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Quality by Design Based Optimization and Development of Cyclodextrin Inclusion Complexes of Quercetin for Solubility Enhancement

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Abstract: The purpose of the current work was to enhance the solubility of Quercetin (QUE) by developing inclusion complexes. The current research focused on preparing ternary cyclodextrin inclusion complexes of Quercetin (ICQs) with tween-80 as surfactant employing quality by the design tool. The concentration of hydroxypropyl-β-cyclodextrin, tween-80, and preparation method was taken as critical process parameters and evaluated to get the final product with desired solubility and dissolution rate as the responses. The central composite design was used for the systemic development of ICQs. The % yield and drug content of the prepared ICQs were found to be greater than 90% & 98%, respectively. X-ray diffraction and differential scanning calorimetry results showed the QUE was converted into an amorphous form after the formulation of ICQs. Improved solubility and dissolution rate was observed for the prepared ICQs than pure drug. The design method was optimized, and design validation studies were also performed. All the factors significantly influenced both solubility and dissolution at $p < 0.05$. Based on the suggested combinations by overlay plot of graphical optimization, a new formulation was developed and evaluated for solubility and dissolution, which were impressively increased from pure QUE.

Keywords: quercetin; cyclodextrin inclusion complexes; quality by design; critical process parameters; central composite design; optimization.

1. Introduction

Quercetin (QUE) is a well-known anti-oxidant, naturally occurring flavonoid present abundantly in routine dietary supplements like apples, red wine, green tea, and berries. Chemically, QUE is known as 3, 5, 7, 3', 4'- Penta hydroxyl flavone, which has anti-inflammatory properties. As an anti-oxidant, QUE is much more effective in treating cardiac-related diseases, helps in controlling the stickiness of bad cholesterol to arteries by preventing its accumulation in arteries, helps to prevent obesity, and is also helpful in killing chemo cells in humans [1]. QUE is a BCS Class-IV compound due to its low solubility and low permeability; excessive 1st pass metabolism before reaching the bloodstream and its instability in the gastrointestinal environment make QUE poorly bioavailable.

As QUE has many therapeutic advantages but hurdles in administration as medicine, science needs to find a novel technique to improve its solubility [2] followed by its

bioavailability instead of using routine methods like co-solvency, pH modification, solid dispersion, etc. Among various research works, solid dispersion is one of the broadly used approaches to improve the solubility [3] of QUE by converting the crystalline form into an amorphous form. Solid dispersions can be formulated using either spray drying, hot melt extrusion, or by any other method using polymers like cellulose derivatives or polyvinyl pyrrolidone or with other polymers [4]. Co-solvency is also one of the well-known technologies to improve the solubility of QUE using solvents like propylene glycol (PG), polyethylene glycol (PEG), glycerin, etc. Different grades are available from the class of PEGs that can be used as either co-solvents (PEG-200, PEG 400, and PEG 600) or as water-miscible/ dissolving solvents (PEG 4000& PEG 6000). Along with the above-mentioned techniques, pH modification is also used as one of the solubility improvement methods to get desired bioavailability.

Along with the methods mentioned above, cyclodextrin inclusion complexes are the emerging techniques to enhance solubility, followed by the bioavailability of QUE. Cyclodextrins (CDs) are a group of sugars made up of different size rings [5]; these rings are named glucopyranosides. CDs are differentiated based on the number of glucopyranosides ring present. The CDs with 6 rings, 7 rings, and 8 rings are named α, β, and γ– CD, respectively [6]. CDs are desirable polymers to enhance solubility and bioavailability by having a lipophilic core and hydrophilic outer surface. Among all the CDs, the β form is more useful for pharmaceutical applications due to its abundant availability, cost-effectiveness, and ability to entrap various drug molecules in its cavity [7]. Various reports reported different applications for CD complexes, such as an increase in drug solubility, to improve stability and mask the drug's unpleasant taste [8]. As a higher quantity of CDs is required to provide the drugs with the desired fold increment in solubility, it is very difficult to accommodate the drugs with high doses in CD complexes. To get the desired drug solubility with a lesser amount of CD, either modification must be done to this oligomer, or any other solubility enhancer like surfactants need to be added along with the polymer [9].

Quality by Design (QbD) is a tool to design the dosage forms with desired quality with less experimentation [10]. Various elements like critical quality attributes (CQA), which define the required quality of the product, must be spotted and assessed, which will directly impact the product's desired quality [11]. Critical material attributes (CMA) and formulation parameters which are types under Critical process parameters (CPP), need to be selected based on previous experiences and literature to know their impact on the CQAs [12].

Current research focuses on preparing QUE CD inclusion complexes using tween-80 as a surfactant by employing QbD. The concentration of hydroxypropyl-β-CD (HPβ-CD), the concentration of tween-80, and the preparation method were taken as the CPPs. Their influence on the solubility and dissolution rate which were taken as the CQAs was evaluated statistically using the design of experiments (DoE). Central composite design (CCD) was employed to limit the number of trials needed to get formulation with desired characteristics [13]. The Inclusion Complex of Quercetin (ICQs) developed by kneading and solvent evaporation methods [14] was evaluated by subjecting to thermal analysis, X-ray diffraction (X-RD) analysis, solubility, and dissolution testing. The design was also optimized to get ICQs with the desired CQAs [15].

2. Materials and Methods

2.1. Materials.

QUE was acquired from Hetero Labs Private Limited, Hyd. Methyl β-CD, HPβ-CD, and Tween-80 were bought from Sigma Chemicals Co., and all other materials of analytical reagent grade were employed.

2.2. Preparation of cyclodextrin complexes of quercetin (ICQs).

The ICQs were formulated systemically by applying the Central composite design of QbD using Stat-Ease Design Expert Software to formulate the products with desired quality [16]. Based on the prior experience and available literature CQAs, formulation parameters as the material attributes under CPPs were selected to know their impact on the desired quality of the ICQs.

2.2.1. Quality Target Product Profiling (QTPP).

QTPP of prepared ICQs should be rapidly dissolving in nature to overcome the dissolution-limited bioavailability of QUE.

2.2.2. Critical Quality Attributes (CQAs).

Critical Quality attributes are the output qualities of the process and also have a direct impact on QTPP. Solubility and dissolution rate constant were selected as CQAs as they are the direct measures of drug dissolution.

2.2.3. Critical Process Parameters (CPPs).

From the detailed literature review, the concentration (% w/w) of Hydroxypropyl β-cyclodextrin (HPβ-CD) with respect to the total weight of the QUE and HPβ-CD, Concentration (% w/w) of Tween 80 with respect to the weight of the QUE, Method of preparation at two levels were selected as CPPs.

2.2.4. Experimental design.

CCD was selected as the best suitable experimental design based on the levels and nature of the factors, and the possible combinations of the factors are shown in Table 1. Graphical optimization was done to identify the design space (a combination of the optimum levels of the factors to give the desired CQA values).

2.3. Preparation of ICQs by the kneading method.

Requisite quantities of QUE and HPβ-CD were weighed in a mortar and triturated thoroughly with the pestle until the formation of a uniform mixture. Dispense the required quantity of tween-80, add to the prepared uniform mixture and mix thoroughly until the formation of a uniform mixture. Convert the prepared uniform mixture into the uniform paste by adding a small amount of solvent mixture (dimethyl sulfoxide (DMSO) and water at a 1:1 ratio) in a dropwise manner under continuous mixing. Dry the formed wet ICQs by placing at 70°C until the solvent gets removed completely. The formulated ICQs were labeled and stored properly until further usage [17].

2.4. Preparation of ICQs by solvent evaporation.

Required quantities of HPβ-CD and tween 80, as per Table 1, were dispensed and dissolved in water; QUE was dissolved in DMSO under vortexing until the formation of clear solutions. Both the solutions were slowly mixed, and this mixture was subjected to evaporation at 60°C in Rotavapor until the entire solvent was evaporated, resulting in dry powder [18]. The formulated ICQs were labeled and stored properly until further usage.

Table 1. Combinations of the factors and their levels according to CCD for developing ICQs.

Std. order	Run order	Code	Level of Factors			Response: Solubility (mg/mL)*
			A (Conc. of HPβ-CD, % w/w)	B (Conc. of Tween 80, % w/w)	C (Method)	
13	1	ICQ1	33.30	2.00	K.M	0.07 ± 0.01
8	2	ICQ2	60.00	2.00	K.M	0.22 ± 0.03
10	3	ICQ3	33.30	5.00	K.M	0.16 ± 0.04
6	4	ICQ4	60.00	5.00	K.M	0.35 ± 0.05
16	5	ICQ5	27.77	3.50	K.M	0.08 ± 0.01
18	6	ICQ6	65.53	3.50	K.M	0.39 ± 0.02
5	7	ICQ7	46.65	1.38	K.M	0.09 ± 0.02
7	8	ICQ8	46.65	5.62	K.M	0.27 ± 0.04
2	9	ICQ9	46.65	3.50	K.M	0.15 ± 0.03
9	10	ICQ10	33.30	2.00	S.E.M	0.26 ± 0.05
1	11	ICQ11	60.00	2.00	S.E.M	0.71 ± 0.08
3	12	ICQ12	33.30	5.00	S.E.M	0.31 ± 0.02
17	13	ICQ13	60.00	5.00	S.E.M	0.74 ± 0.04
15	14	ICQ14	27.77	3.50	S.E.M	0.25 ± 0.03
14	15	ICQ15	65.53	3.50	S.E.M	0.82 ± 0.06
11	16	ICQ16	46.65	1.38	S.E.M	0.43 ± 0.01
4	17	ICQ17	46.65	5.62	S.E.M	0.49 ± 0.02
12	18	ICQ18	46.65	3.50	S.E.M	0.44 ± 0.02

2.5. Characterization of the ICQs.

2.5.1. Determination of % yield.

The % yield of the ICQs was represented as a ratio of the quantity of ICQs obtained to the total quantity of components taken, expressed in percentages.

2.5.2. Total drug content.

ICQs' drug content was quantified using an in-house developed UV- spectroscopic method. Briefly, 100 mg QUE equivalent prepared ICQs were dispersed with 100 mL of 0.1N NaOH solution using an orbital shaker for 2 h. Now filtered, the mixture and analyzed the filtrate with suitable dilution in methanol, and the final volume was made up to 10 ml and filtered through a 0.45µm syringe filter [19]. The resultant solutions were scanned by using UV-spectrophotometer, and the percent drug content was calculated from the obtained absorbances [20].

2.5.3. Differential scanning calorimetry (DSC).

DSC for the formulation as well as the API alone was performed to know the physical nature of the QUE before and after complexation. About 5 mg samples were analyzed for DSC by placing them in aluminum pans with crimp-on lids. DSC was performed using a thermal analyzer by raising the temperature at 10°C/ min from 50–400°C under a nitrogen atmosphere [21].

2.5.4. X-Ray Diffraction (XRD).

Powdered XRD characterization investigations were performed for the pure drug and the prepared ICQs by each method to know the crystalline nature after complexation. The appearance of sharp peaks with more intensities indicates the presence of crystallinity, whereas broad peaks or peaks with more noise or less intensity indicate the presence of amorphous molecules [22].

2.5.5. Solubility.

Solubility for the prepared ICQs was determined using the shake flask method [23]. Briefly, 10ml of water was placed in 25ml conical flasks. The excess amount of ICQs was added to the media and continued shaking for 24 hours; the presence of undissolved material confirmed saturation. Then, the flasks were removed from the shaker and mixed thoroughly using a vortex for 20 min. and filtered with the help of Whatman filter paper, the filtrate was subjected to centrifugation at 13000 rpm for 10 minutes, and the collected supernatant was diluted with water and scanned against water in a spectrophotometer at its wavelength maximum, 256 nm [24]. The absorbance values were placed in a standard calibration curve for quantification.

2.5.6. *In vitro* Dissolution.

A dissolution test was accomplished for the formulated ICQs using the USP Type-II apparatus. 100 mg equivalent ICQs were dispensed and placed in a dissolution apparatus fitted with type-II paddle apparatus filled with 900 mL of water. A dissolution study was performed at 100 RPM, and 5 mL of samples were collected every 5 minutes up to 30 minutes. The collected samples were filtered and subjected to spectrophotometrical quantification [25] to determine the % drug dissolved at a particular time.

2.6. Design validation and optimization of ICQs.

The selected CCD was validated by the Stat-Ease design expert software. The responses, solubility (R1), and dissolution rate constant (R2) of the variables were analyzed using the Sequential model sum of squares analysis [26] to find out the best-suited statistical design to elucidate the influences of the factors on the responses. Analysis of variance (ANOVA) was applied to know the suitability of the selected model and assess the factors' influences on the responses. Finally, graphical optimization was performed to identify the best possible combination(s) of the selected factors to achieve the desired QTPP (improve the solubility and dissolution rate) of the practically insoluble QUE.

3. Results and Discussion

The ICQs were formulated according to the CCD design and were evaluated for % yield, % total drug content, solubility (mg/mL), and the dissolution rate constant (min^{-1}). Results are shown in Table 2.

Table 2. Results of characterization studies of the ICQs.

ICQ code	Yield (%)	Total drug content (%)	Solubility (R1, mg/mL)	Dissolution rate constant (R2, min^{-1})
IC1	95.6 ± 2.9	99.1 ± 1.3	0.07 ± 0.01	0.03 ± 0.002
IC2	96.2 ± 1.6	98.6 ± 2.2	0.22 ± 0.03	0.08 ± 0.007

ICQ code	Yield (%)	Total drug content (%)	Solubility (R1, mg/mL)	Dissolution rate constant (R2, min^{-1})
IC3	97.9 ± 2.7	98.9 ± 1.8	0.16 ± 0.04	0.06 ± 0.003
IC4	98.1 ± 1.4	99.5 ± 1.6	0.35 ± 0.05	0.14 ± 0.010
IC5	97.4 ± 2.1	102.4 ± 2.7	0.08 ± 0.01	0.05 ± 0.006
IC6	98.3 ± 0.9	100.8 ± 0.9	0.39 ± 0.02	0.15 ± 0.020
IC7	97.6 ± 2.5	101.3 ± 1.5	0.09 ± 0.02	0.06 ± 0.008
IC8	97.3 ± 1.7	98.6 ± 2.3	0.27 ± 0.04	0.13 ± 0.021
IC9	98.5 ± 1.2	100.5 ± 1.9	0.15 ± 0.03	0.08 ± 0.009
IC10	97.4 ± 2.5	99.2 ± 3.6	0.26 ± 0.05	0.06 ± 0.011
IC11	95.3 ± 3.5	98.0 ± 2.8	0.71 ± 0.08	0.14 ± 0.023
IC12	96.8 ± 2.7	98.4 ± 1.5	0.31 ± 0.02	0.11 ± 0.017
IC13	93.4 ± 4.2	101.7 ± 3.7	0.74 ± 0.04	0.20 ± 0.034
IC14	94.9 ± 2.9	100.3 ± 0.8	0.25 ± 0.03	0.08 ± 0.009
IC15	95.2 ± 3.3	99.7 ± 1.2	0.82 ± 0.06	0.21 ± 0.025
IC16	93.6 ± 4.1	100.9 ± 2.4	0.43 ± 0.01	0.11 ± 0.018
IC17	94.1 ± 4.6	102.1 ± 1.1	0.49 ± 0.02	0.18 ± 0.022
IC18	95.7 ± 2.8	98.6 ± 2.5	0.44 ± 0.02	0.13 0.036

3.1. DSC.

DSC graphs are illustrated in Figure 1 involving the pure drug and the ICQs made using two different methods. The pure DSC curve of QUE showed an endothermic peak at around 120°C, which might be because of the evaporation of the water of hydration entrapped in QUE. This indicated that the QUE took the hydrate form. A sharp endotherm was observed at 321°C corresponding to the melting of the crystalline QUE. However, ICQs prepared using kneading and solvent evaporation methods devoid of any sharp peak at the same temperature due to the conversion of the crystalline nature of the drug to amorphous form owing to the solvent action in the selected method of preparation. These results were correlated with those testified by Sathishkumar P *et al.* [27] and Manta K *et al.* [28] Kim J [29]. This could further indicate that complete miscibility of the QUE in HPβ-CD was possible in solvent evaporation technique than in kneading. The XRD studies further corroborated the results.

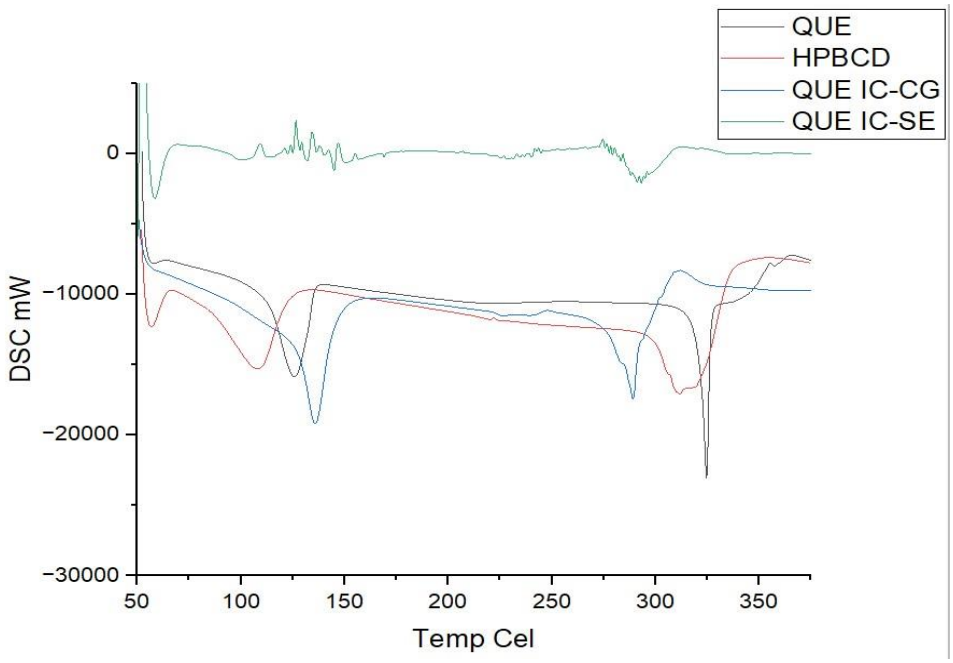

Figure 1. DSC thermograms of pure QUE, HPβ-CD and the ICQs prepared by kneading and solvent evaporation techniques.

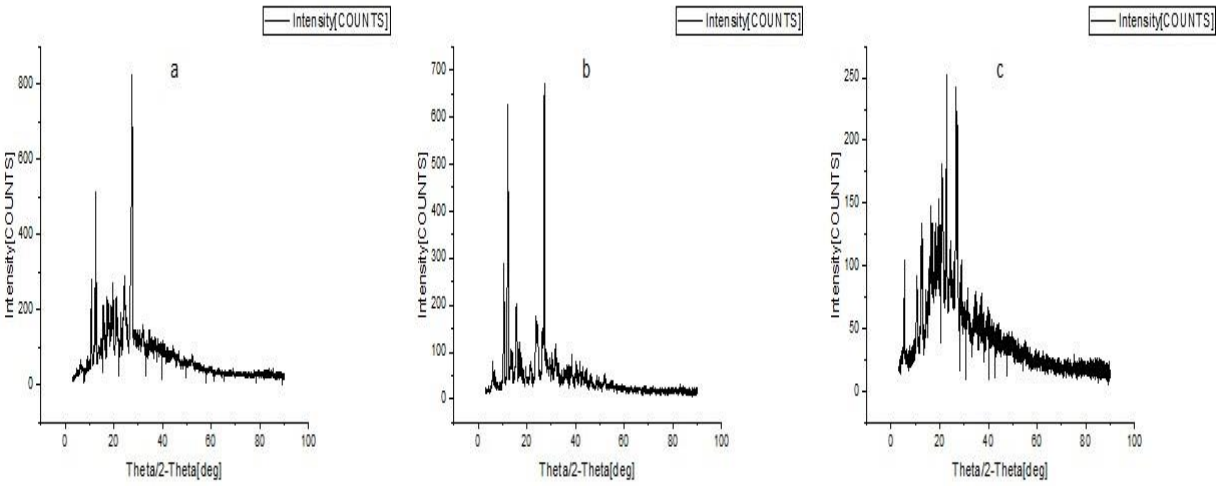

Figure 2. X-RD spectra of (**a)** Pure QUE; (**b)** ICQs prepared by the kneading method; (**c)** the ICQs prepared by the solvent evaporation method.

3.2. XRD studies.

X-Ray diffraction studies were performed for pure drugs and after the preparation of ICQs. Sharp, high-intensity peaks were observed for the pure QUE, indicating the pure QUE's crystalline nature. Whereas the XRD graphs of ICQs prepared by kneading or solvent evaporation method exhibits halo (less intense peaks), which indicates the possible conversion of crystalline QUE into amorphous form (Figure 2). This result was supported by the previous DSC analysis and also correlated well with those reported by Kim J [29].

3.3. Solubility and its Design of experiments (DoE) analysis.

In the case of all the ICQs, the solubility of QUE was found to be increased. The possible mechanism behind the solubility enhancement could be majorly due to the conversion of the crystalline QUE to its amorphous form, which was designated by the DSC and X-RD studies. Further, the influences of the formulation factors on the solubility were discussed as follows. The solubility results of the ICQs prepared according to the selected CCD were subjected to the sequential model sum of squares analysis in the software to identify the model of the influence of the factors on this response R1, solubility. The Quadratic model was found to be significant and suggested for this response R1.

Table 3. Results of the sequential model sum of squares for selecting a model for solubility (R1).

Source	Sum of squares	Degrees of freedom	Mean square	F value	p-value	Inference
Mean vs. Total	2.16	1	2.16			
Linear vs. Mean	0.80	3	0.27	49.98	< 0.0001	
2FI vs. Linear	0.058	3	0.019	12.21	0.0008	
Quadratic vs. 2FI	0.014	2	0.0072	21.89	0.0003	**Suggested**
Cubic vs. Quadratic	0.00081	5	0.00016	0.30	0.8886	Aliased
Residual	0.0021	4	0.00053			
Total	3.03	18	0.17			

Table 4. Results of ANOVA test for the quadratic model for the Solubility (R1).

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value[d] (Prob>F)	Inference
Model	0.87	8	0.11	333.48	< 0.0001	Significant
A-HPBCD Conc.	0.38	1	0.38	1159.98	< 0.0001	Significant

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value[d] (Prob>F)	Inference
B-Tween 80 Conc.	0.026	1	0.026	78.08	< 0.0001	Significant
C-Method	0.40	1	0.40	1210.21	< 0.0001	Significant
AB	0.00005	1	0.00005	0.15	0.7050	
AC	0.051	1	0.051	157.35	< 0.0001	Significant
BC	0.006	1	0.006	18.32	0.0020	Significant
A^2	0.012	1	0.012	36.00	0.0002	Significant
B^2	0.0009	1	0.0009	2.78	0.1299	
Residual	0.0029	9	0.00033			
Cor Total	0.88	17				

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

ANOVA test was applied for the suggested model, and the F values (Table 4) were also calculated for different variables. The selected model was significant as its F value for the selected model was obtained as 333.48. In the present model, variables A, B, C, AC, BC, A^2 were significant, whereas the variables with *p* values more than 0.05 are non-significant.

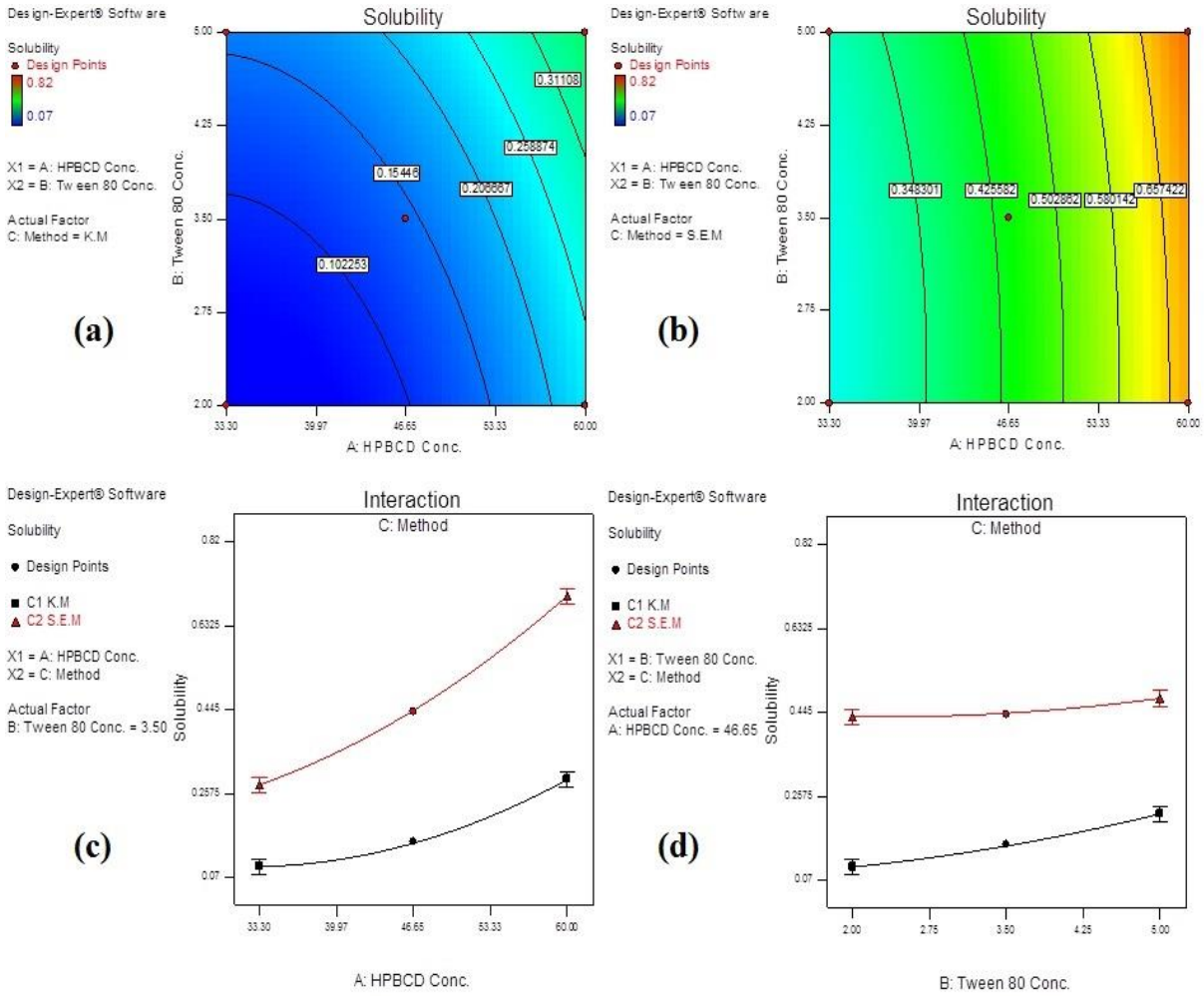

Figure 3. Contour plots showing the effects of factors A and B on solubility in the case of **(a)** the kneading method; and **(b)** the solvent evaporation method. Interaction plots show the interaction effect of **(c)** AC on solubility; and **(d)** BC on solubility.

The solubility results of the prepared ICQs are shown in Table 2. Figure 3 displays the contour and interaction plots of response variable R1, showing the powerful relationship between the factors. The plots illustrated that the increased concentration of HPβ-CD was proportionally improving the solubility of QUE in both the preparation methods. This can be

attributed to the availability of more host molecules at a higher concentration for the complexing of more guest drug molecules, which increases the QUE solubility. The obtained results were in accordance with a previously published report by Manta K *et al.* [18]. The surfactant was also found to have a positive effect on the solubility, i.e., the solubility was found to be increased upon the increase in its concentration. This might be because of the decreased interfacial tension between the host and guest during complexation and between any un-complexed drug and water during solubilization. These solubility results further designate that at a fixed concentration of HPβ-CD, an increase in the tween 80 concentration showed a noteworthy enhancement in the solubility. This confirmed that the presence of surfactant alongside HPβ-CD could significantly contribute to the solubility enhancement. Therefore, further increase in HPβ-CD concentration may not be necessary to get higher solubilities; hence, the weight of the ICQs equivalent to one dose of QUE is under control. The results were found to be correlated with those reported by Chowdary KPR *et al.* [30].

The interaction effects of AC and BC were found to be significant from ANOVA and were shown in the interaction plots. The AC interaction plot indicated that the effect of factor A was more prominent in the solvent evaporation method than in the case of the kneading method. This could be attributed to the possible conversion of the total amount of HPβ-CD into free guest molecules because it was dissolved completely in a sufficient amount of solvent [31]. So, more amounts of guest molecules can be possibly complexed, thus resulting in more solubility. But, in the case of the kneading method, the amount of solvent used during complexation is relatively less, and the maximum utilization of the HPβ-CD could not have occurred. Hence, the solubility was increased to a greater extent in the solvent evaporation method than in the kneading method at the same HPβ-CD concentration. The overall results indicated that the solvent evaporation method was found to increase solubility to a greater extent than the kneading method. The results were found to be in line with those reported by Okoye EI *et al.* [32].

The BC interaction plot illustrated that factor B has a more prominent effect in the kneading method than in the case of solvent evaporation. This could be because of the possible association of the surfactant Tween 80 with the drug that enhanced the solubility during solubilization. But, in the case of the solvent evaporation method, the Tween 80 was dissolved in the solvent (DMSO + Water). This could cause the interaction of the Tween 80 molecules with the drug as well as HP-β-CD and could be precipitated in the same manner. Hence, during the solubilization of the drug complex, the effect of surfactant on the drug could be relatively less compared to that of the kneading method [30,31].

3.4. In-vitro Dissolution its Design of experiments (DoE) analysis.

In-vitro dissolution was performed for all the ICQs formulated using kneading as well as the solvent evaporation method. Among all the formulations prepared by a solvent evaporation method, IC15 & 13 showed the faster drug release of almost 100% (99.48, 99.32, respectively) within 25 minutes. Among all the formulations prepared by the kneading method, IC6 & 4 showed the faster drug release of almost 100% (98.97, 98.84 respectively) within 30 minutes. From the *in-vitro* dissolution studies, it was evident that the formulations with a combination of higher polymer and higher surfactant concentrations could provide faster drug release for ICQs prepared by either the kneading method or a solvent evaporation method. Drug dissolution kinetics of zero and first order were applied for the dissolution data obtained, and the dissolution rate constant was also calculated. The data is shown in Table 5. The data showed

that almost all the formulations followed first-order drug release, which indicated that the dissolution rate was concentration-dependent.

Table 5. Dissolution kinetics of ICQs.

S. No.	Inclusion complex	Regression value		Dissolution rate constant (k, min^{-1})
		Zero-order	First-order	
1	IC1	0.973	0.996	0.03
2	IC2	0.904	0.975	0.08
3	IC3	0.828	0.99	0.06
4	IC4	0.699	0.981	0.14
5	IC5	0.92	0.994	0.05
6	IC6	0.61	0.979	0.15
7	IC7	0.919	0.991	0.06
8	IC8	0.737	0.965	0.13
9	IC9	0.879	0.974	0.08
10	IC10	0.887	0.99	0.06
11	IC11	0.612	0.984	0.14
12	IC12	0.745	0.988	0.11
13	IC13	0.631	0.978	0.2
14	IC14	0.913	0.934	0.08
15	IC15	0.637	0.963	0.21
16	IC16	0.772	0.985	0.11
17	IC17	0.514	0.984	0.18
18	IC18	0.708	0.988	0.13

The results of the dissolution rate constant of the ICQs prepared according to the selected CCD were subjected to the sequential model sum of squares analysis (results shown in Table 6) in the software to identify the model of the influence of the factors on this response R2. Among all models, a linear model was found to be significant and suggested for this response R2. At the same time, models like 2-factorial interaction, quadratic and cubic models were not suitable for response R2.

Table 6. Results of the sequential model sum of squares analysis for selecting model.

Source	Sum of squares	Degrees of freedom	Mean square	F value	p-value	Inference
Mean vs. Total	0.22	1	0.22			
Linear vs. Mean	0.045	3	0.015	91.99	< 0.0001	***Suggested***
2FI vs. Linear	0.00065	3	0.00022	1.45	0.2803	
Quadratic vs. 2FI	0.000096	2	0.000048	0.28	0.7608	
Cubic vs. Quadratic	0.0001	5	0.00002	0.058	0.9961	Aliased
Residual	0.00143	4	0.00036			
Total	0.27	18	0.015			

ANOVA test was applied for the suggested model, and the F values (Table 7) were also calculated for different variables. The selected model was significant as its F value for the selected model was obtained as 91.99. In the present model, variables corresponding to the main effects of all three factors were observed to be significant.

Table 7. Results of ANOVA test for the quadratic model for the Dissolution rate constant (R2).

Source	SS[a]	Df[b]	MSS[c]	F value	p-Value	Inference[d]
Model	0.045	3	0.015	91.99	< 0.0001	**Significant**
A-HPBCD Conc.	0.024	1	0.024	149.56	< 0.0001	**Significant**
B-Tween 80 Conc.	0.0099	1	0.0099	60.59	< 0.0001	**Significant**
C-Method	0.011	1	0.011	65.83	< 0.0001	**Significant**
Residual	0.0023	14	0.00016			
Cor Total	0.047	17				

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

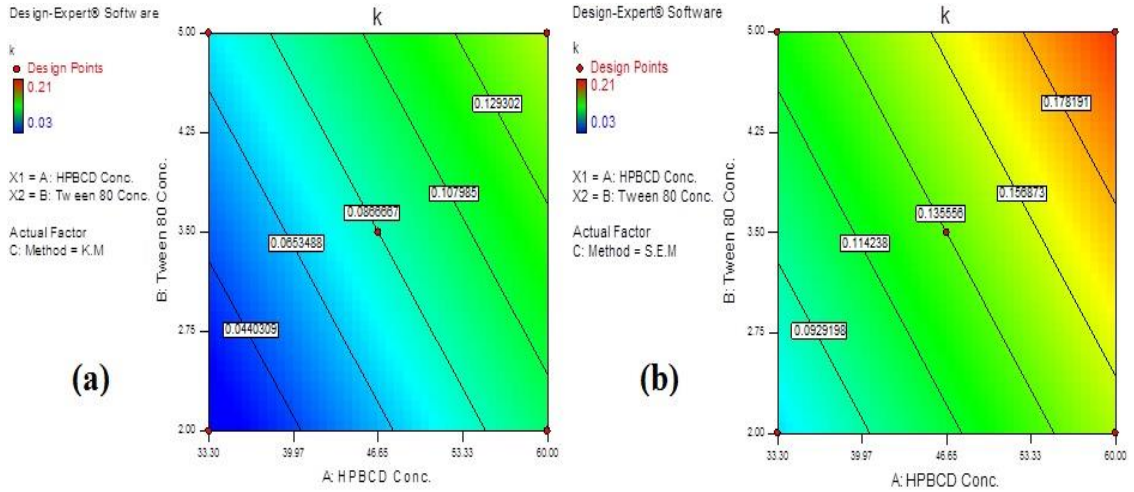

Figure 4. Contour plots displaying the impact of factors A & B on the dissolution rate constant of the ICQs **(a)** in the case of the kneading method; and **(b)** in the case of the solvent evaporation method.

Figure 4 illustrates the contour plots of response variable R2, which shows the powerful relationship between the factors and this response R2. Upon increasing the concentrations of both HPβ-CD and tween 80, the dissolution rate of the QUE from the ICQs prepared by both methods was found to be increased. Similar to the solubility discussion, this influence could be due to more complexation of QUE at higher concentrations of HPβ-CD; decreased interfacial tension, and increased affinity towards the water at higher concentrations of tween 80. Further, the dissolution was found to be better in the case of the solvent evaporation method than in the kneading method. This could again be attributed to the effective utilization of the hydrophilic carriers, which were completely activated in their molecular state in the presence of sufficient solvent in solvent evaporation. But unlike solubility, no interaction effects were observed, and the factors had only linear effects. This could be due to the availability of a large quantity of the media in dissolution, which might eliminate the possible difference among the factorial interactions. The results are correlated with those reported by Rodde MS *et al.* [33] and George SJ *et al.* [34].

3.6. Design validation.

In response R1, the adjusted and predicted R-Squared values were obtained as 0.9936 and 0.9869, respectively; in response R2, these were found to be 0.9414 and 0.9180, respectively. As both these values were close and differences were less than 0.2, it signifies that the selected quadratic model for response R1 and linear model for response R2 were suitably fit to the obtained values of the responses. Further, the model F values from the corresponding ANOVA testing (as shown in Table 4 for R1 and Table 7 for R2) were significant at $p < 0.05$, which also confirmed that the selected models for the responses from the CCD design were significant. Model evaluation plots, like predicted versus actual plots, indicate any necessary modification to the response data before subjecting them to DoE analysis. These plots for both responses are shown in Figure 5. All the data points in the predicted versus actual plots were uniformly distributed around the 45^o line. Hence, these two plots indicated no requirement to transform the response data for the DoE analysis. Altogether these diagnostic results indicated that the selected models for the responses could be navigated to develop the design space.

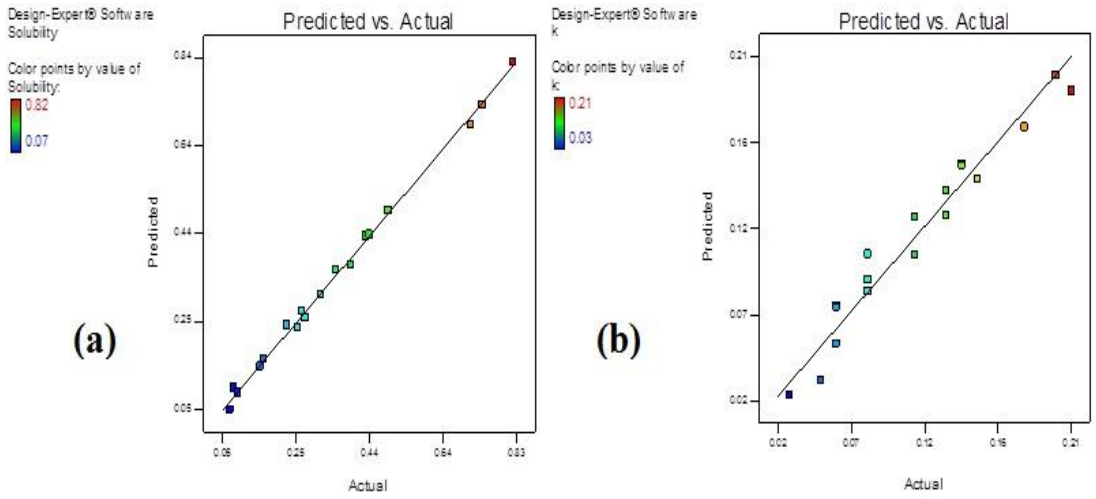

Figure 5. Predicted versus Actual plots of the responses **(a)** Solubility, R1; and **(b)** Dissolution rate constant, R2.

3.7. Graphical optimization.

Graphical optimization was performed to identify the best possible combination(s) of the selected factors' levels to achieve the desired QTPP (improved solubility and dissolution rate) of the practically insoluble QUE. The desirability criteria or constraints for the optimization were taken as maximizing the solubility above 0.5 mg/mL and maximizing the dissolution rate above 0.16 min^{-1}. The results of the graphical optimization are illustrated in Figure 6. The yellow color area in that plot indicates the design space (in the case of solvent evaporation method) inside which any combination of factors A and B would yield ICQs with maximum solubility and dissolution rate. Point prediction by the software identified one such combination. The combination of the factors and predicted response results are shown in Table 8. A fresh ICQ was prepared at the suggested combination and tested for solubility and the dissolution rate constant. The observed results (Table 8) were correlated with those predicted by design; hence, this combination was considered the optimum formulation of the ICQs. The solubility of pure QUE in the present experiment was found to be 0.003 mg/mL at 28°C, which was correlated with the reported value of 0.002 mg/mL at 25.6°C by Srinivas K *et al.* [35]. Hence, QUE is considered to be practically insoluble in water. So, in the present work, the developed ternary inclusion complexes of QUE using HPβ-CD and tween 80 using the QbD approach successfully improved the solubility and dissolution of QUE.

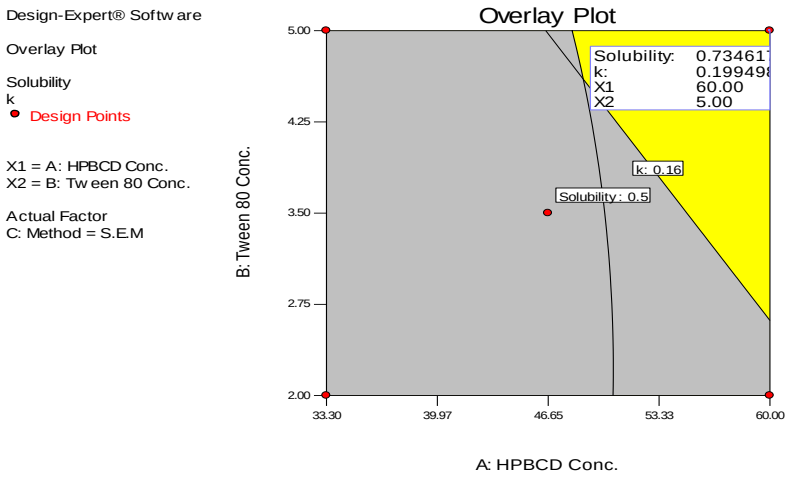

Figure 6. Overlay plot presenting the design space for the set desirability criteria.

Table 8: Predicted and observed values of the responses for the optimized ICQs

Factors combination	Responses	Predicted values	95% CI low	95% CI high	Observed values
A: HPBCD Conc. (60 % w/w) B: Tween 80 conc. (5 % w/w) C: Method (S.E.M)	R1: Solubility (mg/mL)	0.735	0.71	0.76	0.746
	R2: k (min^{-1})	0.199	0.19	0.21	0.193

4. Conclusions

ICQs were developed using HPβ-CD and tween 80 with the objective of improving the solubility and dissolution of QUE. The possible hypothesis behind the objective was that converting the crystalline QUE to its amorphous form upon complexation and including the drug QUE inside the cyclodextrin hydrophilic cavities could enhance the solubility of the drug. In addition, the incorporation of tween 80 could further improve QUE's hydrophilicity, thereby decreasing the amount of HPβ-CD required for enhancing the solubility. Solvent evaporation and kneading method were selected to test the stated hypothesis by adopting CCD design under the QbD approach. ICQs were prepared as suggested by the CCD design and evaluated for physicochemical characterization. The DSC and XRD studies revealed that the crystalline QUE was converted into an amorphous form which could be the major mechanism behind the solubility enhancement of QUE. The obtained results of the solubility and dissolution were statistically evaluated to check the possible model of the influence of the factors. From the ANOVA studies of the respective models, all three factors were found to influence solubility and dissolution significantly. The combination at 60% w/w HPβ-CD and 5% w/w tween 80 by solvent evaporation was the optimized ICQ. The obtained solubility and dissolution results of this optimized ICQ were found to be correlated with the values predicted by design. This designated that QbD was successfully applied for the development of ICQs. Further, it was inferred that the inclusion of tween 80 as a surfactant could successfully reduce the concentration of HPβ-CD in attaining the desired solubility. All these results and their statistical inferences revealed that the formed hypothesis was proved correct and the set objectives were successfully achieved.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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