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Development and Optimisation of Quercetin Cubosomes Incorporated in Glycerylmonooleate Aided by Design Expert Software

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ABSTRACT

Cubosomes are in a liquid-crystalline state which is an intermediate between an ordered crystal and a disordered isotropic liquid. Topical administration of antioxidants such as Quercetin provides an efficient way to enrich the endogenous cutaneous protection system. The objective of the study was to incorporate Quercetin into the glyceryl monooleate which is a polar unsaturated monoglyceride also known as monoolein; they form thermodynamically stable self- assembled structures in presence of aqueous phase. The cubosomes was prepared by thermally assisted ultrasonication method. The dose of Quercetin was determined using the brine shrimp lethality assay. Optimisation performed using design expert software 9.0.5.1. The particle size of optimized cubosomes was found to be 195.6 ±0.36 nm. The Polydispersity index (PDI) was 0.346±0.12. The cuboidal structure of the nanoparticles was confirmed by TEM analysis. The entrapment efficiency of optimized Cubosomes was found to be 87.43 ±0.07 % and the flux of optimized formulation was found to be 0.0383µg $cm^{-2}min^{-1}$ which were in close agreement with the value predicted by Design expert software. The stability studies confirmed the stability of the product. The encouraging results obtained from this study states that Quercetin cubosomes could be proposed as a skin protection against free radial associated damage and may be incorporated into gel or cream base.

INTRODUCTION

Cubosomes have attracted significant attention due to their potential improvements in physicochemical stability, improved skin retention, and hydrophobic drug loading. Due to its improvement in the physicochemical stability drugs and plant extracts can be entrapped and may have a better action potential than its crude form. Several studies have shown that nanosized particles tend to reside in the outer layers of the stratum corneum and epidermis, with negligible penetration into the dermis, which is beneficial in topical drug delivery [1].

The cubosomes have the property of a crystal as well as liquid and they are highly ordered, also are free to diffuse in a random way. In general liquid crystals, systems can be classified into two categories, i.e. thermotropic and lyotropic mesophases. Thermotropic liquid crystal phases are formed by a thermal change, whereas lyotropic phases are formed when it is mixed with a solvent. The lyotropic cubosomes are of two types, exosomes and cubosomes. The Hexosomes and cubosomes are named after their highly ordered crystalline structure or shape. Quercetin, a topical antioxidant, is known to have the ability to defend ultraviolet radiation-mediated oxidant injury and cell death by scavenging oxygen radicals, by terminating the chain-radical reaction. Glyceryl monooleate is a polar unsaturated monoglyceride. Glyceryl monooleate is a nontoxic, biodegradable, and biocompatible material and is listed in the FDA's Inactive Ingredients Guide. When exposed to the aqueous environment, amphiphilic lipids spontaneously form thermodynamically stable self- assembled structure [2].

The concentration of glyceryl monooleate is of a greater importance in the case of drug delivery. An increased concentration beyond 20% leads to the better entrapment of drug, but the permeability of the drug will be nullified and vice versa. The moreover greater concentration of glyceryl monooleate leads to skin irritations and limited spreadability. Oleic acid is a monounsaturated fatty acid that tends to increase the permeability of lipophilic drugs. Our aim is to optimize the concentration of glyceryl monooleate and oleic acid so that the formulation having least drug permeability and greater drug entrapment efficiency is obtained [3].

MATERIALS AND METHODS

PREFORMULATION STUDIES

Quercetin was purchased from Otto Chemie, Mumbai, Pvt Ltd. All other chemicals used were of the analytical standard.

CHARACTERISATION OF QUERCETIN

1) Solubility

The solubility of Quercetin was determined by taking an excess amount of Quercetin was added to 100mL of the conical flask, which contains 10ml of phosphate buffer PH 7.4 containing 1% tween 20. The flask was shaken occasionally for 12 hr at 37± 2 ^{0}C in magnetic stirrer. The solution was kept to equilibrate for next 24 hrs and then centrifuged for 10 min at 1000 rpm (Remi Instruments Limited, Mumbai India). The supernatant solutions of each flask were filtered through what man filter paper no.41. An aliquot was adequately diluted with distilled water and that appropriate dilution analyzed by spectrophotometric method using a UV-visible spectrophotometer JASCO V-630 at 256 nm [4].

2) Melting point

The melting point of Quercetin was determined using melting point apparatus. The drug sample was taken in a small capillary tube and placed in the melting point determination apparatus. The melting point of the sample was examined visually through the window and the sample-melted temperature was noted.

3) FT-IR Spectroscopy

Fourier- transform infrared (FT-IR) spectrum of Quercetin was obtained. The sample was made into pellets with Potassium bromide and FT-IR model Thermo Nicolet, Avatar 370 was used for obtaining spectra. The spectrum obtained was compared with reported data of Quercetin [5].

CHARACTERISATION OF GLYCERYLMONOOLEATE

1) Solubility

The solubility of glyceryl monooleate was determined qualitatively in various media, like ethanol, methanol, acetone, petroleum ether and Phosphate buffer saline (PBS) (pH 7.4).

2) Melting point

The melting point of glyceryl monooleate was determined using melting point apparatus. The drug sample was taken in a small capillary tube and placed in the melting point determination apparatus. The melting point of the sample was examined visually through the window and the sample-melted temperature was noted.

3) Viscosity

The viscosity was measured to determine rheological properties of formulations. Brookfield rheometer viscometer RVDVE at 30°C with a CPE 02 spindle at 30 rpm was used for this purpose. Results were taken in triplicate and the average was taken into consideration.

4) FT-IR Spectroscopy

The FT-IR spectrum of glyceryl monooleate was taken and compared with reference spectrum for confirmation. FT-IR model Thermo Nicolet, Avatar 370was used for obtaining spectra. The spectrum obtained was compared with reported data of glyceryl monooleate [6].

QUERCETIN- GLYCERYLMONOOLEATE COMPATABILITY STUDIES

Prior to a formulation, to study the chemical compatibility of Quercetin with glyceryl monooleate was determined. The following studies were conducted on drug and glyceryl monooleate mixtures. The ratio of drug and Glyceryl monooleate was selected as 1:1.

FT-IR Spectroscopy

The FT-IR spectrum of the drug-glyceryl monooleate mixture was obtained. FT-IR model Thermo Nicolet, Avatar 370was used for obtaining spectra. The spectrum obtained was compared with reported data of drug and with spectra of glyceryl monooleate.

DETERMINATION OF CYTOTOXIC DOSE OF QUERCETIN BY BRINE SHRIMP LETHALITY ASSAY

Brine shrimp eggs were hatched in a shallow rectangular dish filled with seawater under constant aeration and illumination. Two days were allowed for the shrimps to hatch and mature as nauplii.10mg of Quercetin was dissolved in 100ml of methanol (stock solution). 10, 20, 30, 40, 50 µg/ml of Quercetin was prepared from the above stock solution.

Ten brine shrimps were added to the test tube containing 4.5ml of brine solution using a pipette.0.5 ml of different concentration of Quercetin was added to these test tubes. 0.5ml of potassium dichromate was taken as a positive control and was made up to 5ml with brine solution.0.5ml of absolute methanol was taken as a negative control and was made up to 5 ml with the brine solution. All the test tubes were maintained under illumination for 24 hrs.

After 24 hours, survivors were counted with the aid of a magnifying lens against a lighted background. Nauplii were considered dead if they were lying immobile at the bottom of the vials and the percentage of deaths for each dose and for the control were determined. Lethal concentration (LC_{50}) was determined using probit analysis method described by Finney [7].

FORMULATION DESIGN AND DEVELOPMENT

Formulation of glyceryl monooleate entrapped cubosomes dispersion of Quercetin by ultrasonication method

The required amounts of glyceryl monooleate (10-15%), propylene glycol, and oleic acid was taken and melted in a water bath at 60 °C followed by Quercetin addition with continuous stirring to complete dissolution. The concentration of Quercetin was determined from brine shrimp lethality assay; It was fixed as 30 µg/ml. The required amount of distilled water was gradually added to the melted mixture with vortexing for 10 min followed by ultrasonication for 1 hr. The cubosomes dispersion prepared was stored at room temperature for further studies.

Optimisation of glyceryl monooleate entrapped cubosomes dispersion of Quercetin using Design expert software 9.0.5.1 by response surface method [8].

Optimisation of glyceryl monooleate entrapped cubosomes dispersion of Quercetin was done using Design expert software 9.0.5.1 by response surface method. The concentration of glyceryl monooleate is of a greater importance in the case of drug delivery. An increased concentration beyond 20% leads to the better entrapment of drug, but the permeability of the drug will be nullified and vice versa. The moreover greater concentration of glyceryl monooleate leads to skin irritations and limited spreadability. Oleic acid is a monounsaturated fatty acid that tends to increase the permeability of lipophilic drugs. Our aim is to optimize the concentration of glyceryl monooleate and oleic acid so that the formulation having least drug permeability and greater drug entrapment efficiency is obtained.

1) Determination of drug entrapment efficiency

For determination of drug entrapment efficiency, the cubosomes dispersion was centrifuged at 15,000 rpm for 30 min using Remi CFC free centrifuge CZCI- 8899.The supernatant was spectrophotometrically analyzed using JASCO V-630 spectrophotometer at 256 nm. The entrapment efficiency was calculated using the formula [9]

$$W_{SYS} - W_{SUP} / W_{SYS} * 100$$

$W_{SYS=}$ Total amount of Quercetin in the dispersion

$W_{SUP=}$ Amount of Quercetin in the supernatant

2) Determination of in-vitro skin permeability

The *in-vitro* skin permeation studies were carried out in glass Franz type diffusion cell with a diffusion area of 0.785 cm2. The goatskin samples were placed between the donor and the receptor compartments of the cell, with the dermal side in contact with the receptor medium. The formulations studied (1ml) was placed in the donor compartment. The receptor chamber was filled with 5 ml of phosphate buffer of pH 7.4 and kept at 32 ± 0.5°C by a circulating water jacket. Samples of 1 ml were withdrawn from the receptor compartment at 0.5, 1, 2, 3 and 4 hours and replaced the same volume with phosphate buffer of 32°C. Sink conditions were maintained in all cases. The withdrawn samples were spectrophotometrically analyzed at 256 nm [10].

The corresponding concentration in receptor compartment was found out using the equation. The graph of the cumulative amount of drug diffused Vs time was plotted.The slope of the terminal part gives flux (J).

Formulation of optimized glyceryl monooleate entrapped cubosomes dispersion of Quercetin by ultrasonication method

Optimisation of glyceryl monooleate entrapped cubosomes dispersion of Quercetin was done using Design expert software 9.0.5.1 by response surface method. From the 15 runs, 110 solutions were suggested by the software. Out of these solutions, the solution with maximum drug entrapment and minimum skin permeability (Flux) was chosen as the optimized formulation.

The required amounts of glyceryl monooleate (1.5 g), propylene glycol (0.27 ml), and oleic acid (0.51) ml was taken and melted in a water bath at 60 °C followed by Quercetin addition with continuous stirring to complete dissolution. A required amount of distilled water was gradually added to the melted mixture with vortexing for 10 min followed by ultrasonication for 1 hr.

CHARACTERISATION OF OPTIMISED CUBOSOME DISPERSION

1) Particle size and zeta potential

Photon Correlation Spectroscopy (PCS) using a Malvern Zetasizer Ver .7.01, at a fixed angle of 90°C and at 25°C. Determined the average diameter of cubosomes. The cubosomes dispersions were diluted with distilled water before analysis. The polydispersity index (PDI) was also calculated.The zeta potentials of the samples were determined at 25^{o}C after suitable dilution with distilled water [11].

Transmission electron microscopy

The morphology of the nanoparticles was examined using Jeol/JEM 2100 at 70 kV after dilution with the original dispersion medium of the cubosomes; the samples were negatively stained with 1% (w/v) phosphotungstic acid for observation [12].

2) Entrapment efficiency

For determination of drug entrapment efficiency, the cubosomes dispersion was centrifuged at 15,000 rpm for 30 min using Remi CFC free centrifuge CZCI-8899.The supernatant was spectrophotometrically analyzed using JASCO V-630 spectrophotometer at 256 nm.

3) *In-vitro* drug release study

The *in-vitro* drug release was evaluated by Franz diffusion cell using Spectra/Por® regenerated cellulose membrane of the Molecular weight cut off(MWCO)10,000 g/mol (surface area of 0.785 cm^2). The speed was maintained at 37±2ºC at 100 rpm using a magnetic stirrer. Liquid crystalline dispersion equivalent to 30 µg of the drug was placed in the donor compartment, and receptor compartment was filled with 5 ml of phosphate buffer pH 5.4 s to maintain sink condition. Samples were withdrawn at fixed time intervals 0, 0.5, 1, 2, 3, 4, 5, 6,8 hrs from the receptor compartment. Samples were then analyzed by UV spectroscopic method at 256 nm.

4) *Invitro* skin permeability study

The permeation studies were carried out in glass Franz type diffusion cell with a diffusion area of 0.785 cm2. The goatskin samples were placed between the donor and the receptor compartments of the cell, with the dermal side in contact with the receptor medium. The formulations studied (1ml) was placed in the donor compartment. The receptor chamber was filled with 5 ml of phosphate buffer of pH 7.4 and kept at 32 ± 0.5°C by a circulating water jacket. Samples of 1 ml were withdrawn from the receptor compartment at 0.5,1, 2, 3 and 4 hours and replaced the same volume with phosphate buffer of 32°C. Sink conditions were maintained in all cases. The withdrawn samples were spectrophotometrically analyzed at 256 nm [13].

The graph of the cumulative amount of drug diffused Vs time was plotted.The slope of the terminal part gives flux (J).At the end of 24 hrs, the skin was cut into small pieces and extracted with isopropyl alcohol. It was analyzed spectrophotometrically at 256 nm

STABILITY STUDIES

Accelerated stability studies

According to ICHQ1A (R2) guidelines for drug products intended to be stored at room temperature, the accelerated stability studies are to be carried out at controlled temperature and humidity conditions of 40 ± 2°C and humidity 75 ± 5% RH. Thus for the stability evaluation of the solid lipid nanoparticles, the samples were stored at a temperature of 40 ± 2°C and humidity 75 ± 5% RH. The samples were withdrawn at 0, 30, 60 days and the drug content and *Invitro* drug release were determined. The zero time samples were used as controls [14].

(a) Particle size and zeta potential: Samples were withdrawn at regular intervals of time and were analyzed for drug content.

(b)*In-vitro* drug release: samples were withdrawn at regular intervals of time and were analyzed for in vitro release.

RESULTS

PREFORMULATION STUDIES

CHARACTERISATION OF QUERCETIN

1) Solubility

The solubility of Quercetin in phosphate buffer pH 7.4 containing 1% tween 20 was found to be 27.45µg/ml.

2) Melting point

The melting point of Quercetin was found to be 3140C. The results observed were in close agreement with reported data.

3) FT-IR Spectroscopy

The FTIR spectrum for the pure sample of Quercetin exhibited absorbance bands in the range of 3409-941 cm^{-1} as given in Figure 1. The low-intensity absorbance bands arising from Quercetin were not much affected by dilution in dry potassium bromide; therefore, in the present study, we have used dry potassium bromide as the diluent. The most prominent absorbance band

corresponding to the carbonyl group centered in the range of 1710–1703cm^{-1}. The results are discussed in Table 1.

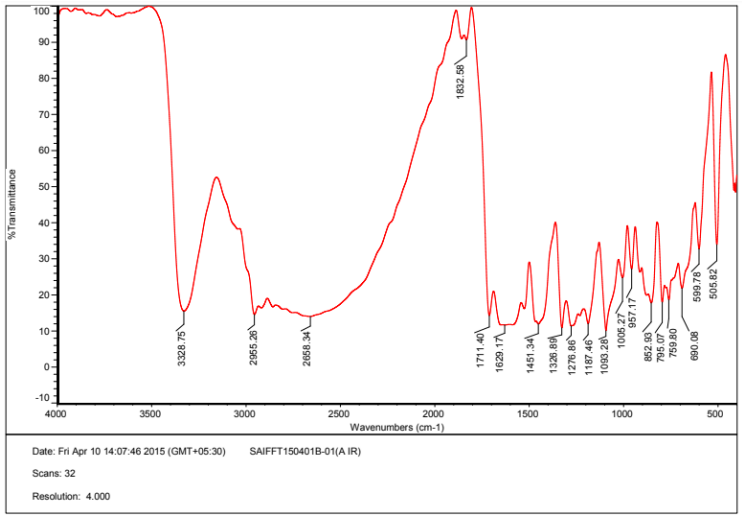


Figure 1 FT-IR spectra of Quercetin

CHARACTERISATION OF GLYCERYLMONOOLEATE

1) Solubility

Glyceryl monooleate was soluble in chloroform, ethanol, methanol, petroleum ether. It was insoluble in water.

2) Melting point

The melting point of glyceryl monooleate was found to be 380C.The results observed were in close agreement with reported data.

3) Viscosity

The viscosity of glyceryl monooleate was found to be 220±0.14 Centipoise (Cp).The test was performed in triplicate and expressed as the Mean ± Standard deviation (SD) (n=3)

4) FT-IR Spectroscopy

The FT-IR spectrum for a pure sample of glyceryl monooleate exhibited absorbance bands in the range of 3429-931 cm^{-1} as depicted in Figure 2. The most prominent absorbance band corresponding to the carbonyl group centered in the range of 1710–1703 cm^{-1}. The results are discussed in Table 2.

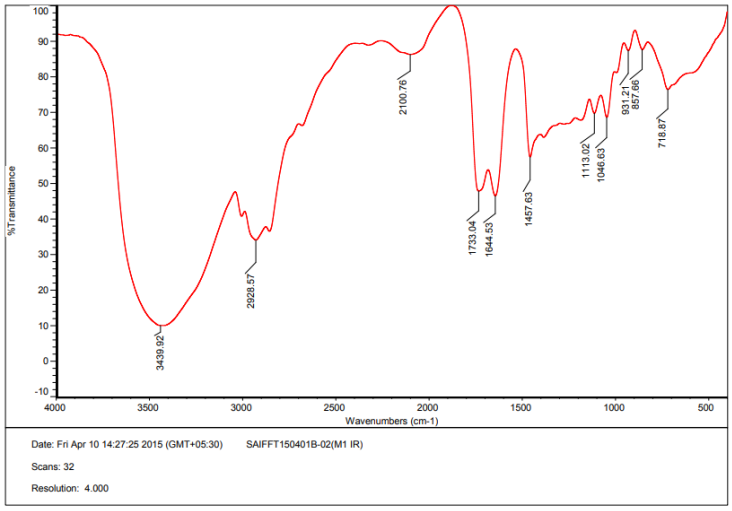


Figure 2 FT-IR spectra of GlycerylMonooleate

QUERCETIN- GLYCERYLMONOOLEATE COMPATABILITY STUDIES

FT-IR Spectroscopy

The FTIR spectrum for sample mixture exhibited absorbance bands in the range of 3409-722 cm^{-1}. The most prominent absorbance band corresponding to the carbonyl group centered in the range of 1710–1703 cm^{-1}as shown in Figure 3. The results are explained in Table 3.From the spectrum interpretation no chemical reactions or major shift of spectrum was observed, indicating the compatibility.

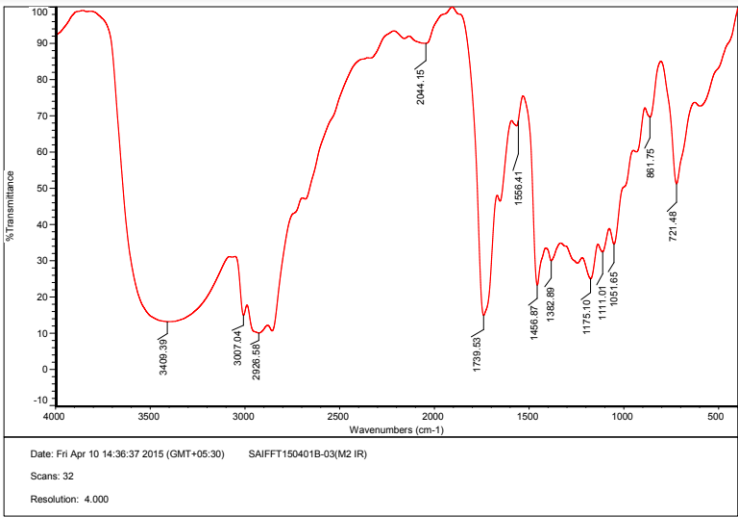


Figure 3 FT-IR spectra of Glyceryl monooleate and Quercetin in the ratio (1:1)

DETERMINATION OF CYTOTOXIC DOSE OF QUERCETIN BY BRINE SHRIMP LETHALITY ASSAY

The percentage mortality of brine shrimp increased with the increase in the concentration of Quercetin. An approximate linear correlation was observed when logarithmic concentration vs. percentage mortality was plotted Figure 4. The results are discussed in Table 4 and LC_{50} was found to be 32.57µg/ml.

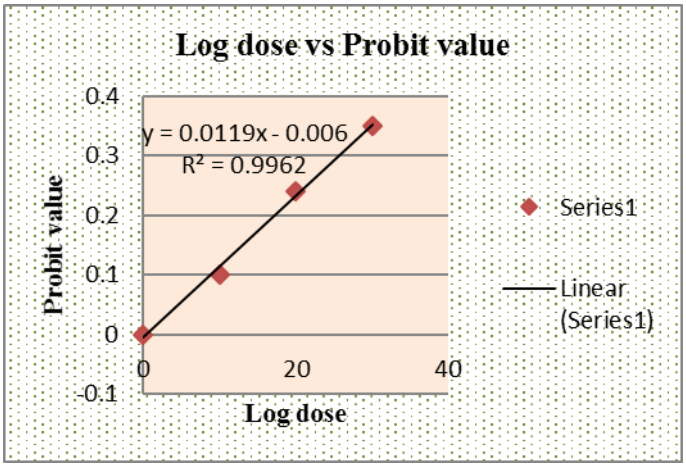
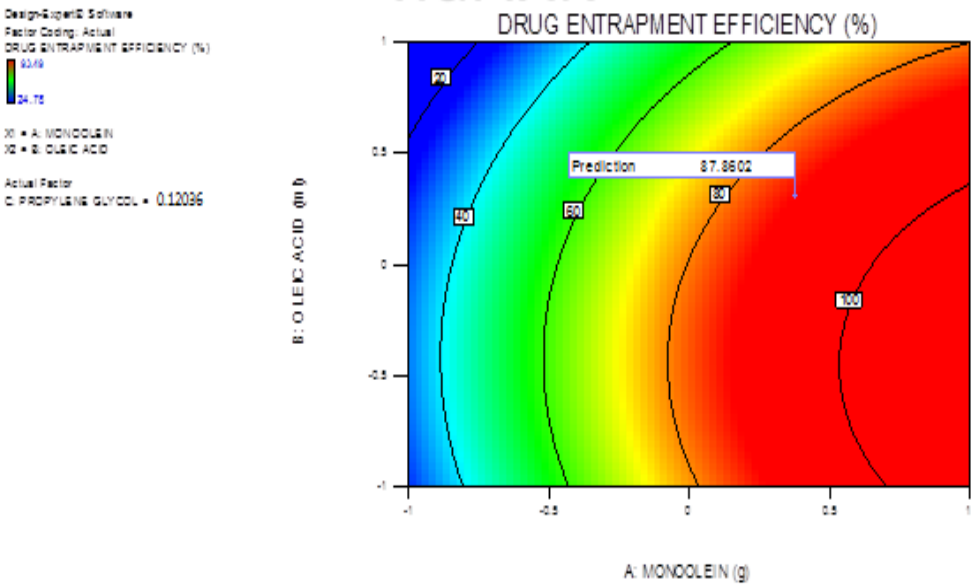


Figure 4 Probit analysis plot

Corrected percentage: For the 0 % dead : 100(0.25/n) For the 100% dead: 100 [(n-0.25)/n] n is the number of animals in the group, (Probit value was found from probit table) LC_{50} was found from the Log does Vs Probit value graph, By interpolating Probit value 5, using equation from the graph (y=mx+c)

OPTIMISATION OF CUBOSOMES

Three parameters at five levels and two responses were chosen. Central composite design with Quadratic design model was fixed by the software. The design had total runs of 15.The value for '0' was fixed from the literature reviews. The design summary is given in Table 6 *Invitro* skin permeability (flux) and Drug entrapment efficiency was the responses determined. The maximum and minimum for the proposed factors and responses were determined by the software as shown in Table 7 and 8.Three levels were set for the experiment ie 0,1.41421 and -1.41421 and were randomized for 15 runs, given in Table 9.The cubosomes were formulated using the assigned values calculated as suggested by the software, respective formulas are given in Table10.The formulated products were evaluated for the responses like Response 1 and Response 2 and the data were tabulated and given in Table 11.The software with respect to the responses shown in Figure 5 and Figure 6 designed contour plots and overlay plots.



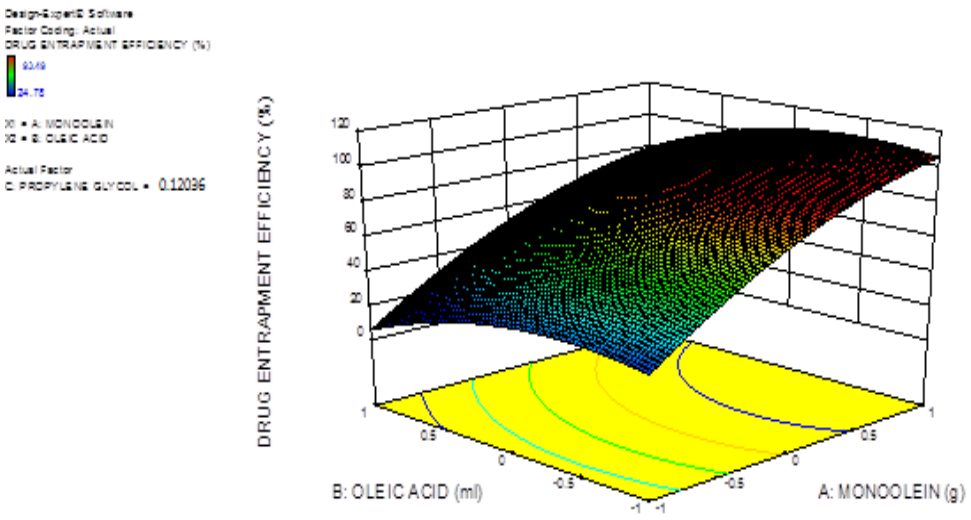
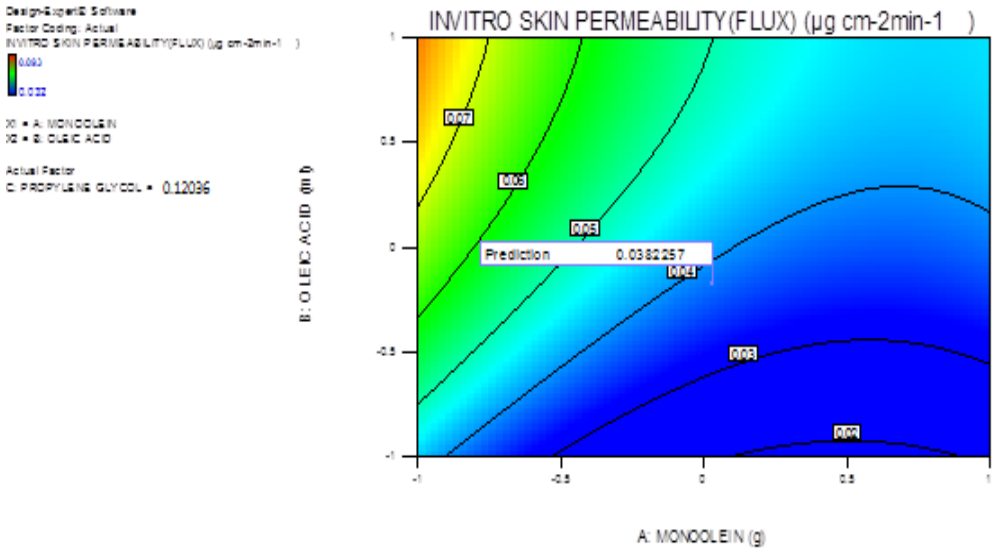


Figure 5 Contour and overlay plot of Drug entrapment efficiency

The figure shows the predicted region of maximum entrapment efficiency, the desired output either maximum or minimum can be set in the software, for which the entrapment efficiency was set the maximum. The areas in overlay plot showing the same are shaded as red.



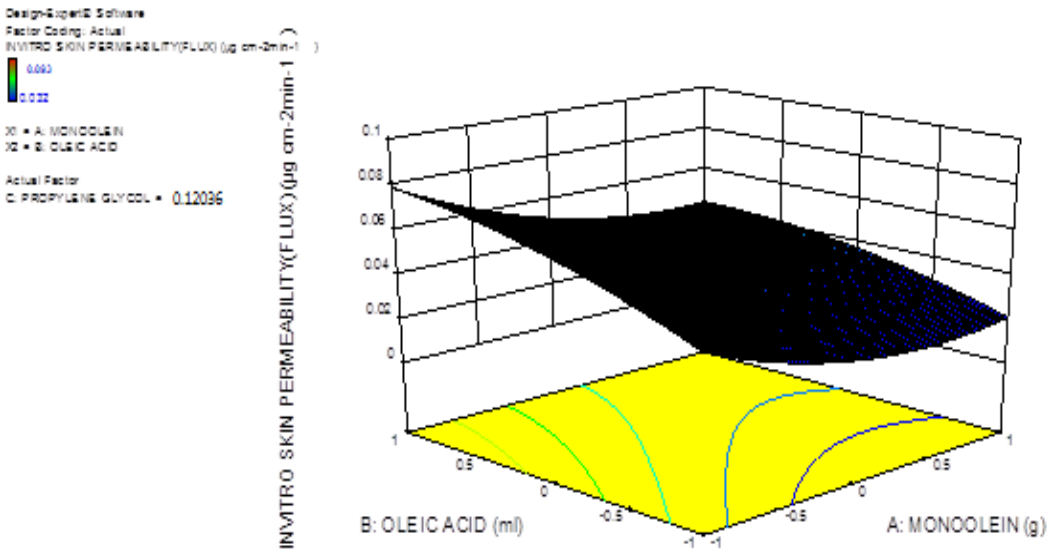


Figure 6 Contour and overlay plot of In vitro skin permeability (Flux)

The figure shows the predicted region of minimum flux, the desired output either maximum or minimum can be set in the software, for which the flux was set the minimum. The areas in contour plot showing the same are shaded as blue.

CHARACTERISATION OF OPTIMISED CUBOSOMES DISPERSION

1) Particle size and zeta potential

The particle size of optimized cubosomes was found to be 195.6 ±0.36 nm. The PDI was 0.346±0.12, Zeta potential of cubosomes was determined.It could be seen that Zeta potential was -23.5±0.06 mV,

2) Transmission electron microscopy

The morphology of the optimized cubosomes was examined the cuboidal structure of the cubosomes was confirmed as shown in Figure 7.

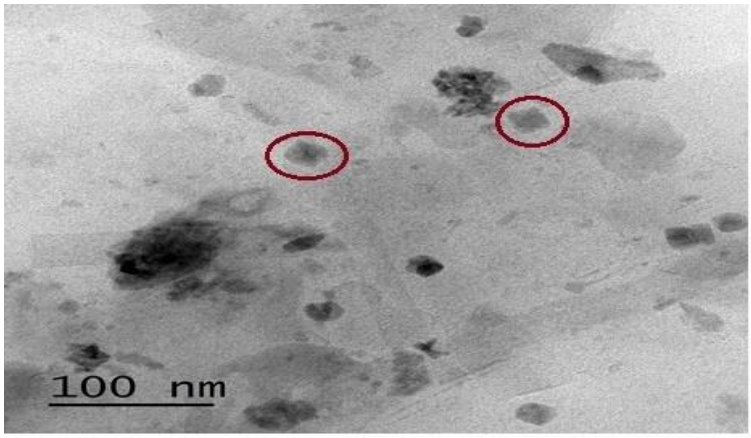


Figure 7 TEM image of Cubosomes

3) Drug entrapment efficiency

The test was performed in triplicate. Percentage entrapment efficiency expressed as Mean ± SD (n=3) the entrapment efficiency of optimized cubosomes was found to be 87.43 ±0.07 % which was in close agreement with the value predicted by Design expert software.

4) *In-vitro* drug release study

The in vitro drug release was performed in a Franz diffusion cell. Withdrawn samples were analyzed for drug release in UV Spectrophotometer at 256nm. Cumulative % drug release Vs time is plotted as shown in Figure 8. The results are explained in Table 12.

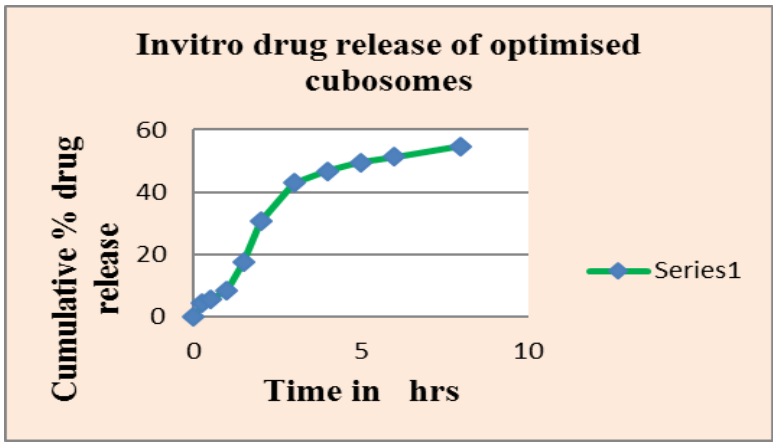


Figure 8 Cumulative percentage drug release of optimized cubosomes.

The graph shows a steady increase in drug release over time, And it nearly approaches steady state at the 8th hour.

Kinetics of drug release

Various kinetic models were used for analyzing the in vitro drug release. Cumulative Percentage Drug release versus square root of Time (Higuchi Model); Log Cumulative Percentage Drug release versus Log Time (Korsmeyer Model).kinetics is discussed in Table 13.

Higuchi plot is the amount of drug released against the square root of time. A linear graph indicates that diffusion is the most prominent process of release. A correlation coefficient of 0.929 was observed for the cubosomes. Korsmeyer model is the plot of the log cumulative % drug release Vs log time. The value less than 1 indicates nonfiction diffusion and for cubosomes, it was observed as 0.819.

5) *Invitro* skin permeability study

The graph of the cumulative amount of drug diffused Vs time was plotted.The slope of the terminal part gives flux (J).The flux of optimized formulation was found to be 0.0383µg cm $^{-2}$min^{-1}as given in Table 14.The value was in close agreement with the proposed value by design expert software. The concentration on skin was found to be 19.14 µg/ml.

STABILITY STUDIES

Accelerated stability studies

The stability studies were carried out on the optimized cubosomes.The stability studies were conducted in a stability chamber at 40±2°C and 75±5% R.H. for a period of 2 months.

a) Zeta potential

Zeta potential of cubosomes dispersion stored at desired temperature and humidity over 60 days was found to be -23.5±0.06 mV. The results show no significant changes in the zeta potential as given in Table 15, indicating stability of the product without formation of coalescence. Sufficient zeta potential helps to prevent coalescence of the droplets by electrostatic repulsion.

b) *In-vitro* drug release

The in-vitro drug releases were performed for a time period of 6 months. The results as shown in Figure 9 clearly indicate that there is no remarkable deviation in the drug release over the period as given in Table 16, proving the better stability of the product upon storage.

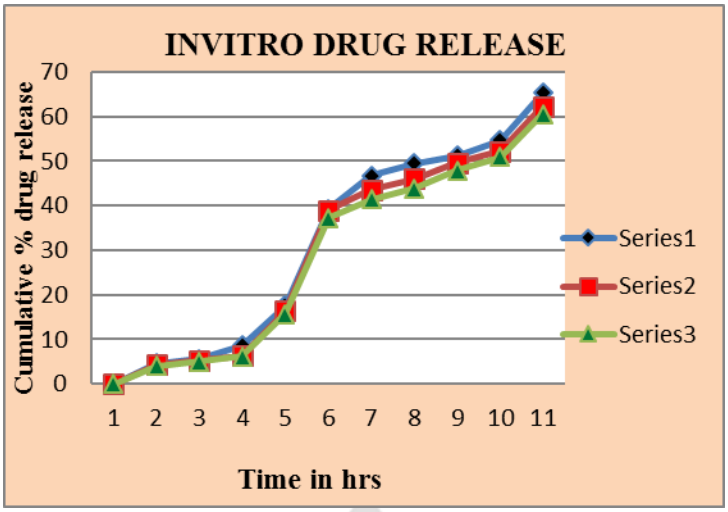


Figure 9 Stability studies

Evaluation of stability of the cubosomes for different time periods of 0, 3 and 6 months. The series (1) Indicating the drug release at 0 months, Series (2) Drug release at 3rd month, Series (3) Drug release at 6th month.

DISCUSSION

Topical administration of antioxidants provides an efficient way to enrich the endogenous cutaneous protection and thus may be a successful strategy for diminishing ultraviolet radiation-mediated oxidative damage to the skin.

Initially, the characterization of Quercetin and glyceryl monooleate were done spectrophotometrically. The results observed were in close agreement with reported data. Prior to formulation, the chemical compatibility of Quercetin with Glyceryl monooleate was also performed, which showed no significant interaction.

Optimisation of glyceryl monooleate entrapped cubosomes dispersion of Quercetin was performed using Design expert software 9.0.5.1 by response surface method**.** The central composite design was chosen with 15 experimental runs [15].The optimization parameters like drug entrapment efficiency and in-vitro skin permeability were evaluated. The software provided 110 solutions for an optimized formulation, out of which the one with maximum desirability was chosen (>0.9). The model predicted 87.86 % of drug entrapment and flux of 0.0382 µg cm^{-2} min^{-1}. The optimized product was formulated by using the optimized formula provided by the software by ultrasonication method. The characterization of an optimized product was performed. It showed particle size of 195.6±0.36 nm. The PDI liquid crystalline formulation was 0.346 ±0.12, which is less than one, indicating a narrow size distribution. Zeta potential was found to be -23.5±0.06 mV resulting in lower aggregation and narrow distribution of particle size. Sufficient zeta potential helps to prevent coalescence of the droplets by electrostatic repulsion The formulation had a skin friendly pH of 5.13 ±0.12. The scanning electron microscopy and transmission electron microscopy confirmed the cuboidal morphology of nanoparticles.

The stability studies were carried out on the optimized cubosomes. The stability studies were conducted in a stability chamber at 40±2°C and 75±5% RH and were found to be stable upon storage at the specified temperature and humidity for 60 days. The present study demonstrates that drug release, permeation, and retention of Quercetin cubosomes were controllable by the amount of glyceryl monooleate and additives.

CONCLUSION

Cubosomes have attracted significant attention due to their improvements in physicochemical stability, improved skin retention and loading of hydrophobic drugs. The aim of the study was to formulate and evaluate Quercetin entrapped glyceryl monooleate based liquid crystalline nanoparticles for topical drug delivery

The Quercetin entrapped nanoparticles have a promising effect as the topical antioxidant and anti-inflammatory agent. The encouraging results obtained from this study states that liquid crystalline system could be proposed as a UV protectant or may be incorporated into gel or

cream base. Incorporation of such nanoparticles may offer greater stability than the other conventional topically applied products.

This finding may open the new door to formulate cubosomes for potential applications in dermo-cosmetic and pharmaceutical fields. The further thorough study is required for comparing, the Quercetin entrapped cubosomes with available topical formulations.

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Table 1 FT-IR data of Quercetin

Wave number cm^{-1}	**Functional Group**
The broad peak at 3409	Hydroxyl group stretching
The sharp peak at 1664	The C=O stretch of coumarin
The sharp peak at 2922	Aromatic C-H stretching

Table 2 FT-IR data of glyceryl monooleate

Wave number cm^{-1}	**Functional Group**
The broad peak at 3439	Hydroxyl group
Sharp peak at1733	Carbonyl group
The sharp peak at 2928	C-H stretching
The sharp peak at 1644	C=C stretching
The sharp peak at 931	C-H bend out of the plane

Table 3 FT-IR data of Quercetin – the Glyceryl monooleate mixture

Wave number cm^{-1}	**Functional Group**
The broad peak at 3409	Hydroxyl group stretching
The sharp peak at 2925	Aromatic C-H stretching
The sharp peak at 1740	Keto group
The sharp peak at 1457	Methyl group stretching
The sharp peak at 935	C-H bending out of the plane
The sharp peak at 1650	C=C stretching

Table 4 Effect of different concentration of quercetin on percentage mortality in brine shrimp lethality assay

Concentration in µg/ml	10	20	30	40	50	Negative control	Positive control
No. of brine shrimps	10	10	10	10	10	10	10
No of live brine shrimps after 24 hrs	9	8	6	4	0	0	10
No of dead brine shrimps after 24 hrs	1	2	4	6	10	10	0
Percentage mortality	10	20	40	60	100	100	0

Table 5 Conversion of percentage mortality to probit value

Sl no	Dose µg/ml	Log dose	Dead/Total	Percentage mortality	Corrected percentage	Probit value
1	10	1	1/10	10	10	3.27
2	20	1.30	2/10	20	20	4.16
3	30	1.47	4/10	40	40	4.75
4	40	1.60	6/10	60	60	5.25
5	50	1.69	10/10	100	97.5	6.96

Table 6 Design model, type, and Runs of the study using Design expert software 9.0.5.1

Design Summary			
File Version	9.0.5.1		
Study Type	Response Surface	**Runs**	15
Design Type	Central Composite	**Blocks**	No Blocks
Design Model	Quadratic		

Table 7 Proposed factors and their deviations

Factor	Name	Units	Type	Subtype	Minimum	Maximum	Std. Dev.
A	GLYCERYL MONOOLEATE	g	Numeric	Continuous	-1.41421	1.41421	0.755929
B	OLEIC ACID	ml	Numeric	Continuous	-1.41421	1.41421	0.755929
C	PROPYLENE GLYCOL	ml	Numeric	Continuous	-1.41421	1.41421	0.755929

Table 8 Proposed responses and their deviations

Response	Name	Units	Obs	Analysis	Minimum	Maximum	Mean	Std. Dev.
R1	DRUG ENTRAPMENT EFFICIENCY	%	15	Polynomial	24.78	93.49	65.866	22.4259
R2	INVITRO SKIN PERMEABILITY (FLUX)	g cm-2min-1	15	Polynomial	0.032	0.086	0.045	0.0139027

Table 9 Design expert optimization Runs, Factors, and levels

Runs	Factor 1: a Glyceryl monooleate (g)	Factor 2 :b Oleic acid (ml)	Factor 3:c Propylene glycol (ml)
1	-1	-1	-1
2	0	-1.41421	0
3	0	1.41421	0
4	0	0	0
5	0	0	1.41421
6	1.41421	0	0
7	-1.41421	0	0
8	0	0	0
9	1	-1	1
10	0	0	-1.41421
11	1	1	-1
12	0	0	0
13	0	0	0
14	-1	1	1
15	0	0	0

Table 10 Assigned values for each level

Values	Glyceryl monooleate (g)	Oleic acid (ml)	Propylene glycol(ml)	Water Added (ml)
0	1.5	0.25	0.15	8.1
1	2.0	0.30	0.20	7.5
-1	1.0	0.20	0.10	8.7
1.414	2.41	0.71	0.61	6.2
-1.414	0.586	-	-	9.4

Table 11 Responses of 15 runs using the desired responses

Runs	Response 1 Drug entrapment efficiency ±SD(%)	Response 2 *Invitro* skin permeability±SD(Flux) (µg $cm^{-2}min^{-1}$)
1	27.15±0.014	0.0473±0.009
2	77.34±0.31	0.0323±0.024
3	87.07±0.078	0.0937±0.0024
4	54.50±0.14	0.0527±0.001
5	53.32±0.27	0.0722±0.0057
6	93.49±0.15	0.0644±0.0051
7	27.32±0.06	0.0916±0.0033
8	56.91±0.01	0.0511±0.0051
9	77.37±0.24	0.050±0.008
10	78.36±0.37	0.0398±0.014
11	24.78±0.12	0.0499±0.0027
12	58.82±0.03	0.0564±0.039
13	57.15±0.08	0.0548±0.097
14	39.41±0.18	0.0509±0.006
15	59.75±0.35	0.0524±0.003

All the tests were performed in triplicate and expressed as Mean ± SD(n=3)

Table 12 The predicted values and actual values obtained from the 15 runs, the Residual value is the deviation from the predicted value.

Runs	Actual value	Predicted value	Residual value
1	0.0473	0.0451	0.002
2	0.0323	0.0342	-0.0019
3	0.0937	0.0928	0.0009
4	0.0527	0.0456	0.0071
5	0.0722	0.0655	0.0067
6	0.0644	0.0531	0.0113
7	0.0916	0.0890	0.0026
8	0.0511	0.0463	0.0048
9	0.050	0.0641	-0.0141
10	0.0398	0.0402	-0.0004
11	0.0499	0.0536	-0.0037
12	0.0564	0.0503	0.0061
13	0.0548	0.0535	0.0013
14	0.0509	0.0556	-0.0047
15	0.0524	0.0614	-0.009

Table 13 In vitro drug release study of the optimized cubosomes The test was performed in triplicate. Cumulative percentage drug release expressed as Mean ± SD(n=3)

Formulation Code	Time (hr)	Cumulative % drug release ± sd
Optimized cubosomes (CA)	0	0
	0.25	4.62±0.24
	0.5	5.50±0.36
	1	8.54±0.48
	1.5	17.55±0.57
	2	30.56±0.62
	3	42.87±0.37
	4	46.76±0.84
	5	49.49±0.45
	6	51.23±0.47
	8	54.65±0.95

Table 14 Kinetics of drug release of optimized Cubosomes

Time (hr)	Log Time	The square root of time	Percentage drug release	Log percentage drug release	Log percentage drug to be released
0	-	0	0	-	2
0.25	0.6020	0.5	4.62	0.6646	1.979
0.5	-0.3013	0.7	5.50	0.7403	1.975
1	0	1	8.54	0.9331	1.961
1.5	0.1760	1.2	17.55	1.244	1.916
2	0.3013	1.4	30.56	1.485	1.841
3	0.4771	1.7	42.87	1.632	1.756
4	0.6020	2	46.76	1.669	1.726
5	0.6989	2.2	49.49	1.694	1.703

Table 15 Observation and calculation table of the flux of Cubosomes

Time in min	Absorbance (256 nm)	Concentration µg/ml	The cumulative amount of drug diffused µg cm^{-2}	Flux(J) µg $cm^{-2}min^{-1}$
30	0.0702	5.201	4.641	
60	0.09258	6.643	6.114	
120	0.1081	7.645	7.145	0.0383
180	0.1404	9.729	9.289	
240	0.1631	11.193	10.783	

Table 16 Zeta potential of Cubosomes at 0, 30, 60 days

Number of days	Zeta potential mv
0	-23.5
30	-23.2
60	-23.1

Table 17 Cumulative percentage drug release of cubosomes

Time (h)	Cumulative % drug release		
	0 month	1st month	2nd month
0	0.05±0.01	0.03±0.01	0.02±0.01
0.25	4.62±0.24	4.07± 0.84	3.95 ± 0.34
0.5	5.50±0.36	5.27 ± 0.61	4.96 ± 1.92
1	8.54±0.48	6.39 ± 0.24	6.24 ± 0.9
1.5	17.55±0.57	16.87 ± 1.3	15.62 ± 2.23
2	39.56±0.62	38.75 ± 2.48	37.83 ± 1.45
3	42.87±0.37	43.58± 3.52	41.36 ± 3.14
4	46.76±0.84	45.99± 2.24	43.84 ± 3.2
5	49.49±0.45	48.43 ± 3.25	47.94 ± 4.7
6	51.23±0.47	49.27 ± 1.85	48.89 ± 1.2
8	54.65±0.95	52.78 ± 1.8	51.57 ± 1.85

All the tests were performed in triplicate. Percentage drug release expressed as Mean ± SD(n=3).