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Original Article

Quality by Design based Quercetin Hydrate Nanoemulsions for Enhanced Solubility by Reducing Particle Size

Lakavath Sunil Kumar[1,*], Hindustan Abdul Ahad[2]

[1]Department of Pharmacy, Jawaharlal Nehru Technological University, Ananthapuramu, Andhra Pradesh, INDIA.
[2]Department of Industrial Pharmacy, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)- Autonomous, K.R. Palli Cross, Chiyyedu (P), Ananthapuramu, Andhra Pradesh, INDIA.

ABSTRACT

Aim/Background: The oral-based drug delivery system has a great pace in this era of novel discoveries. The globe is running toward new medical dosage forms, but from the primer days of drug discovery to now, a major issue faced by pharmaceutical scientists is solubility and bioavailability issues. The nanoemulsions are the best suitable formulations which can upsurge the bioavailability of the insoluble drugs. In the past three years, many research activities have been conducted due to the pandemic situation. Almost all nations have concentrated on scientific and medical research during this process. **Materials and Methods:** In recent days, quercetin hydrate has been found to have anti-malarial activity for which the bioavailability can be uplifted by using Nanoemulsion formulations. The authors used the high-energy process for formulating the nanoemulsions with the support of design expert software, where it was easy to find the number of trials to be performed. Various tools are used for the optimization of formulations for novel drug delivery systems. These tools have been found advantageous as they lead to a reduction in the number of experiments and less wastage of costly reagents. The purpose of the selection of a Central Composite Design (CCD) was that it required fewer runs over various other designs. **Results:** According to the design expert, CCD software was accessible for 17 runs, which corresponded to 17 groupings or formulations. Batches produced by the experimental design were formulated and assessed for globule size and dispersibility. **Conclusion:** Even though quercetin hydrate has been approved as a remedy for the treatment of various disorders, its poor oral bioavailability due to poor aqueous solubility and variable absorption is still a challenge in its clinical applications. Quercetin hydrate-loaded nanoemulsion fabricated with *Opuntia ficus indica* seed oil, PEG400, tween 80, and ethanol resulted in getting nano-sized particles that help in drug solubility and bioavailability. This work illustrated the importance of nanoemulsion to enhance the bioavailability of Quercetin hydrate.

Keywords: Quercetin hydrate, Bioavailability, Design expert, Globule size, Nanoemulsion.

Correspondence:
Lakavath Sunil Kumar, B.Pharm, M.Pharm
Research Scholar, Department of Pharmacy, Jawaharlal Nehru Technological University, Ananthapuramu-515002, Andhra Pradesh, INDIA.
Email: lakavath.sunil@gmail.com

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INTRODUCTION

In this modern era, a high scope has been given to lipid-based preparations to enhance the oral Bioavailability (BA) of weak aqueous soluble drugs.[1] The most commonly used methods involve combining the lipophilic drug with hydrophobic transporters such as oils, emulsifier dispersions, emulsions, liposomes, micro or Nanoemulsions (NEs),[2] and self-emulsifying formulations. All these improve the surface area of the lipophilic drugs to raise their solubility performance. From this outlook, oral drug formulations based on lipids are measured to improve the solubility and absorption of lipophilic drugs.[3]

Many scientists have investigated a range of approaches similar to Self-Nano Emulsifying Drug Delivery Systems (SNEDDS), liposomes, and pro-liposomes to advance the BA of Quercetin Hydrate (QH).[4] Amongst the different lipid-based formulations, NEs have gained an edge and have been exploited vastly for improving the aqueous solubility,[5] thereby BA. They are capable of delivering the drug in its molecular form. Additionally, these are kinetically steady systems and their nanosized globules are liable for their stability as they are insensible to gravitational force. For an extensive range of weakly aqueous soluble drugs such as medicine,[6] breviscapine,[7] and itraconazole,[8] NEs have been used for upgrading oral BA. The advantages of preparing NEs include their utilisation to incorporate lipophilic drug molecules into the oil phase, hence increasing their solubility.[9] Generally, high-energy emulsification and low energy emulsification are the two basic methods employed for making NEs, and both methods

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can produce stable NEs. High mechanical energy (disruptive force) is utilised to disrupt the phases, resulting in the formation of small-sized droplets in the energy emulsification procedure.[10] The key advantages of this method are its simplicity and ease of implementation.

QH is a flavonoid component that can be found in a variety of foods, including onions, grapes, berries, cherries, broccoli, and citrus fruits.[11] It is a versatile antioxidant known to possess protective abilities against tissue injury induced by various drug toxicities.

In the present investigation, an effort has been made to develop QH-loaded NEs using *Opuntia ficus indica* (OFI) as the oil phase, Polyethylene Glycol (PEG)-400 as a surfactant, and tween-80 as a co-surfactant to increase QH solubility. High-pressure NEs have been made by subjecting a coarse emulsion to high-pressure homogenization.[12] A Central Composite Design (CCD) has been engaged to explore the effect of Surfactant/co-surfactant mixture (S_{mix}) concentration, handing out load, and several cycles on Globule Size (GS).

MATERIALS AND METHODS

Materials

Quercetin hydrate was procured from TCI Chemicals in Guntur, India. Ethanol, Tween 80, and PEG 400 were procured from SD Fine Chemicals. *Opuntia ficus indica* seed oil was procured from Deve's Herbs, New Delhi, India. Double distilled water is used in the entire operation.

Screening and selection of oils

Oils with different properties are examined for the solubility of QH. The solubility of QH in oils was estimated by the addition of an additional quantity of QH to 1 mL of oil (placed in a vial). These samples were set aside at 25±0.5°C in an isothermal shaker. After 72 hr, the samples were collected and centrifuged. The supernatant was carefully taken out and the concentration of QH was determined using a UV-spectrophotometer (Shimadzu UV 1800) at 369 nm.[13]

Screening and selection of surfactants and co-surfactants

The solubility of QH in a range of surfactants was unwavering in the identical way as mentioned in the selection of oils. Surfactant selection was also based on miscibility investigations with certain oils. For this, the surfactant was mixed with oil in a 1:1 ratio in a vortex shaker and investigated visually. The basic criterion for the assortment of co-surfactants was the miscibility of co-surfactants with selected oils in a 1:1 ratio. The combination that appeared clear was measured for the research of Nes.[14,15]

Formulation and optimization of NEs by experimental design

Two factors, three levels of Quality by Design (QbD) arithmetic devices, were opted to optimise QH loaded NEs. We chose a S_{mix} range of 10–20% and a homogenization pressure range of 1000–2500 bar.[16] The range of homogenization cycles was taken as 5–15. The constraints for GS and Polydispersibility Index (PDI) were set at least (that helps in the drug release), and the constraints were located at their utmost. QbD software was employed to evaluate the effects of S_{mix} concentration, dispensation force, and several cycles on the GS and PDI.[17] Several NEs were made as per the design described in Table 3 for 9 runs each and were explored for drop size, PDI, and transmittance as the response variables.[18] O/W is loaded with QH and was set by an elevated power emulsification system. Momentarily, crude emulsions (10 mL) were developed by combining oil, S_{mix}, water, and *Opuntia ficus indica* seed oil (OFISO) using a magnetic stirrer and a bath sonicator.[19] The NEs were made from transient coarse emulsions by a high-pressure homogeniser (Table 1).

Characterization of NEs

Determination of solubility characteristics of Quercetin

The study of QH and its complex solubility was carried out by adding a surplus of the samples to 5mL of water/n-octanol in a preserved glass container at room temperature, shaking for 24 hr, and centrifuging at 6000 rpm (15 min). The supernatant was filtered and analyzed spectrophotometrically at 369 nm.[19]

Preparation of the standard curve of Quercetin

The standard stock solution of QH was formulated by dissolving 100 mg of QH in 100 mL of 7.4 pH PBS (i.e., 1000 g/mL). This solution was then sonicated to complete the dissolution of the drug. Absorbances were taken in the UV-visible spectrophotometer at 369 nm against 7.4 PBS as a blank. From these absorbances, the standard curve was plotted.[20]

Globule size and PDI

The GS and PSD of the NEs were determined using a photon correlation spectrometer. To establish the GS, all developments were diluted about 200 times with distilled water followed by vigorous shaking to reduce numerous scattering effects to an extent. Every model was then examined by a nanoparticle analyzer for Particle Size Distribution (PSD) and average GS. Light scattering was restrained at 25°C using a 90° angle. PDI, a measure of PSD in the sample, was calculated in triplicate.[21]

Table 1: Various batches of QH nano-emulsions.

Formulations	A: OFIS oil (mL)	B: T80 (mL)	PEG-400 (mL)	Water (mL)
QNE-1	10	10	10	Up to 100
QNE-2	20	10	10	Up to 100
QNE-3	10	20	10	Up to 100
QNE-4	20	20	10	Up to 100
QNE-5	7.92893	15	10	Up to 100
QNE-6	22.0711	15	10	Up to 100
QNE-7	15	7.92893	10	Up to 100
QNE-8	15	22.0711	10	Up to 100
QNE-9	15	15	10	Up to 100

Table 2: Different nano-emulsions studied.

Formulations	Factor 1	Factor 2	Response 1
	A: OFIS oil (mL)	B: T80 (mL)	PS (nm)
QNE-1	10	10	294
QNE-2	20	10	295
QNE-3	10	20	292
QNE-4	20	20	305
QNE-5	7.92893	15	293
QNE-6	22.0711	15	301
QNE-7	15	7.92893	297
QNE-8	15	22.0711	298
QNE-9	15	15	304

Table 3: ANOVA for a Quadratic Model (Response 1: PS).

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	172.55	5	34.51	13.90	0.0275
A-OFIS oil	80.10	1	80.10	32.26	0.0108
B-T80	11.08	1	11.08	4.46	0.1251
AB	36.00	1	36.00	14.50	0.0318
A^2	39.56	1	39.56	15.93	0.0282
B^2	34.38	1	34.38	13.84	0.0338
Residual	7.45	3	2.48		
Cor Total	180.00	8			

Zeta potential

The Zeta potential of the optimised formulation was assessed with a nanoparticle analyzer and Zetasizer (Horiba Scientifics India). Before measurement, the sample was diluted to 10 mL with distilled water.[22]

RESULTS

Solubility characteristics of Quercetin

The phytosomal complex exhibited greater solubility (35.6±1.24 µg/mL) when compared to pure phytoconstituent (3.23±0.82 µg/mL). This is owing to the effective complexation of QH with phospholipids, whereas in the case of n-octanol, the solubility was set up to be 53.46±1.48 µg/mL and 54.12±1.11 µg/mL for QH and its complex, respectively.

The standard curve represents a slope value of 0.0044x+0.0946 with a regression value of 0.999 (Figure 1).

UV-spectra of Quercetin hydrate

A variety of tools are applied to the optimization of formulations for innovative drug delivery systems. These tools have been found to save time and money by reducing the number of tests needed

and reducing reagent waste. The choice of CCD was based on the fact that it required fewer runs than other systems. The CCD programme presented 17 runs, according to the design expert, which was comparable to 17 combinations or formulations. Batches were produced by the CCD and tested for GS and PDI. The information gathered for the experiment (Table 2). An Analysis of Variance (ANOVA) was applied to govern the model's significance (Table 3). The quadratic polynomial models described Statistical data suggested that (p=0.05) and model terms (p=0.0001) were highly significant, corresponding to all four responses and showing a good fit to the quadratic model. The value of R^2 must be close to 1, as this indicates a good fit. The predicted R^2 is said to be in reasonable agreement with the adjusted R^2 if the difference is < 0.2. For any term in the models, a high F-value and a small p-value would indicate a more significant effect on the respective response variable.

The F-value of 13.90 for the model indicates that it is significant. An F-value of this magnitude has a 2.75% chance of occurring due to noise. Model terms with p-values <0.05 are significant. In this case, A, AB, A^2, and B^2 are significant model terms. The model terms are not important if the value is >0.10. Model reduction may improve your model if there are many inconsequential model terms (not including those required to support the hierarchy). The final equation in terms of coded factors was illustrated as PS= +304.00 +3.16 A +1.18 B +3.00 AB -3.69A^2-3.44 B^2

The equation in terms of coded factors can be used to anticipate how each element will respond to different levels. High levels of the components are coded as +1 by default, whereas low levels are represented as -1. By comparing the factor coefficients, the coded equation can be used to determine the relative impact of the components.

Plots like this show the sway of two factors on the response at the same time. The contour plot and response surface plot (Figure 2) show an equivalent increase with the concentration of the OFISO.

Particle size results

The study revealed that the PSD ranged from 292 to 305. QNE-3 formulation with 10 mL OFISO had the smallest particle size (292 nm), while QNE-4 formulation with 20 mL OFISO had the largest particle size (305 nm) (Figure 3). On the other hand, the PSD in the optimized formulation (QNE-3) was found to be 292nm (Figure 4).[23]

DISCUSSION

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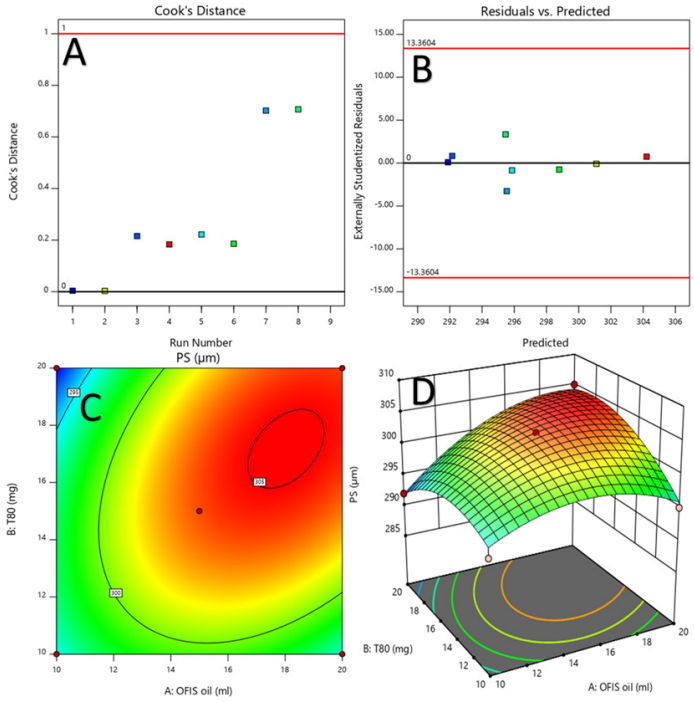

Figure 3: Cooks distance, residual vs predicted, and contour response surface plots showing the effect of OFISO and tween 80.

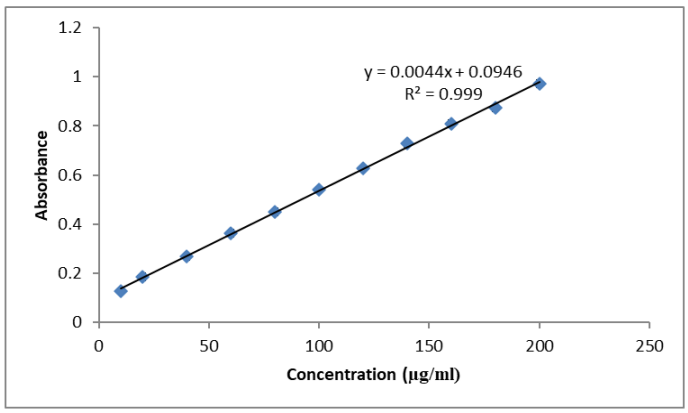

Figure 1: Standard Curve of QH in 7.4 PBS.

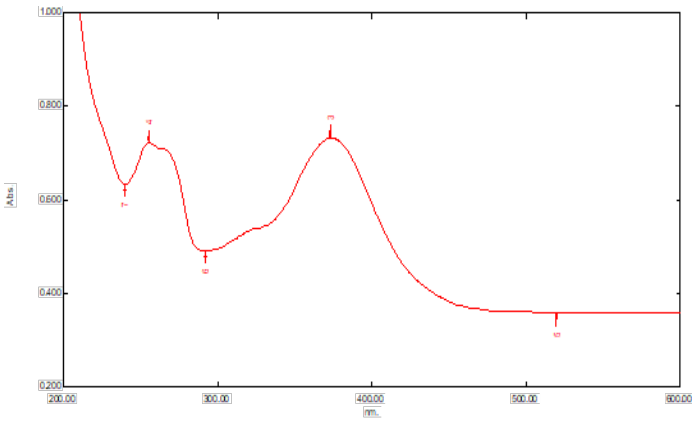

Figure 2: UV-spectra of Quercetin hydrate.

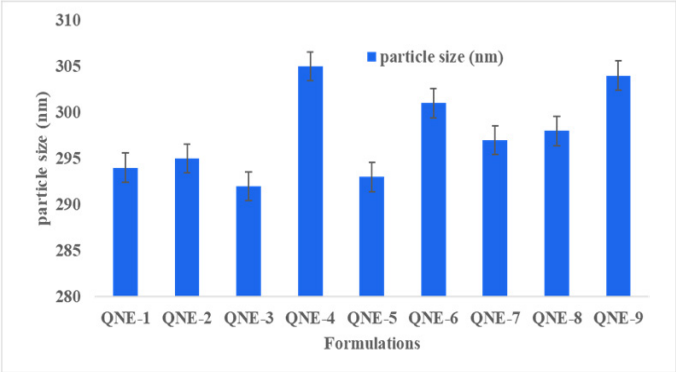

Figure 4: PSD of various QNEs.

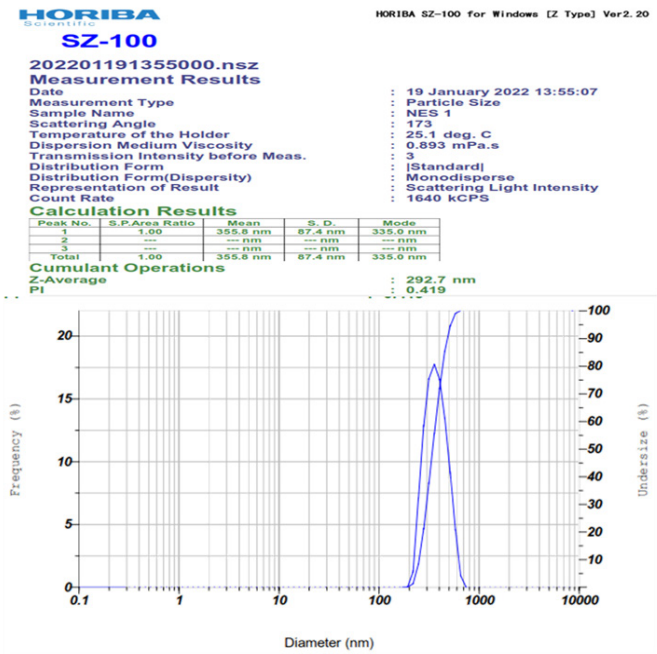

Figure 5: PSD in the optimized formulation (QNE-3).

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Central composite design and data analysis

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CONCLUSION

Even though quercetin hydrate has been approved as a remedy for the treatment of various disorders, its poor oral bioavailability due to poor aqueous solubility and variable absorption is still a challenge in its clinical applications. Quercetin hydrate-loaded nanoemulsion fabricated with Opuntia ficus indica seed oil, PEG400, Tween 80, and ethanol resulted in nano-sized particles that help in drug solubility and bioavailability. This work illustrated the importance of nanoemulsion to enhance the bioavailability of quercetin hydrate. Moreover, quercetin hydrate-loaded nanoemulsion can be a promising oral delivery system for quercetin hydrate with enhanced oral bioavailability.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

CCD: Central Composite Design; **BA:** Bioavailability; **NEs:** Nanoemulsions; **QH:** Quercetin hydrate; **OFI:** *Opuntia Ficus indica;* **GS:** Globule size; **QbD:** Quality by Design; **PDI:** Poly dispersibility index; **QNE:** Quercetin Nano emulsion.

SUMMARY

Poor bioavailability of various biological drugs which are having high efficacy in treatment of disorders actively has high solubility issues to overcome this the presented research work gave a solution for formulating these compounds by Nanoemulsion method. Quercetin hydrate drug was used in this formulation and particle size was achieved which promotes easy absorption and increased bioavailability. There is more scope in this area of research to overcome solubility issues of drugs.

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