6 and Figure 5.

Table 6: Results of various pharmacokinetic parameters after non-compartmental analysis

S. No.	Pharmacokinetic parameter	Plasma concentration* (µg/mL)		*P*-value[$#]
		QUE Suspension	Opt. QUE OTFs	
1	C_{max} (µg/mL)	0.87 ± 0.17	1.65 ± 0.16	0.004
2	T_{max} (h)	1.67 ± 0.29	1.00 ± 0	0.016
3	AUC_{0-t} (h.µg/mL)	4.10 ± 0.90	7.33 ± 1.05	0.015
4	$AUC_{0-\infty}$ (h.µg/mL)	4.39 ± 1.03	7.96 ± 1.22	0.018
5	K_{el} ($^{h-1}$)	0.24 ± 0.03	0.21 ± 0.01	0.16
6	$t_{1/2}$ (h)	2.88 ± 0.36	3.34 ± 0.14	0.11
* Indicated as mean ± standard deviation for n = 3				
[$] For one way ANOVA at α = 0.05				
[#] *P*-value less than 0.05 indicates significant difference				

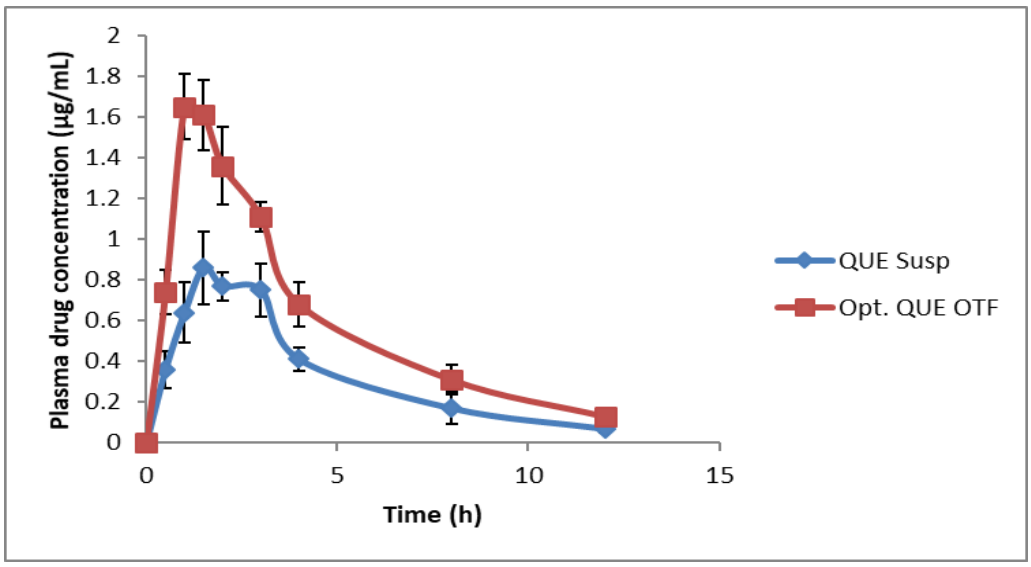


Fig 5: Plasma QUE concentration – time profiles of QUE from the aqueous suspension and the optimized OTFs

The HPLC method was developed using Carvedilol as an internal standard. The calibration curve was constructed with a correlation coefficient of 0.9998. It was observed that the C_{max} and the $AUC_{0-\infty}$ found to be increased ($p < 0.05$); and t_{max} was decreased ($p < 0.05$) for the optimized QUE films in comparison to QUE aqueous suspension. Due to the rapid disintegration followed by rapid dissolution of the QUE from the films, the drug absorption rate was found to be increased. The C_{max} and $AUC_{0-\infty}$ were found to be increased by 89.6% and 78.8% respectively. The increase in solubility and dissolution rate of Quercetin from the inclusion complex based films was observed in GIT which might be due to abundant availability of highly soluble form. As the type of formulation can only impact the absorption rate of the drug through GIT, the elimination rate constant was found not to be differed significantly (P>0.05). From the pharmacokinetic parameters it was concluded that the optimized ICQF formulation was found to have more bioavailability than the original Quercetin.

CONCLUSION

The OTFs for the complexed form of the QUE were developed and characterized. BBD was employed to elucidate the influences of the factors related to formulation viz. film former, plasticizer and disintegrant on the disintegration and dissolution character of the OTFs. The results were statistically analyzed and observed that all the three factors had significant effect on the responses. The optimized OTF was found to have a DT of 32.9 sec and dissolved 82.4% of QUE within 10 min. this optimized OTF was tested for in vivo bioavailability in comparison with QUE aqueous suspension. The found results indicated that the bioavailability of QUE from the OTF was significantly improved over the pure QUE. Hence, the formulation and statistical approach adopted in this work lead to successful achievement of the set objectives of the work.

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Conflict of interest:
The authors affirm that there are no conflicts of interest.

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