

ONE POT DEVELOPMENT OF LIPID-BASED QUERCETIN SPHERICAL AGGLOMERATES FOR BIOAVAILABILITY ENHANCEMENT: *IN VITRO* AND *IN VIVO* ASSESSMENTS

RAKESH MISHRA^{1*} , SHWETA KULKARNI², AKASH AHER³

^{1,2,3}Department of Pharmaceutics, Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune, India

*Email: mishrarakesh287@gmail.com

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ABSTRACT

Objective: Quercetin, a wonder flavanoid despite numerous pharmacological actions, has limited clinical applications due to solubility and permeability issues and additionally having shorter biological half-life. The goal of the current work was to design Quercetin lipid-based spherical crystals, to improve its oral bioavailability and sustain its *in vivo* plasma levels.

Methods: An anti-solvent precipitation method was employed to prepare quercetin spherical agglomerates using ethanol and distilled water as good and bad solvents, respectively. As bridging liquid chloroform, dichloromethane, hexane and gelucire 43/01, compritol 888 as lipid carrier were screened. The drug-to-lipid polymer proportion and stirring speed effect were optimized by 3-level, 2-factor, experimental design. Numerical optimization function was employed to identify the optimum level of independent variables. Spectroscopic, micromeritic, surface morphology, size distribution, saturated solubility, *in vitro* dissolution, *in vivo* pharmacokinetic and stability studies were performed.

Results: Surface morphology studies indicated the agglomeration of quercetin needle-like fragments into a spherical shape, which further showed smooth surfaces due to entrapment of QC in lipid carrier. The spherical agglomerates of quercetin showed a four-fold improvement in aqueous solubility compared to pure drug and showed 92.13% release in 8 h. The optimised formulation showed a 3.69-fold enhancement in relative bioavailability in contrast to the marketed preparation in an *in vivo* pharmacokinetic analysis in male Wistar rats.

Conclusion: The obtained lipid-based spherical crystals of quercetin with enhanced bioavailability could be effectively used for its various potential pharmacological applications. The designed system can also be utilized to deliver other phytochemicals with poor bioavailability due to limited solubility and permeability.

Keywords: Quercetin, Spherical crystallization, Anti-solvent precipitation, Solubility, Permeability, Gelucire 43/01, Lipid-based agglomerates

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INTRODUCTION

Quercetin (QC) is a well-known plant flavonoid having wide appearance in our regular diet like onions (red and white), red apples, grapes, red wine, green/black tea, cranberries, green hot peppers, kale, blueberries, pears and spinach [1]. QC is derived from the Latin word quercetum, which meaning "oak forest" or "Quercus" (oak). It's a flavonoid polyphenol that belongs to the flavonol subclass. The scientific community is interested in QC due to its potent anti-oxidant effect responsible for its various pharmacological activities [2-4]. In addition, numerous scientific reports have confirmed the potential clinical applications of QC including antiviral, anti-arthritis, anti-obesity, anti-diabetic, anti-inflammatory, cardiovascular disease, antioxidant antimicrobial, Alzheimer's disease, anti-asthmatic and anti-cancer [5-10].

Even though QC demonstrated promising therapeutic effect, it exhibits poor oral bioavailability due to reduced aqueous solubility, and permeability and additionally having shorter biological half-life of 2-2.5 h [11-13]. Numerous attempts were employed to enhance solubility and permeability of QC, including micronization, solid dispersion, inclusion complexes, pro-drugs, nano-emulsion, polymeric micelles, liposomes, and microspheres. Among them the lipid-based systems confirmed significant enhancement in solubility and permeability but having issues of either poor drug loading ability, long-term stability and toxicity [14-19].

Spherical crystallization a particle engineering approach in which crystallization-agglomeration occurs concurrently in single step. Spherical agglomeration is one technique that have been widely employed for improvement in solubility, flow properties, dissolution profile and bioavailability of drugs [20-22]. It easily allows precipitation, agglomeration and encapsulation of drug in polymer into spherical particles in one step, which can be explored for solubility, permeability enhancement and drug release modification to improve patient compliance [23].

Hence, lipid-based spherical agglomerates of QC can be designed and developed to improve solubility and permeability, minimize administration frequency, and improve the micromeritic characteristics of needle-shaped raw QC. The formulation of QC lipid-based spherical agglomerates can also circumvent the drawbacks associated with the earlier developed QC systems [24].

The present study aims to prepare spherical agglomerates of QC by anti-solvent precipitation method using ethanol and water as good and bad solvents, respectively. As bridging liquid chloroform, dichloromethane and hexane were screened. Gelucire 43/01 was used as polymer lipid carrier. The drug: polymer lipid ratio and stirring speed effect was also studied and optimized using a 3²-factorial design.

MATERIALS AND METHODS

Materials

Quercetin was acquired from Himedia Laboratories, Mumbai, India., Gelucire 43/01 and Compritol 888 obtained from Gattefosse Mumbai, India. The other reagents and solvents were of analytical quality.

Methods

Quercetin spherical agglomerates preparation

Anti-solvent precipitation method was employed to prepare QC spherical agglomerates using ethanol and distilled water as a good solvent and bad solvent, respectively [25, 26]. Chloroform, dichloromethane and hexane were screened as bridging liquid. Batches (B1-B7) were prepared to optimize the type and amount of bridging liquid (table 1) and subjected to saturated solubility studies using orbital shaking incubator (Remi Cis-18 Plus) [27]. Further batches were prepared by incorporation of polymer lipid carrier. Gelucire 43/01 and Compritol 888 screened as lipid carrier. The

weighed amounts of QC 500 mg were dissolved in 5 ml of ethanol, than a sufficient amount of distilled water was added under continuous stirring using a propeller type agitator. When fine crystals of QC began to form, bridging liquid and polymer lipid

carrier were added drop-wise to obtain spherical agglomerates. A controlled speed stirrer was used to stir the mixture for 0.5 h (150, 300 and 450 rpm). The agglomerates were filtered and dried at room temperature for 24 h.

Table 1: Formulation of spherical agglomerates

Batch number	Bridging liquid	Bridging liquid amount (ml)	Stirring speed (Rpm)
B1	Chloroform	1	300
B2	DCM	1	300
B3	Hexane	1	300
B4	Chloroform	0.5	300
B5	Chloroform	1.5	300
B6	Chloroform	1	150
B7	Chloroform	1	450

Optimization of formulation

A 3²-experiment factorial design was adopted for optimization. The independent variable selected was drug: polymer ratio (X1), stirring speed (X2) and drug release in 8 h (Y1) as dependent variable (table 2). The 09-run experiment indicated in (table 3), was analysed using Design Expert (Stat-Ease, version 10).

Evaluation of QC spherical agglomerates

Calibration curve of QC

QC stock solution of 100 µg/ml prepared in media containing 0.2% w/v SLS and a spectrophotometric calibration curve in the range of 2-20 µg/ml at 374 nm was determined (Schimadzu 1700, Japan).

Table 2: Independent variables levels in the current study

Factors	Coded value			Actual value		
	Low	Medium	High	Low	Medium	High
Drug: Polymer lipid ratio	-1	0	+1	1:0.5	1:1	1:1.5
Stirring speed (rpm)	-1	0	+1	150	300	450

Table 3: Experimental formulation batches of lipid-based spherical agglomerates

Batch number	Drug: polymer lipid ratio (Gelucire 43/01)	Stirring speed (Rpm)
B8	1:0.5	150
B9	1:1	150
B10	1:1.5	150
B11	1:0.5	300
B12	1:1	300
B13	1:1.5	300
B14	1:0.5	450
B15	1:1	450
B16	1:1.5	450

*Each batch containing 500 mg QC, ethanol, water as good and bad solvent, respectively with 1 ml Chloroform as bridging liquid

Fourier transform infrared (FTIR) spectroscopy

FTIR scans of QC, gelucire 43/01 and physical mixture of drug and excipient were performed (Shimadzu, 8400S, Japan) between 4000 to 4000 cm⁻¹ to determine the compatibility of drug and lipid polymer [28].

Optical microscopy

The external morphology and shape of spherical agglomerates was studied by optical microscopy using Saglo software (SGL-11, India). The drug and agglomerates sample was taken on glass slide and is observed under 45x magnification.

Micromeritic and size distribution analysis

The prepared agglomerates Carr's index and Hausner's ratio were calculated. By taking a small amount of material on a slide, the size determination assessed by optical microscopy containing calibrated eye piece with stage micrometre. The mean diameter of about 50 spherical agglomerates was computed after they were measured [29-31].

Drug content and percentage yield

Each batch drug content was evaluated by dissolving agglomerates equivalent to 25 mg of QC in enough methanol. To achieve thorough

drug extraction, the mixture is sonicated for 0.5 h and then filtered. The drug content was evaluated spectrophotometrically at 374 nm (Shimadzu 1700, Japan) (n = 3). For each batch, the percentage yield of prepared agglomerates was calculated.

Scanning electron microscopy (SEM)

SEM was employed to inspect the surface morphology of drug and QC agglomerates (NOVA, NanoSEM, NPEP 303, USA). At a voltage of 20 kV, photomicrographs of the agglomerates were taken.

Crystallinity of QC spherical agglomerates

Differential scanning calorimetry (DSC) (Metler Toledo, USA) was used to detect the crystallinity of QC, Gelucire 43/01 and prepared QC spherical agglomerates. 1-2 mg of sample was placed in aluminium pan with a nitrogen flow rate of 50 ml/min. The temperature range was from 30 to 350 °C with heating rate of 10 °C/min. The powder X-ray diffraction (PXRD) pattern of the samples were obtained on Philips 1830 X-ray diffractometer (Philips, Almelo, The Netherlands) using a CuK α source at a ($\lambda=1.5406 \text{ \AA}$).

In vitro dissolution study

Using a USP Type-I dissolution equipment, the release of QC from the spherical agglomerates was investigated (Veego-VDA-8D, India). Spherical agglomerates containing 25 mg of QC were filled into

capsules and placed in 900 ml 0.1 N HCl for two hours before being transferred to PBS 6.8 for another four hours at $37 \pm 0.5 \text{ }^\circ\text{C}$ at 100 rpm. Sink conditions were maintained while 5 ml samples were taken at predefined time intervals (0.5, 1, 2, 3, 4, 5, 6, 7, and 8 h). At 374 nm, samples were filtered and spectrophotometrically examined [28]. The optimised batch obtained cumulative drug release data was also analyzed with different mathematical models to reveal release kinetics [32-35].

Statistical optimization

Design-expert® software was used to analyse the data for the dependent variable, which was the percent drug release after 8 h for all formulations. Polynomial models were created for all of the responses. The recommended model was chosen and subsequently analysed using ANOVA to discover relevant model terms. The numerical optimization function based on the desirability technique was also employed in the study [36, 37].

Stability studies

The optimized QC spherical agglomerate batch was subjected to six month accelerated stability testing following ICH criteria. The agglomerates were stored in 05 ml glass vials at $40 \text{ }^\circ\text{C}$ and 75 percent relative humidity before being evaluated for drug concentration and release at predetermined time interval [38, 39].

In vivo pharmacokinetic studies

In vivo pharmacokinetic studies conducted to assess and compare the oral bioavailability of QC and marketed preparation. The investigations carried out in male Wistar rats (~ 200 g) were permitted by Institutional Animal Ethical Committee (DYPIPSR/IAEC/Jan/21-22/P-32) and in accordance with CPCSEA, India. Two groups were randomly formed, consisting of 06 rats in each group. Before experimentation, rats have fasted overnight. Orally, QC spherical agglomerates and a marketed formulation (Health vit quercetin) equivalent to 50 mg/kg of QC were given to the respective groups [36]. The samples were dispersed in 0.1 % CMC prepared in distilled water and administered to rats by oral gavage 18G. Blood samples (0.5 ml) were taken at preset time intervals (0.25, 0.5, 1, 2, 4, 8, 12, and 24 h) after the oral dose. Plasma was extracted and stored at $-20 \text{ }^\circ\text{C}$ until analysis by centrifuging at 4000 rpm for 15 min. The plasma concentrations of QC were estimated by reversed-phase HPLC on a Shimadzu HPLC LC20 AD (Shimadzu Corporation, Japan). The mobile phase was made up of acetonitrile and 0.1 % formic acid (60:40). In the concentration between 5 to 100 $\mu\text{g/ml}$ calibration curve were obtained and utilized to assess plasma QC concentration. The QC concentration and the peak areas plot showed linear relationship with a good correlation coefficient ($r^2 = 0.993$). A 100 μl plasma sample was mixed with 900 μl acetonitrile, vortexed and centrifuged for 15 min at 10000 rpm to assess QC content in rat plasma. The resulting supernatant filtered through 0.2 μm filter before being injected into HPLC column in a volume of 20 μl . The flow rate was set aside at 1 ml/min, and the UV detector was adjusted to 374 nm [40, 41]. The major pharmacokinetic parameters (C_{max} , AUC, T_{max} , MRT) and relative oral bioavailability were calculated and statistically analyzed by PK solver software (version 2.0).

Anti-oxidant activity of QC spherical agglomerates

To determine the enhancement in the antioxidant effect of QC spherical agglomerates (batch, B-14) compared to pure drug anti-peroxidation and antiradical activity was evaluated. The anti-peroxidation effect was determined by estimating the percentage inhibition of lipid peroxidation employing the method of Pryor and Stanley, 1975 [42]. The antiradical activity was measured by DPPH inhibition method (Mensor LL *et al.*, 2001) [43].

RESULTS AND DISCUSSION

Preliminary screening results

Initially, QC agglomerates batches (B1-B7) were prepared to optimize the type and amount of bridging liquid. The optical microscopy study suggested that no agglomeration occurs in batches prepared with dichloromethane and hexane (B2 and B3) used as

bridging liquid. On the other side chloroform as bridging liquid in the same amount was able to form the QC agglomerates (batch B1). In batches, B4 and B5 amount of chloroform varied (i.e. 0.5 and 1.5 ml) and microscopic studies were conducted. Incomplete agglomeration was observed in batch B4. The microscopic studies suggested that 1 ml chloroform is sufficient for agglomerate formation for the working batch size. Additionally, to check the effect of stirring speed on agglomerate formation, batches (i.e. B6 and B7) were taken with 1 ml chloroform at different speeds. The microscopic findings indicated the impact of speed on size and shape of agglomerates (fig. 1). The size of QC spherical agglomerates found between 190 μm to 400 μm at different stirring speeds employed in current study. Optical microscopic images of the pure QC show needle-like crystals. To determine the improvement in solubility of QC saturated solubility studies were conducted in different media at room temperature. Nearly four fold enhancement in aqueous solubility of QC agglomerates (batch B1, $0.012 \pm 0.001 \text{ mg/ml}$) was observed compared to pure QC ($0.003 \pm 0.0002 \text{ mg/ml}$).

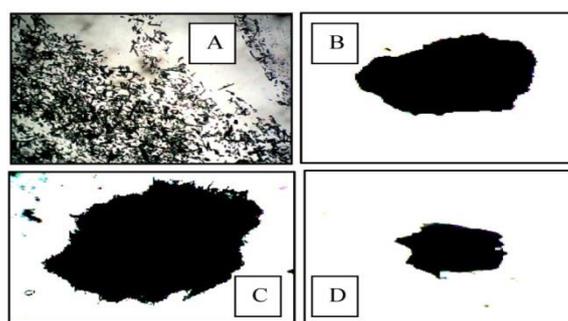


Fig. 1: Optical microscopy of A. Pure QC, B. Batch B1, C. Batch B6, D. Batch B7 at 45 x

Further batches were prepared by incorporating polymer lipid carriers to sustain the *in vivo* plasma levels of QC. Gelucire 43/01 and Compritol 888 screened as lipid carrier. The preliminary *in vitro* release studies depicted that Compritol 888 could not delay drug release for more than 4 h (data not shown). Subsequently a 3^2 factorial design employed to study QC: Gelucire 43/01 ratio and stirring speed effect on drug release in 8 h for optimization.

Evaluation of spherical agglomerates

UV spectroscopic studies

The λ_{max} of QC in different media was observed at 374 nm. The calibration curves of QC taken in range of 1-20 $\mu\text{g/ml}$ following Beer-Lamberts law shown in fig. 2.

Fourier transform infrared (FTIR) spectrum

The FTIR spectra of QC showed characteristic peaks at 677.04, 806.27, 931.65 cm^{-1} (C-H bending), 1012.66 cm^{-1} (C-O), 1273.06 cm^{-1} (C-CO-C stretching), 1384.94 to 1012.66 cm^{-1} (in-plane =C-H bending), 1529.60, 1599.04 and 1662.69 cm^{-1} (aromatic C=C stretching), 1662.69 cm^{-1} (C=O aryl ketonic stretching), 1384.94 cm^{-1} (OH bending), 3421.83 and 3176.87 cm^{-1} (OH stretching) indicated in fig. 3. The major peaks in the IR spectrum are identical to functional groups of QC. The characteristic peaks for Gelucire 43/01 are 3,695.59 to 3,190.72 cm^{-1} (OH stretching), 2,877.11 cm^{-1} (C-H stretching), 1,446.73 to 1,174.32 cm^{-1} (C-H bending), 1,728.99 cm^{-1} (C=C stretching), 1,124.27 cm^{-1} (C-O-C stretching), 1,174.69 cm^{-1} (C-O stretching), 842.71 cm^{-1} (C-C stretching) and 727.97 cm^{-1} (C=C bending). The characteristic peaks for QC SA with gelucire 43/01 are at 644.29, 806.27, 964.44 cm^{-1} (out of plane C-H bending), 1196.91 cm^{-1} (C-CO-C stretching), 1340.57, 1196.91 cm^{-1} (in plane =C-H bending), 1514.17 and 1612.54 cm^{-1} (aromatic C=C stretching), 1612.54 cm^{-1} (C=O aryl ketonic stretching), 3386.18 and 3257.88 cm^{-1} (OH stretching). The major peaks of QC found in the IR spectrum of the physical mixture with a minor shift in wave numbers indicated that the drug and the lipid carrier were compatible.

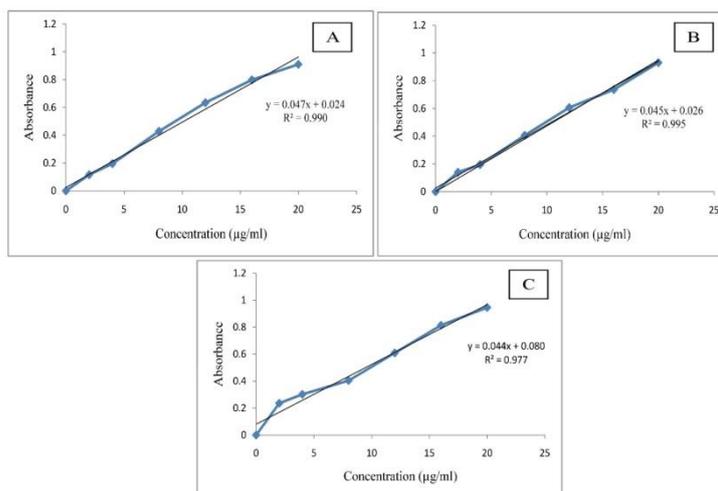


Fig. 2: Calibration curve of QC in A. Distilled water, B. 0.1 N HCl (pH 1.2), C. PBS (pH 6.8) at 374 nm

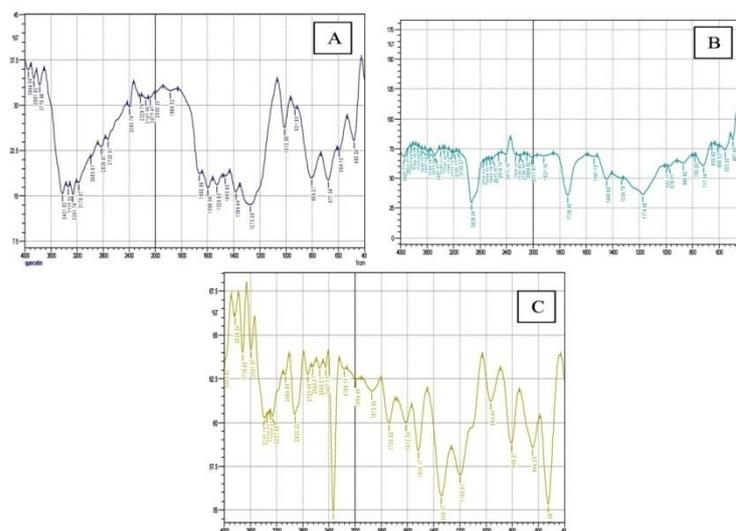


Fig. 3: FTIR scans of A. Quercetin, B. Gelucire 43/01, C. Physical mixture of QC and gelucire 43/01

Micromeritic and size distribution analysis

Micromeritic studies of spherical agglomerates revealed that all formulation batches had good flow characteristics, with Carr’s index ranging from 12.61±1.22% to 18.43±0.8%, and Hausner’s ratio between 1.14±0.02 to 1.19±0.03. The large decrease in inter-particle friction owing to their spherical form and minor electric static

charge could be attributed to the improved flowability of agglomerates. Normal size distribution curve was plotted for size distribution studies of batches (B8–B16) prepared at different stirring speeds (fig. 4). Batch (B8–B10), (B11–B13), (B14–B16) agglomerates size obtained were between 371-390 µm, 311-330 µm and 191-200 µm respectively. The results indicated the decrease in size of agglomerates with increasing stirring speed.

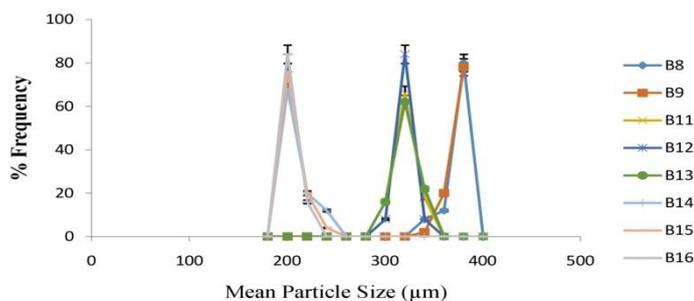


Fig. 4: Normal size distribution curve for QC spherical agglomerates batches (B8–B16)

Drug content and percentage yield

UV spectroscopy was used to evaluate the drug concentration in all of the spherical agglomerates formulations. The content uniformity

of QC in prepared spherical agglomerates was in between 96.40±1.3 to 98.46±1.7 %, indicating fine content uniformity. The percentage yield for all batches was found in the range of 83±2.6 to 90±1.9 %.

Scanning electron microscopy

The surface appearance of pure QC, QC spherical agglomerate batch (B1) and QC spherical agglomerate with lipid carrier batch (B14) was studied. The uneven flake shapes of pure QC crystals with needle-like fragments were observed in photomicrographs shown in fig. (5A). The surface morphology studies indicated the agglomeration of QC needle-like fragments into a spherical shape, which further showed smooth surfaces due to lipid carrier deposition indicated in fig. (5B and 5C).

Crystallinity of QC spherical agglomerates

DSC and PXRD studies were performed to study the crystallinity of prepared spherical agglomerates. The QC DSC curve showed characteristic onset endothermic peaks at 103.30° and 308.12 °C as a result of the evaporation of bonded water and melting of drug. The Gelucire 43/01 showed characteristic onset endothermic peak at

42.81 °C. However, spherical agglomerates did not show characteristic endothermic peaks of pure drug (fig. 6). The disappearance of QC endothermic fusion peak in the formulation indicated the conversion of it into an amorphous state. The QC exhibits sharp crystalline peaks at 2θ value of 27.52°, 27.30°, 27.24°, 23.84°, 23.82°, 23.72°, and 23.74° depicting crystalline behaviour. Gelucire 43/01 also depicted slight crystallinity as two characteristic peaks were observed at 2θ value of 19.55°, 23.82° and 22.13°, 23.73° respectively. The sharp crystalline peaks of QC disappeared in the spherical agglomerates formulation (fig. 7). The reduction in intensity and disappearance of QC peak in the formulation indicated that QC is no longer present as a crystalline material but is converted into an amorphous state in the agglomerate formulation. The results of DSC and PXRD collectively signifying that the QC is present in a molecularly dispersed state in the agglomerate formulation.

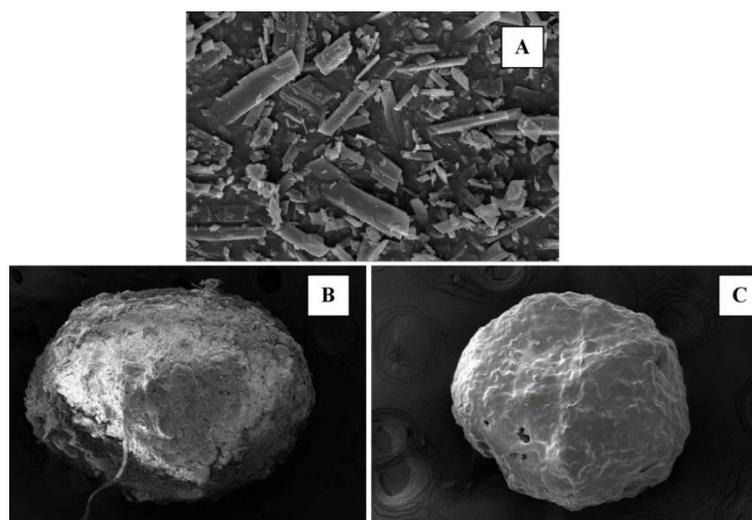


Fig. 5: Scanning electron microscopy photographs of A. Pure drug QC, B. QC spherical agglomerates (batch-B1), C. QC spherical agglomerate with lipid carrier batch (batch-B14) at 250 x

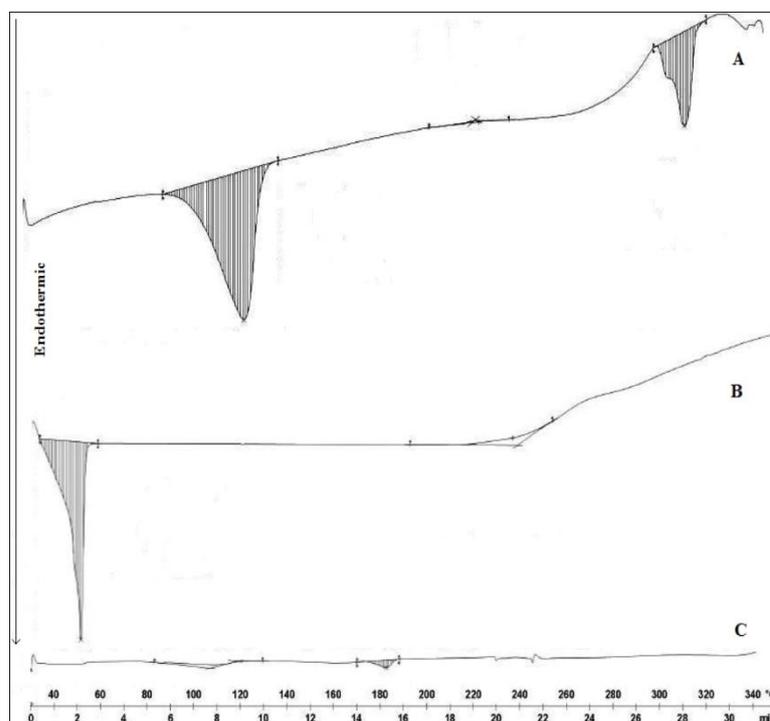


Fig. 6: DSC thermograms of (A) QC, (B) Gelucire 43/01 (C) QC Spherical agglomerates

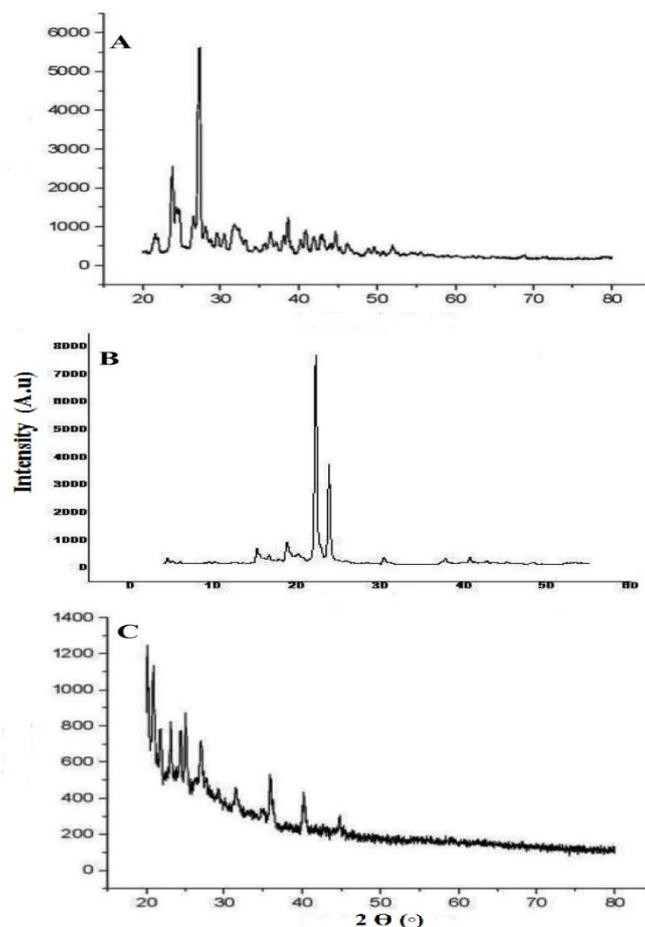


Fig. 7: Powder X-ray diffractograms of (A) QC, (B) Gelucire 43/01 (C) QC Spherical agglomerates

In vitro drug release studies

The *in vitro* dissolution profile of pure QC drug and prepared QC spherical agglomerates without lipid carrier formulation (batch—B 1) was estimated in 0.1 N HCl fig. 8 A. In 2 h, the pure QC and QC spherical agglomerates released about 22.14% and 87.57% percent of the drug, respectively. Due to QC low intrinsic solubility, the pure drug had a slow dissolving rate. The solubility of pure drug compared to drug spherical agglomerates (batch B 1) was found to be in distilled water (0.003 mg/ml, 0.012 mg/ml), 0.1 N HCl pH 1.2 (0.001 mg/ml, 0.0045 mg/ml), phosphate buffer pH 6.8 (0.006 mg/ml, 0.041 mg/ml) respectively.

As more than 4 fold enhancement in solubility of QC agglomerates (batch B1) was observed compared to pure QC responsible for increased dissolution rate fig. 8 B. The agglomeration of QC needle-like crystal into a spherical shape could be the probable reason of improved solubilisation and dissolution. Further, the *in vitro* release profile of QC spherical agglomerates containing lipid carrier was also

determined batches (B8-B16). The dissolution studies revealed the significant effect of lipid carrier gelucire 43/01 and stirring speed on drug release. As the amount of lipid carrier increases with lower level of stirring speed, significant drug release retardation was observed. This could be attributed that with the increasing amount of lipid carrier and increased mean size of agglomerates at lower stirring speed, medium penetration reduces, leading to sustain drug release. The batches (B8 to B16) prepared at different stirring speed with varying drug to lipid carrier gelucire 43/01 ratio showed sustained release for 8 h in the range of 63.3%±1.77 to 94.13%±1.98 shown in fig. 9. Various mathematical models were also applied to the optimized formulation batch (B14) to estimate the QC release kinetics. The regression values obtained for Zero order, First order, Higuchi release, and Korsmeyer-Peppas (n value) were 0.996, 0.901, 0.993 and 0.73, respectively. The obtained regression coefficients found highest fit for Zero order and Higuchi release, indicating both dissolution and diffusion mechanism responsible for drug release.

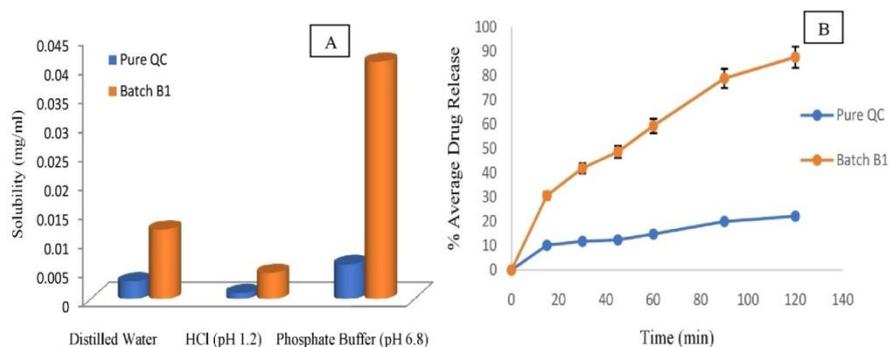


Fig. 8: A. Solubility and B. *In vitro* release profile of pure drug QC and spherical agglomerates (Batch-B1), mean±SD; n = 3

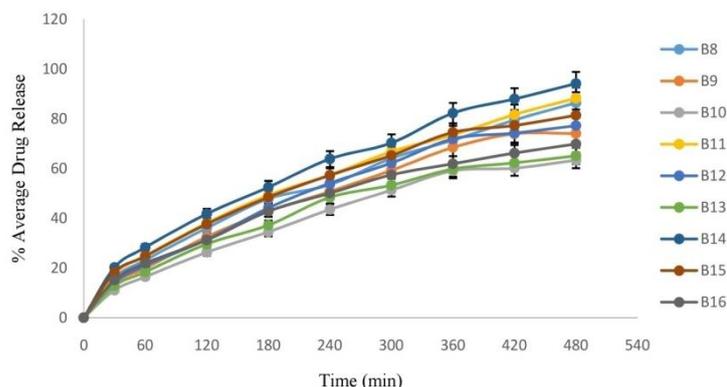


Fig. 9: *In vitro* release profile of lipid-based spherical agglomerates batches (B8-B16), mean±SD; n = 3

Statistical optimization

The response i.e., percentage release in 8 h for batches (B8-B16) were fixed into various models by design expert software. In fit summary quadratic model was suggested and accordingly, ANOVA and statistical parameters results were obtained indicated in table 4. The response had significant p values (<0.05), indicating a significant effect of model terms. The statistical parameter analyses showed a reasonable agreement of adjusted R2 with the predicted R2. The adequate precision also found to be greater than 4, which is desirable, indicating a lower error magnitude. The response surface plot and regression equation was also generated. Response plot showed considerable effect of drug: lipid polymer ratio and stirring speed on release profile. With decreasing levels of gelucire 43/01 and increasing levels of the stirring speed, the drug release rate increases shown in fig. 10. The lipid-based spherical agglomerates prepared at different stirring speed with drug: lipid polymer ratio of 1:0.5 showed 86.37% to 94.13% of drug release in 8 h. However, batches prepared with an increasing amount of polymer ratio i.e., 1:1 and 1:1.5 showed 74.04% to 81.34% and 63.30 % to 69.81 % of drug release in 8 h, respectively. The higher

hydrophobicity of gelucire 43/01 and decreased mean particle size at higher stirring speed level probable reasons behind the QC drug release as predicted by the surface area relationship [44, 45]. The regression equation coefficients (Y1) indicated a prominent negative and positive effect of drug-to-lipid polymer ratio and stirring speed on QC drug release. The predicted vs actual diagnostic plot also revealed reasonable agreement of experimental and predicted values, indicated employed design space is suitable for navigation. Subsequently, the optimum level of independent variables were identified by applying constraints keeping independent variables in range and drug release in 8 h set to maximum. The best formulation was chosen based on the criteria of getting the most drug release in 8 h. The suggested batch is based upon the highest desirability value indicated in table 5. The recommended value of drug release was found to be close to the experimental value.

$$\text{Average drug release (\%)} (Y1) = +77.03 - 11.75 A + 3.59 B + 0.41 A^2 + 1.11 B^2 - 0.31 AB$$

Where, A: Drug: Lipid polymer ratio, B: Stirring Speed

Table 4: Summary of ANOVA and statistical parameters data for response (Y1)

Source	Sum of squares	Degree of freedom	Mean square	F value	p-value prob>F	Remark
Quadratic Model	239.98	5	181.98	985.88	<0.0001	Significant
Parameters-Response-Percentage release in 8 h						
Mean	Standard deviation	Coefficient of variation	R ²	Adjusted R ²	Predicted R ²	Adequate precision
77.56	0.43	0.55	0.998	0.997	0.989	105.12

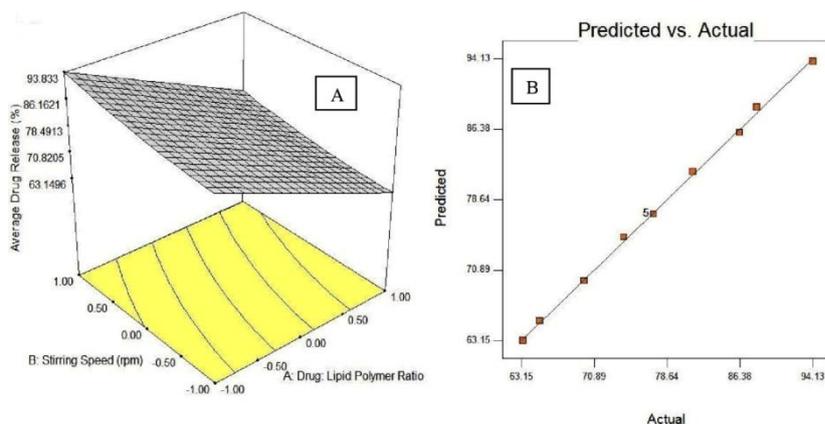


Fig. 10 A) Surface response plot B) Actual and predicted values of % drug release in 8 h

Stability studies

The drug content and percent drug release analysis at 40 °C/75% RH for six months are indicated in table 6. The prepared QC spherical

agglomerates were stable since no significant drug content or release changes were found during storage. The prepared QC agglomerates to maintain excellent stability, can be stored below 40 °C with no significant loss in drug content and percent drug release.

Table 5: Summary of numerical optimization of optimized formulation (B14)

Parameters	Goal	Solution	Desirability	Remark
Independent variables			0.99	Selected
Drug: Polymer lipid ratio	in range	-1 (1:0.5)		
Stirring speed	in range	+1 (450rpm)		
Dependent Variables				
Percent drug release in 8 h	Maximum	93.83 %		

Table 6: Accelerated stability studies data for batch (B14)

Month	Drug content (%)	Average drug release at 8 h (%)
0	98.46±1.73	94.13±1.96
1	97.99±1.38	94.01±2.01
3	97.04±0.99	93.66±1.08
6	96.46±1.56	92.25±1.83

mean±SD; n = 3

In vivo pharmacokinetics-study

The results of *in vivo* pharmacokinetic studies indicated a significant difference ($P < 0.05$) in values of C_{max} , T_{max} , AUC and MRT between QC formulation (batch B14) and marketed preparation. The comparative time vs mean plasma concentration profiles of prepared formulation and marketed preparation of QC are indicated in fig. 11. The C_{max} , T_{max} , $AUC_{0-\infty}$ and MRT for QC formulation (batch B14) found to be $203.44 \pm 2.01^* \mu\text{g/ml}$, $4.00 \pm 0.16^* \text{h}$, $1142.33 \pm 4.32^* \mu\text{g-h/ml}$ and $6.87 \pm 0.21^* \text{h}$ respectively compared to marketed preparation $137.23 \pm 4.01^* \mu\text{g/ml}$, $1.00 \pm 0.09^* \text{h}$, $309.756 \pm 5.46^* \mu\text{g-h/ml}$ and $1.89 \pm 0.11^* \text{h}$. The higher C_{max} , T_{max} and MRT value of the prepared QC formulation indicated sustained *in vivo* plasma levels of drug. Considering the AUC values of QC formulation (batch B14) and marketed preparation, a 3.69-fold enhancement in relative bioavailability was observed.

Anti-oxidant activity of QC spherical agglomerates

The anti-peroxidation and antiradical activity of pure QC and spherical agglomerates (batch, B-14) was compared to determine any significant enhancement in antioxidant capacity. The QC formulation showed highest percentage of peroxidation inhibition 79.21 ± 1.14 compared to pure drug 30.17 ± 2.23 (fig. 12 A). The studies suggested a dose-dependent effect of QC on the percentage inhibition of peroxidation. However decrease in the anti-peroxidation effect of pure drug was observed above $80 \mu\text{g/ml}$ concentration possibly due to occurrence of a pro-oxidant mechanism. The results of DPPH radical scavenging activity revealed that they possessed excellent dose-dependent antioxidant capacity. The scavenging activities of QC spherical agglomerates were found to be 1.5 fold higher compared to pure drug fig. 12 B). The improvement in its overall bioavailability may be the likely cause of QC spherical agglomerates increased antioxidant potential.

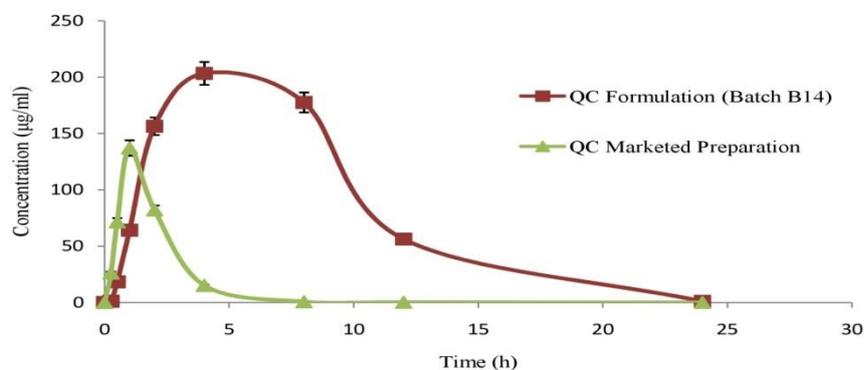


Fig. 11: QC time and mean plasma concentration profiles of QC formulation (batch B14) and marketed preparation, mean±SD; n = 3

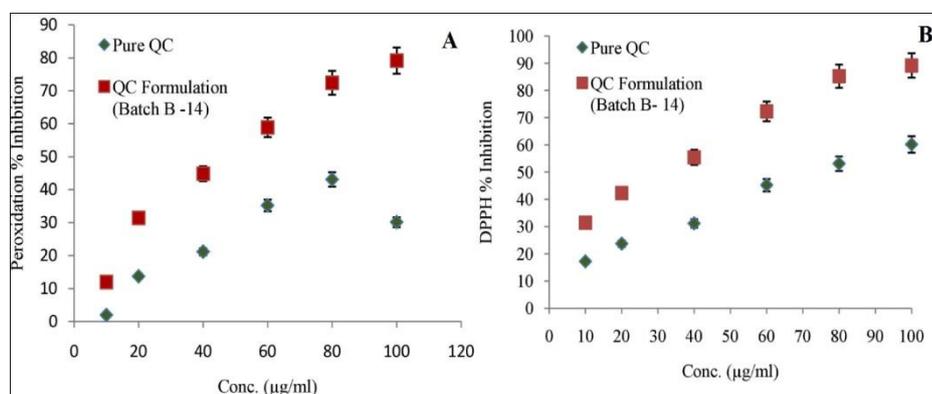


Fig. 12: A. Percentage peroxidation inhibition; B. Percentage DPPH inhibition of QC formulation (batch B14) and pure drug, mean±SD; n = 6

CONCLUSION

In the current study, QC spherical agglomerates were successfully prepared using ethanol and water as good and anti-solvent, chloroform as bridging liquid and Gelucire 43/01 as polymer lipid carrier by anti-solvent precipitation method. The findings of the current investigation demonstrated the application of spherical agglomeration for the bioavailability enhancement of QC. The prepared spherical agglomerates of QC without lipid carrier showed four-fold improvements in aqueous solubility and dissolution compared to pure drug. Batches prepared with incorporation of polymer lipid carrier at different stirring speed showed sustain drug release profile with 94.13 % drug release in 8 h for optimized batch (B14). The numerical optimization under design expert software based on the desirability function identified the optimum level of drug: polymer lipid ratio and stirring speed as 1:0.5 and 450rpm. The microscopic studies confirmed the agglomeration of QC needle shape crystals into a spherical shape. The estimated *in vivo* pharmacokinetics parameters indicated the sustain plasma levels of drug with 3.69 fold increase in relative oral bioavailability compared to marketed preparation. The obtained QC spherical agglomerates can be a suitable choice for its delivery to treat various diseases with improved patient compliance.

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ETHICS APPROVAL

The *in vivo* pharmacokinetic studies carried out in male Wistar rats were approved by the Institutional Animal Ethical Committee (DYPIPSR/IAEC/Jan/21-22/P-32) and all experimentation performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

AUTHORS CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by RM, SK and AA. The first draft of the manuscript was written by RM and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTERESTS

The authors declare no competing interest.

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