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Original Research Article

Quercetin Nanogel for Psoriasis

Design and Optimization of Quercetin-Loaded Nanogel for Topical Application in Psoriasis

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Abstract: Psoriasis is a chronic autoimmune condition that causes rapid skin cell buildup and surface scaling. Psoriatic scales typically appear as thick, red rashes with whitish-silver scales, with this condition typically causing inflammation and redness around the scales. Quercetin has been used to treat many skin disorders in combination with other flavonoid drugs. The novelty of this research describes a lipophilic drug (Quercetin) enclosed in hydrogel for topical application without any combination of drugs. This research aimed to design and optimize the Quercetin-loaded nanogel and evaluate its efficacy by drug loading, entrapment efficiency, and particle size. Quercetin-loaded nanogels have many advantages over other formulations due to their high drug-loading capacity and temporal drug release, making them a convenient delivery system for topical use. The optimized particle size was found to be 34.68 nm, and the Polydispersity index (PDI) was 0.208 with an entrapment efficiency of 6.90 %, which has ideal ranges for topical applications. Studies on homogeneity and extrudability have shown that the nanogel was both homogenous and simple to extrude. Based on the pH data, all the formulation is between 6.1 and 6.9, which is ideal for skin pH. ANOVA analysis implies the quadratic model is significant for studying nanogel formulation. The unique features and low toxicity of nanogel make the nanogel compatible with topical drug delivery.

Keywords: Nanogel, Lipophilic, Psoriasis, Optimization, Nanoemulsion, Nanoparticles

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The fast buildup of skin cells brought on by an autoimmune reaction is a defining feature of the medical disorder psoriasis.[1] On the skin's surface, scaly patches develop as a result. Redness and inflammation are frequently present as well.[2] Usually thick, crimson, and whitish-silver, these patches are noticeable. Purplish or dark brown patches with gray scales occasionally crack, and those with darker skin may experience bleeding. Plaques are a common skin condition that often appears on the elbows and knees, but they can also develop in other areas such as the scalp, palms, soles of feet, and genitals. Unlike eczema, psoriasis tends to be located on the outer surface of the joint[3,4]. The different forms of psoriasis –

1.1 Plaque psoriasis

It is the most prevalent type of psoriasis. It is characterized by round or oval-shaped plaques, which can also be coin-sized. Patients may develop flat, red macules or papules that spread outward and eventually merge into larger plaques measuring up to several centimeters in diameter.[5]

1.2 The term "guttate" in Guttate Psoriasis

Guttate comes from the Greek word "gutta," meaning droplet. This type of psoriasis is characterized by the sudden appearance of numerous small psoriasis lesions that typically measure 2-10mm in diameter. The lesions are mainly distributed centrally but can also appear on the head and limbs.[2,1]

1.3 Inverse Psoriasis,

Psoriasis that affects the body's flexural areas, such as the inframammary, perineal, and axillary regions, has a different appearance than the typical psoriasis plaques found on other parts of the trunk and limbs.[2]

1.4 Erythroderma

It can occur as a symptom of unstable psoriasis triggered by factors such as infections, tar, medication, or discontinuation of corticosteroids. When erythroderma affects the skin, it can interfere with the skin's ability to regulate body temperature, leading to complications such as hypothermia, high output cardiac failure, and metabolic changes, such as hypoalbuminemia and anemia caused by the loss of iron, vitamin B12, and folate.[2]Clinical significance of Psoriasis is that Scaling papules and plaques are the characteristic features of papulosquamous diseases. Psoriasis lesions are unique, distinguishing them from similar diseases like tinea infections, pityriasisrosea, and lichen planus. The psoriasis lesions are typically circular and well-defined, with a distinct red color and a dry, gray, or silvery-white scale. The distribution of psoriasis lesions is usually symmetrical and commonly found on the scalp, elbows, knees, lumbosacral area, and body folds.[6]Current treatment approaches for psoriasis include - Topical medications are frequently the first line of treatment for psoriasis that only affects a small portion of the body (less than 3-5% of the surface). Corticosteroids, analogs of vitamin D3, calcineurin inhibitors, keratolytic, and topical applications in addition to vitamin D3 are some examples. These topical treatments can often include creams, ointments, foams, or gels.[7]Targeted phototherapy is another option for treating limited psoriasis. This therapy uses ultraviolet radiation, which has a local immunosuppressive effect. It can directly impact Langerhans' cells (type of WBCs), inhibit dermal proliferation and formation of new blood vessels, and selectively reduce cutaneous T cells through apoptosis.[8] Adalimumab, infliximab, etanercept, and certolizumabpegol are four of the eleven biologics currently licensed for treating psoriasis. Golimumab is the only biologic approved for the treatment of psoriatic arthritis. IL-17A and IL-17F are the targets of the bispecific medication bimekizumab, while p19 is inhibited by lebrikizumab. Clinical trials at late stages are now being conducted for both medications. IL-17RC stands for IL-17 receptor, C, and Th17 stands for type 17 helper T cells, which is noteworthy. A combination anti-IL-17A medication is bimekizumab, to finish.[9]Despite the current treatment, which can only reduce the disease, there is a clear need for innovative methods of delivering medication topically[10]. We are closer to achieving a safe and effective treatment for psoriasis due to the development of new nanotechnology therapies and an increased understanding of the disease. Various novel topical carriers such as microemulsion, nanogel, niosomes, liposomal hydrogel, deformable liposomes, and solid lipid nanoparticles (SLNs) have been evaluated to enhance skin penetration of MTX. However, these carriers have limitations such as poor drug encapsulation efficiency, drug expulsion during storage, and highwater content in the formulation.[11] In contrast, nanostructured lipid carriers (NLC) have been extensively studied in topical and cosmetic preparations, as they can overcome many of the drawbacks associated with other nanocarriers. Topical Delivery of drugs for Psoriasis in nanocarriers such as Nanofibre. Its anti-inflammatory qualities make the natural substance curcumin an attractive candidate for topical psoriasis treatment. Cellulose Nanofiber (CNF), on the other hand, is a biocompatible biomaterial with mechanical and film-forming characteristics.[12] Nanoparticles are an appropriate solution for breaking through the skin barrier to increase the distribution of hydrophilic substances. The most popular nanoparticle types for topical medication delivery are dendrimers, solid lipid nanoparticles, metal nanoparticles, and polymeric nanoparticles. Nanoparticles can produce a sustained and regulated release of hydrophobic medications and increased drug concentrations in particular target locations.[13]Nanogel: Small cross-linked polymer networks soluble in water are known as nanogels. They constitute a potential approach for efficient topical medication administration and provide a flexible framework for integrating diverse anti-psoriatic drugs.[14] Gels can be characterized as either chemical or physical depending on the method of manufacture. Molecules are covalently bonded to the fibers throughout the chemical gels, which utilize macromolecules and fibers. In contrast, weak connections like van der Waals forces, hydrogen bonds, or coulombic forces between smaller molecules cause physical gels to form, often referred to as supramolecular gels.[15]

1.5 Drug Profile & Its Physicochemical Properties

Quercetin, apigenin, and baicalein are a few flavonoids investigated for possible effects on psoriasis. Many fruits and vegetables, including onions, apples, and berries, contain quercetin.[3] It has been proven to limit the release of inflammatory cytokines and to lessen oxidative stress, both of which have a main role in the early stages of psoriasis. It has been observed to lessen inflammation and reduce skin cell development, which may be helpful for psoriasis. The various information about the physicochemical nature, chemical structure, and application of Quercetin was shown in Table 1 and Figure 1.[3]

Table 1: Shows physicochemical parameters of Quercetin	
Name	**Quercetin**
Molecular weight	302.48
Chemical formula	C15H10O7
IUPAC	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one
Class	Flavonoid
Sub class	Flavones
Water solubility	0.261 mg/mL
logP	1.81

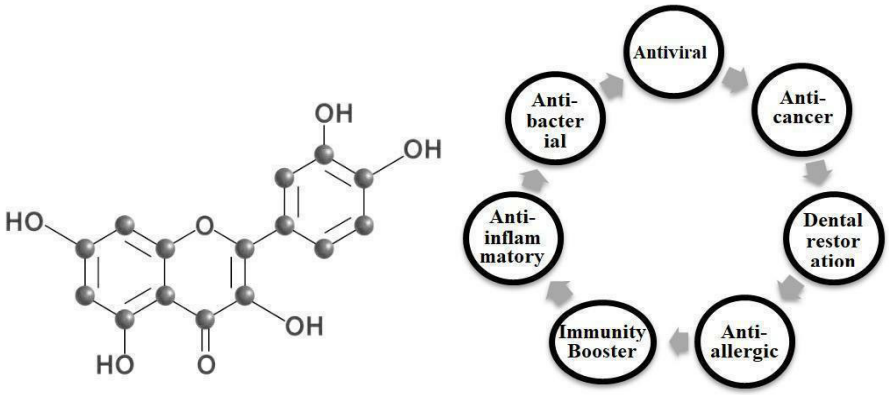

Fig 1: a. Chemical structure of Quercetin b . Shows the Application of Quercetin in various classes

1.6 Biological application of Quercetin with its mechanism of action

Quercetin has a wide spectrum of therapeutic effects in various classes. Being a phytoconstituent, it has fewer side effects and is a more effective constituent for showing therapeutic effects. The various MOAs are shown in the table below.

Table 1: **Biological application of Quercetin with its mechanism of action**	
Biological Activity	**Mechanism of Action**
Anti-oxidant effect	• stimulation of antioxidative enzymes such as GSH • Inhibition of lipid peroxidation • Scavenging of free radicals [16]
Anti-inflammatory effect	• Inhibition of inflammatory markers • Inhibition in release/generation of pro-inflammatory cytokines (IL-3, IL-6)[17]
Anti-neoplastic effect	• Inhibition of angiogenesis • Induction of cellular disruption • Anti-proliferative of abnormal cells[18]
Anti-bacterial and anti-microbial effect	• Destruction of the bacterial cellular membrane • Alteration in cellular morphology • Increases granulation tissue formulation[19]
Anti-viral	• Inhibition of protease enzyme[19]
Anti-aging	• Enhance the production of proteasomes • Restores collagen and keratinocyte cells in cell membrane[19]
Wound healing	• Activation of growth factors[19]

2. METHODOLOGY

2.1. Materials

Quercetin was procured from LOBA CHEMIE Pvt. Ltd. (Mumbai). Coconut oil was received as a gift sample from ULTRA PURE LAB CHEM INDUSTRIES Llp. (Palghar, Mumbai), double distilled water from the institution lab, Ethanol (99.99%) from CGP, Triethanolamine was from Merck specialties, Tween 80 was procured from Merck Specialties Private Limited (Mumbai), Carbopol 940 was procured from LOBA CHEMIE Pvt. Ltd. (Mumbai) were all of the analytical grades.

2.2 Pre-Formulation Studies of the Drug and the Excipients

2.2.1 FTIR

FTIR stands for Fourier transform infrared. ATR technique measures the interaction between the excipients and the drug. In the ATR approach, the sample is in contact with a crystal thinly coated with a reflective substance, such as diamond or zinc selenide. The infrared light is then angled onto the crystal, bouncing off the reflective coating and penetrating the sample.[20,18,21,22] The chemical bonds absorb the light in the sample as it passes through it, and the FTIR device records the resulting spectrum, shown in Figure 2.

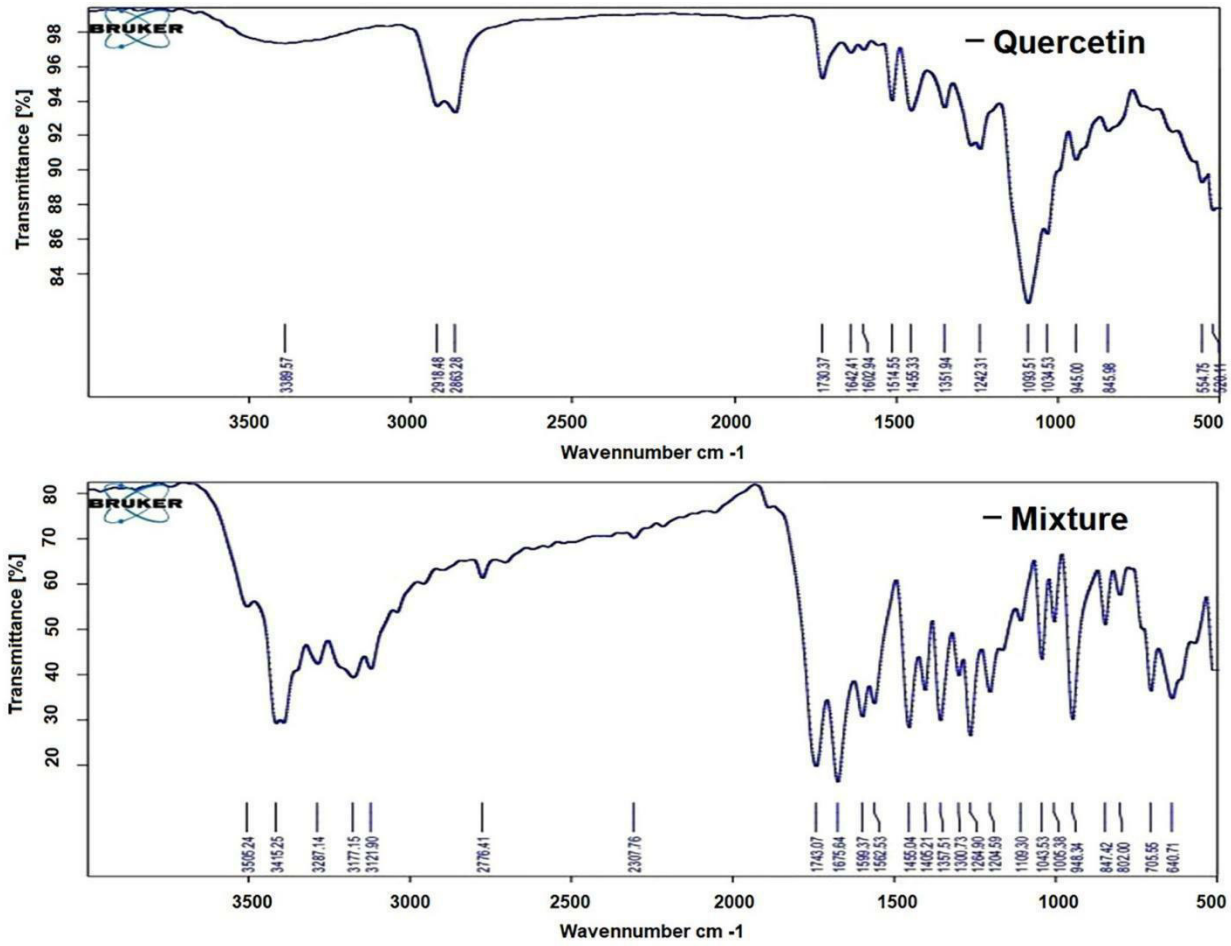

Fig 2: a. FTIR data of quercetin b. FTIR data of quercetin and excipients(mixture)

The FTIR spectrum of the above image shows the peaks from the spectrum of pure drug (Quercetin) and drug + excipients mixture. The prominent peaks for drug, i.e., 1093.51 cm-1 corresponding to C-O stretching; 1730.37 cm-1 corresponding to O-H stretching similarly, the prominent peaks for mixture 1448.04 cm-1 shows stretching corresponding to C-O corresponding to 848.34 cm-1 O-H shows bending. Hence, the functional groups interpreted in the drug and mixture were correlated.

2.2.2 *Solubility*

The solubility studies were done by visual inspection. 1 mg of Quercetin was weighed and mixed with 2 ml lipid solvents such as coconut oil, ethanol, methanol, olive oil, and water. The solution transparency measured the solubility at every interval of 2 hrs. Hence, it was observed that quercetin was insoluble in water, partially soluble in olive oil, fairly soluble in methanol, and fully soluble in coconut oil and ethanol.[23,24]

2.2. *Design of the Experiment*

The pharmaceutical business frequently uses BBD (Box-Behnken Design), a statistical experimental design designed in Design-Expert 7.1.5 software.[25]Using the Box-Behnken statistical design is a quick as well as efficient method of system optimization.[18,23,25,26]The Box-Behnken design (BBD) was used in this study to optimize the QC-NG using Design Expert Software Version 7.1.5. (USA). As shown in Table 2, the amount of coconut oil (Factor A), amount of Tween 80 (Factor B), and sonication time (Factor C) were chosen at two low and high levels, respectively, and served as the independent variables.[4] The response variables were the Polydispersity index (PDI) and particle size. Finding the best combination of variables for attaining the intended result entails methodically altering the amounts of multiple variables.[25,27,28] To achieve the appropriate particle size, stability, drug loading, and release profile while making nanogels, BBD design is frequently used to optimize the formulation variables, such as the concentrations of polymers, crosslinking agents, surfactants, and other excipients**.** The factors were selected based on certain pre-formulation studies.

Table 2: Box- Behnken design of the experiment

Run	Factor 1(ml)	Factor 2 (ml)	Factor 3 (min)	Response 1 PS(nm)	Response 2 (PDI)	Response 3 (%EE)
1	3.50	2	80	56.51	0.427	82.89
2	2	2	40	64.41	0.441	78
3	2	1	80	53.59	0.424	83.63
4	5	2	40	54.3	0.472	80.21
5	5	2	120	34.68	0.208	86.90
6	2	2	120	152.7	0.556	72.78

7	3.50	I	120	65.6	0.435	71.67
8	3.50	3	40	82.8	0.479	79.04
9	5	I	80	115.4	0.549	73.56
10	5	3	80	96.9	0457	75.64
11	3.50	3	120	121.4	0.552	72.39
12	2	3	80	184.4	0.525	74
13	3.50	I	40	148	0.609	79.90

***Table shows all the independent factors and their responses; particle size increases if the amount of oil (factor) increases, and particle size decreases if the sonication time increases. The relation between the Particle size and PDI is inversely proportional; the larger the particle size, the lesser the PDI. The entrapment efficiency depends upon the particle size; a large particle size has less drug entrapment. **Table shows the data from PubMed, ScienceDirect, Google Scholar, and several other databases were utilised to accumulate the related literature using keywords such as "quercetin loaded nanoformulations" and "dermal nanoformulations'. Of quercetin," "quercetin topical formulations," and many others.*

2.3. Preparation of Quercetin loaded Nanogel (QC-NG)

The Quercetin nanogel was prepared by emulsification method, followed by ultrasonication. The 10 mg drug was weighed and mixed with the lipid phase containing coconut oil using magnetic stirrers illustrated in Figure 3. Then, the surfactant is added dropwise and stirred in a magnetic stirrer (Remi 2MLH by Remi Lab World). The Aqueous phase was added to the oil phase solution dropwise while mixing it using a magnetic stirrer under 2000 rpm for 50 mins. After mixing both phases, the solution was kept under sonication (Labman Scientific Instruments Pvt. Ltd.) for 40 mins at 60°C. The homogenous solution was dispersed in a carbopol solution and stirred for proper mixing. Triethanolamine was added dropwise to maintain the formulation's pH and obtain a gel-like consistency. Quercetin-loaded nanogel stored in a refrigerator.[10,18]

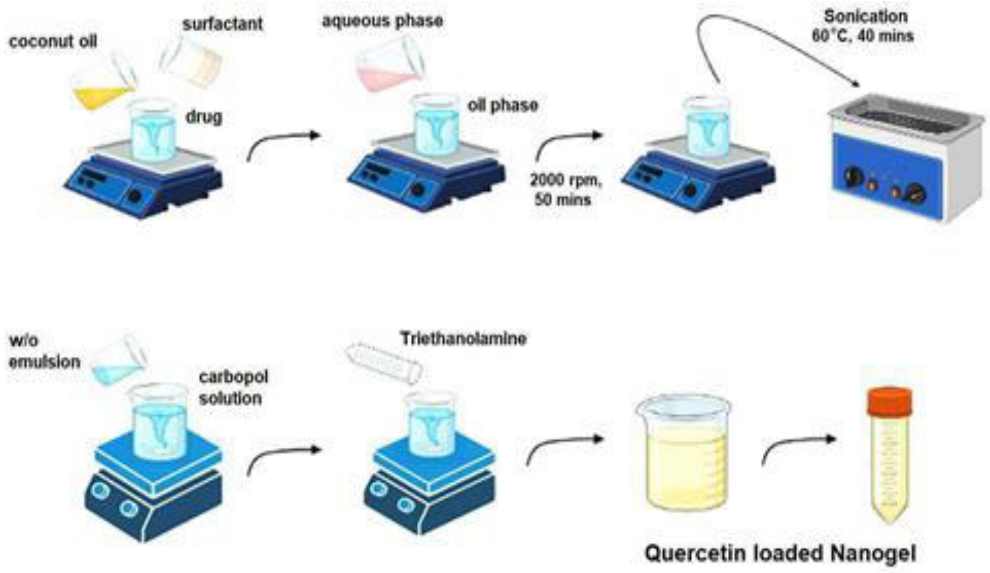

Fig 3: Graphical representation of Preparation of Quercetin-loaded nanogel

***The above graphical representation (Figure III) of the procedure of Nanogel preparation was designed in BioRender software to illustrate the laboratory procedure of nanogel preparation. The aqueous phase contains ethanol and distilled water; the oil phase contains coconut oil with IP grade and drug in powder form. **Triethanolamine (TEA) was added dropwise to maintain the gel consistency and pH.*

3. Characterization

3.1. Physico-chemical evaluation

3.1.1 Homogeneity

The Nanogel formulations (Run 1-Run 13) had good consistency without any lumps, as shown in Table 3. The gels were transparent and free of particles, irregularities, foreign substances, or phase separation, which indicates consistent gel quality.[28]

3.1.2 pH

The pH of the QC-NG was measured with the help of a pH meter. The pH of Run 1 –Run 13 is shown in Table 3.[10,13,]

3.1.3 Spreadability

The method was used to check the gel composition's spreadand extrude abilities. Applying a weighted amount of the product sample to one of the glass slides, compressing it with a second glass slide while applying a 100-gram weight, and then effortlessly removing the top slide after tying it with a 20-gram weight were used to test spreadability. It took 7.50 cm of the upper slide

exactly one second to cross the lower glass slide's thin sheet. The weight required to extrude a 1 cm part of the formulations from collapsible tubes after adding a weighed amount of conventional gel and nanogel was used to gauge the extrude ability of the materials. To ensure accuracy, the analysis was run three times. Spreadability can be calculated by the following formula: -

$$S = W*L/T$$

Where S= spreadability, W = weight tide to upper slide, and T = time is taken to separate the glass slide from each other.[18,20,22,30]

The spreadability of all the nanogel formulations is shown in Table 2

Table 3: Homogeneity, pH, Spreadability, and extrudability of nanogel

Run	Homogeneity	pH	Spreadability (cm)	Extrudability	Viscosity (cps)
1	Homogenous	6.23	5.78	+	3209
2	Homogenous	6.08	5.23	+	3180
3	Homogenous	6.56	5.31	+	3298
4	Homogenous	6.21	6.00	+	3287
5	Homogenous	6.45	6.67	+	3612
6	Homogenous	6.38	5.87	+	3891
7	Homogenous	6.43	5.43	+	3546
8	Homogenous	6.50	5.21	+	3892
9	Homogenous	6.89	5.77	+	3126
10	Homogenous	6.74	5.34	+	3623
11	Homogenous	6.44	5.04	+	3326
12	Homogenous	6.23	5.72	+	3295
13	Homogenous	6.91	5.27	+	3652

***** The table shows the characteristics of the prepared nanogels. Homogeneity, pH, spreadability, Extrudability, and Viscosity were considered. All formulations were homogenous; pH ranges from 6.08-6.89, Spreadability was within the range, and Viscosity was almost the same for all the preparations.***

3.1.4 Determination of Solubility of drug and excipients

The solubility of quercetin is very low in water. Hence, Quercetin solubility is very low in the water, though the solubility was measured on the liquid lipid selection (coconut oil,olive oil, clove oil, soyabean oil, and castor oil. Solubility testing of quercetin with other excipients has been done in two ways: 1. Visual analysis 2. UV analysis

1. Visual analysis

The solubility of quercetin with other solvents (coconut oil, ethanol, olive oil, methanol, and water). The observation was taken in 2 hours after vortexing. The visual observation of solubility data is displayed in Table 4 below.

2. UV-analysis

The solubility of QC with different oils was determined at a wavelength of 276 nm at a scanning range of 200-400 nm in a UV spectrophotometer, and it was seen that quercetin has maximum absorbance with coconut oil among all the solvents and least absorbance with water (±SD) N=3 given in figure IV. Hence, it was observed that the coconut oil solubility of quercetin was more than that of other oils. Based on disease, coconut oil was selected for antipsoriaticnanogel preparation as it provides soothing action in the anti-inflammatory site.

Table 4: Observation of solubility of the drug in the following time intervals

Sl. No.	Solvent	After 30 mins	2 hours	4 hours
1	QC+ olive oil	Freely soluble	Precipitation occurs	Precipitation occurs
2	QC+ coconut oil	Very soluble	Clear solution with no precipitates	Clear solution with no precipitates
3	QC + castor oil	Very soluble	A clear solution appears with no precipitates	A clear solution appears with no precipitates
4	QC + soybean oil	Slightly soluble	Precipitation occurs	Precipitation occurs
5	QC + clove oil	Insoluble	Insoluble	Insoluble

*****Table 4 shows the Solubility of the drug in different oils after some time intervals, and it was seen that after vortexing, the drug particles were precipitated in some oils like clove oil, olive oil, and soybean oil and fully soluble in coconut oil, no residue was seen***

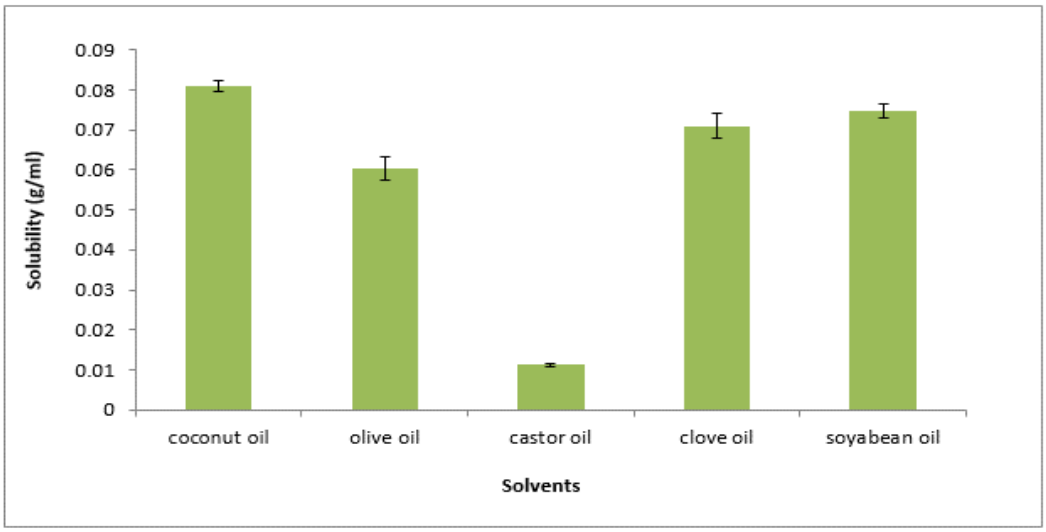

Fig 4: Graph represents solubility of the drug in different organic and inorganic solvent

***Figure IV shows the solubility (g/ml) of Quercetin in different oils as specified. Coconut oil exhibits solubility of Quercetin around 0.08 g/ml, whereas castor oil shows the lowest one (0.01 g/ml)*

3.1.5 Viscosity

Using a Brookfield viscometer (Brookfield Engineering Laboratories, U.S.A. Model No.- LVDVE), the viscosity of optimized QC-NG was measured successfully. The measurements were carried out with spindle number 64 at 20 rpm. The sample was set up on the stage and let to descend to calculate viscosity[30]. The measurements were made three times, and the mean and standard deviation (mean SD) were computed for each. The viscosity of the nanogel formulations is given in Table 3. All the viscosity of QC-NG was found to be in the range of 3200-3500 cP.

3.2. *Stability Studies*

The Quercetin-Loaded nanogel was performed for stability testing by ICH requirements to ensure its stability. The nanogel was kept in weighted volumes of 10 grams in two centrifuge tubes for six months at two different temperatures: low (2-8 °C) and hot (40 °C). Samples were taken and visually inspected for potential physical changes at certain intervals (0, 3, and 6 months).[17] The techniques mentioned earlier were additionally applied to evaluate the drug content and pH levels to evaluate the stability. Nanogel was observed to be physically stable at both temperatures; no cream and cracking was found in the centrifuge tubes, and no colour change of the formulation was found in the tubes. After two months of storage, the nanogel was evaluated for pH and drug loading (%) to check its stability, which is shown in Table 4.[17]

Table 5: Stability data of optimized nanogel formulation

Time (Months)	Drug loading (%)	pH
0	88.98	6.70
3	88.45	6.56
6	87.23	6.68

***The above table shows the stability data of optimized nanogel formulation in six months; there were slight changes in drug loading (%) of nanogel, and the pH of the nanogel was within the range.*

3.3. Morphological studies

3.3.1 Scanning electron microscopy (SEM)

Scanning electron microscopy is a process for determining the size of the particle and the shape of the particles present in QC-NG. The SEM of the Run 5 is shown in Figure IV. Particles were spherical and sized with a narrow range.

3.3.2 Dynamic light scattering (DLS)

DLS is used to characterize the particle size in nanogel formulation. Using Malvern Zetasizer software V7.12, a Malvern Zetasizer Nano ZS with a 633 nm. Samples that had been dialyzed were diluted to 1 mg/mL. Using disposable polystyrene cuvettes with a 1-cm route length, the Z-average diameter was measured between 15 and 55 C with a 600-second thermal acclimatization period. Three measurements were made to obtain the average Z-average diameter and polydispersity index. The distribution of particle size by number and intensity and the Graph of the correlogram of Run 5 (optimized batch) is shown in Figure IV.

3.4. In vitro Drug Diffusion Studies

The Drug diffusion studies of nanogel were performed using the Franz diffusion cell apparatus. A dialysis membrane 50 (Hi-Media) with a receptor compartment capacity of 45 ml and a cross-sectional area of 0.785 cm^2 was carried out using a Franz Diffusion Cell apparatus[32]. The membrane was treated with the created, optimized QC-NG formulation. The membrane was then mended and placed in the donor chamber, with the

membrane facing the receiver compartment. The receiver chamber was filled with a pH 7.4 PBS buffer. Magnetic beads continually stirred the receptor solution, maintaining the temperature at 32 ± 0.5 °C. Following the removal of the sample at various times of 15, 30, 60, 90, 120, 180, 240, 300, 360, and 420 minutes, the samples were assessed under UV at a wavelength of 276 nm. According to all the calculations of drug diffusion parameters, the diffusion pattern of the drug is shown in Figure 5.

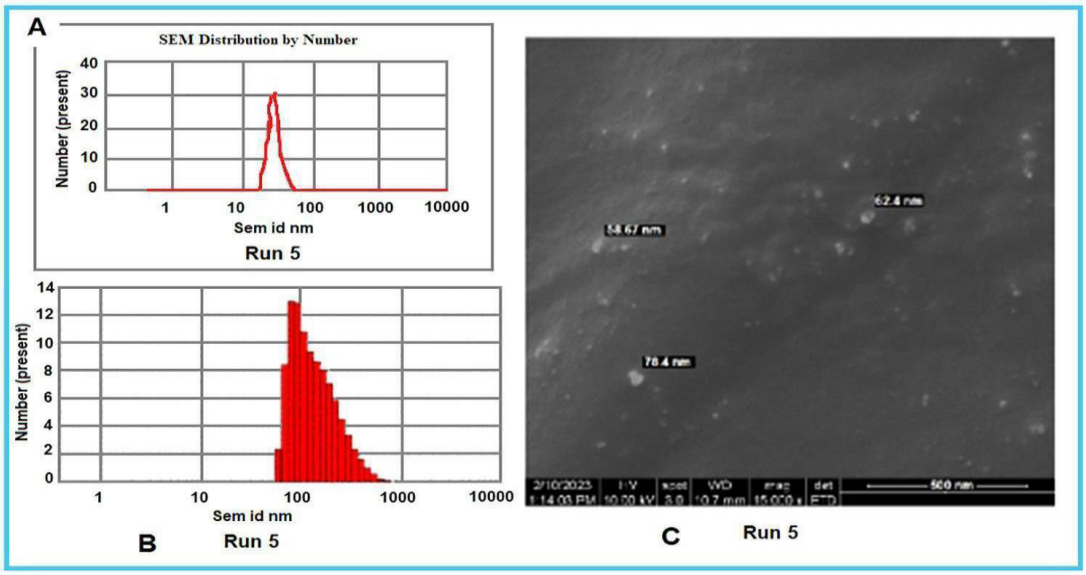

Fig 5: DLS and SEM of Run 5, A. PS Distribution by Number, B. PS Distribution by intensity, C. SEM image

Figure V shows A. Particle size distribution by number in optimized QC-NG (Run 5); the sharp peak in the graph denotes that similar sizes of particles were present in a sample. , B. In a correlogram graph, the curve denotes the flow ability of the particles present in a sample. C. The mean average of the particle size distribution was shown in particle size distribution by intensity. D. SEM image of Optimized QC-NG indicates the particle size is in the nano-range, which confirms the presence of nanoparticles in the optimized QC-NG

4. STATISTICAL ANALYSIS

ANOVA was used to identify any non-significant components, and the data were assessed using Design-Expert Software (7.1.5.). According to the data, the results showed that every dependent variable had a p-value of less than 0.05 (p0.05). The PS, PDI, and EE% Models prove the model's significance. The percentage of the dependent variable's variance that the independent variables can collectively explain is shown by R-squared, a measure of the goodness-of-fit for linear regression models.[18,31]

Table 6: ANOVA for Response Surface Quadratic Model of Particle size

Source	Sum of Squares	Df	Mean Square	F Value	p-value Prob> F	
Model	28891.62	9	3210.18	4.368375	0.0324	significant
A-oil	1776.676	1	1776.676	2.41768	0.1639	
B-Tween80	1334.12	1	1334.12	1.815454	0.2198	
C-sonication time	448.0521	1	448.0521	0.609704	0.4605	
AB	5603.271	1	5603.271	7.624866	0.0280	
AC	5845.367	1	5845.367	7.954308	0.0258	
BC	3660.25	1	3660.25	4.980826	0.0608	
A^2	316.6832	1	316.6832	0.430939	0.5325	
B^2	9640.516	1	9640.516	13.1187	0.0085	
C^2	0.852632	1	0.852632	0.00116	0.9738	
Residual	5144.077	7	734.8681			
Lack of Fit	5144.077	3	1714.692			
Pure Error	0	4	0			
Cor Total	34035.69	16				

The statistical analysis suggests the Model F-value of 4.37 denotes the model's importance. This figure indicates that the possibility of noise producing a "Model F-value" of this magnitude is only 3.24%. Further evidence that the model terms are significant comes from "Prob> F" values less than 0.0500. Particularly important model terms in this instance are AB, AC, and B2, as shown in Table 5. However, values higher than 0.1000 imply that the model terms are unimportant. It might be advantageous to decrease the model to increase its efficacy if many of the terms (apart from those required to maintain hierarchy) are irrelevant.

Table 7: ANOVA for quadratic models indicating-Squared value Model-F value of Particle Size			
Std. Dev.	27.10845	**R-Squared**	0.848862
Mean	82.96059	**Adj R-Squared**	0.654542
C.V. %	32.6763	**Pred R-Squared**	-1.4182
PRESS	82305.23	**Adeq Precision**	7.613305

The table 6 & 7 show the ANOVA analysis of the optimized formulation by Design Expert software version 7.1.5. Table 7 shows all the predicted and adequate R values for the design.

The negative "Pred R-Squared" suggests that the overall mean better predicts your reaction than the present model. The signal-to-noise ratio is measured by "Adeq Precision". The ideal ratio is greater than 4. The ratio of 7.613 shows a strong enough signal, as shown in Table 6. The design space can be explored using this model.

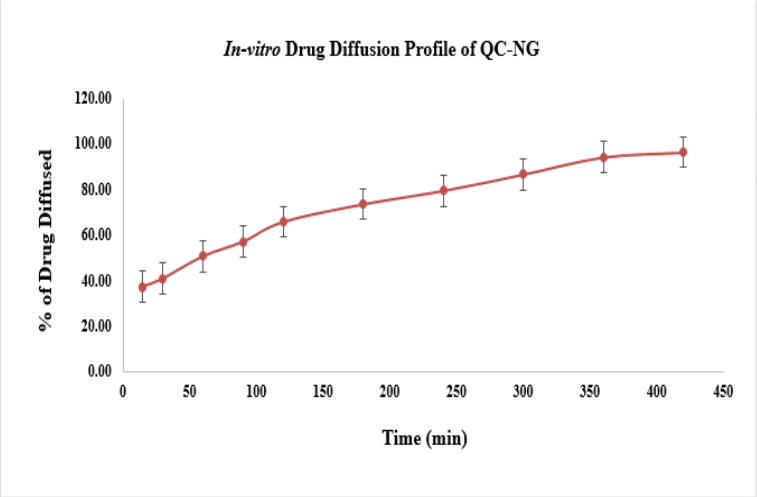

Fig 6: Drug diffusion profile of QC-NG

Figure VI shows the release profile of QC-NG upto 6 hrs, an ideal range for topical formulation for targeted and sustained release. The drug release from the nanogel formulation ideally follows the Higuchi model of release kinetics.

5. RESULTS

5.1 Optimization and statistical analysis of variables

A statistical design using BBD (Box-Behnken Design) was used to develop a Quercetin-loaded Loaded Nanogel. Many experimental design models were applied to fit the data once the experimental design software was used to evaluate the potential effects of independent variables. The quadratic model was discovered to be the best-fitting model (p 0.05). The results of the dependent variables are based on the regression analysis using the quadratic model. [26]

5.2 Effect of Independent Variable on Particle Size of Nanogel Formulation

In a nanogel, the particle size significantly impacts how well the drug diffuses and releases at the site of the infection. As a result, making a Nanogel containing tiny particles is ideal. After comparing different models, it was discovered that the quadratic model (p 0.0001), which had an F value of 4.37, was the most optimized result. Additionally, the difference between the adjusted R2 value (0.8489) and the anticipated R2 value (0.6545) was less than 0.20, showing strong agreement between them. The various types of graphs, such as 3D surface graphs, Contour plots, and perturbation graphs of optimization by particle size, are shown in Figure VII. The formula used in the studyillustrates the influence of independent variables on particle size.

PS = +139.80 + 15.67A 22.19B + 3.25AB 3.09A2 + 1.91B2 Eq.no. (I)

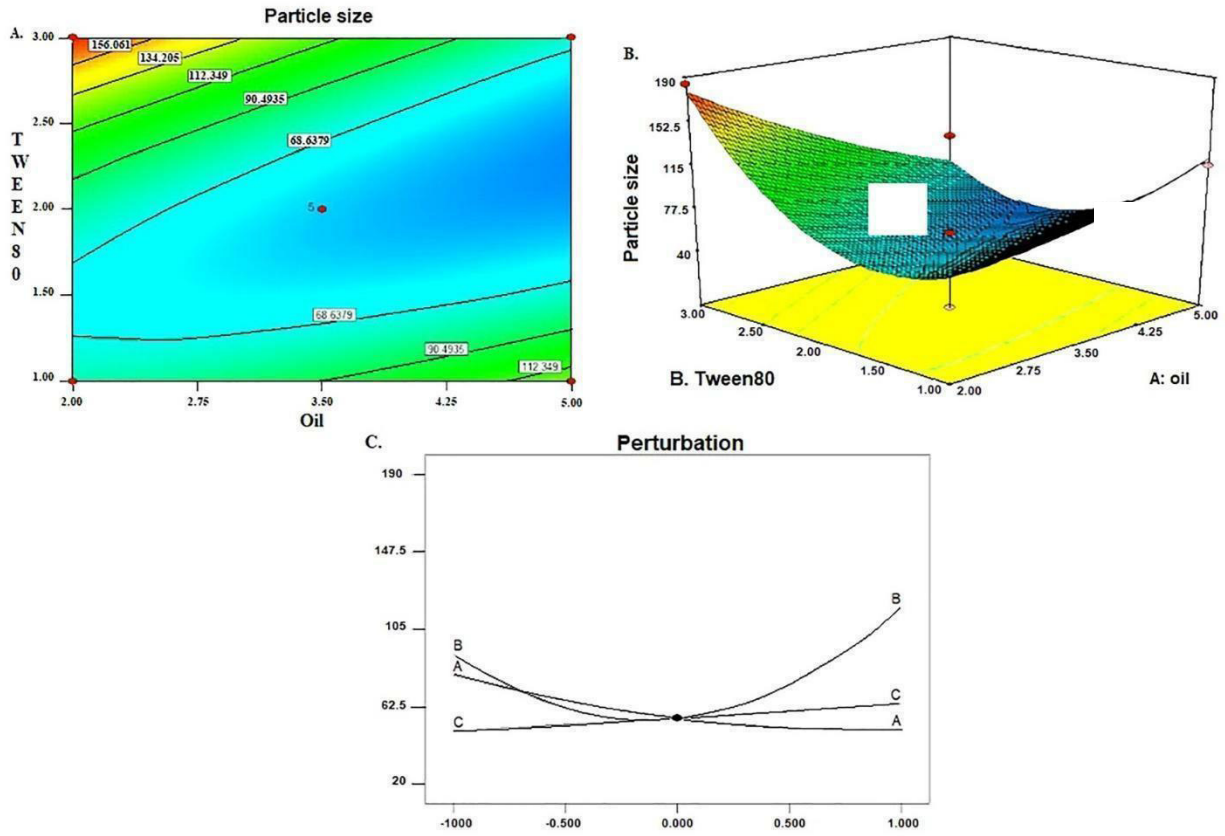

Fig 7: Optimization of Particle size, A. contour plot, B. 3D response surface graph, C. Perturbation graph

Figure shows the various response graphs of QC-NG based on particle size. A. shows the particle size in the 3D response curve; the colour distribution in the graph indicates the highest and lowest levels of the response. The red indicates the maximum particle size due to increasing the oil and Tween. Similarly, the dark blue color has the lowest particle size due to decreasing the amount of Tween and coconut oil. The same is the color distribution of the contour pot of particle size, which indicates the optimum point is inside the experimental design.

4.3 Entrapment Efficiency and PDI

The optimized batch had an entrapment efficiency of 90%, which was in line with the anticipated result. The values obtained for the optimized batch fell within a satisfactory range, according to statistical analysis, which had a 95% degree of confidence in its results. The projected values for particle size and entrapment efficiency were also achieved. Figure VIII and Figure IX display the 3D response surface graph and contour plot for entrapment efficiency and PDI.

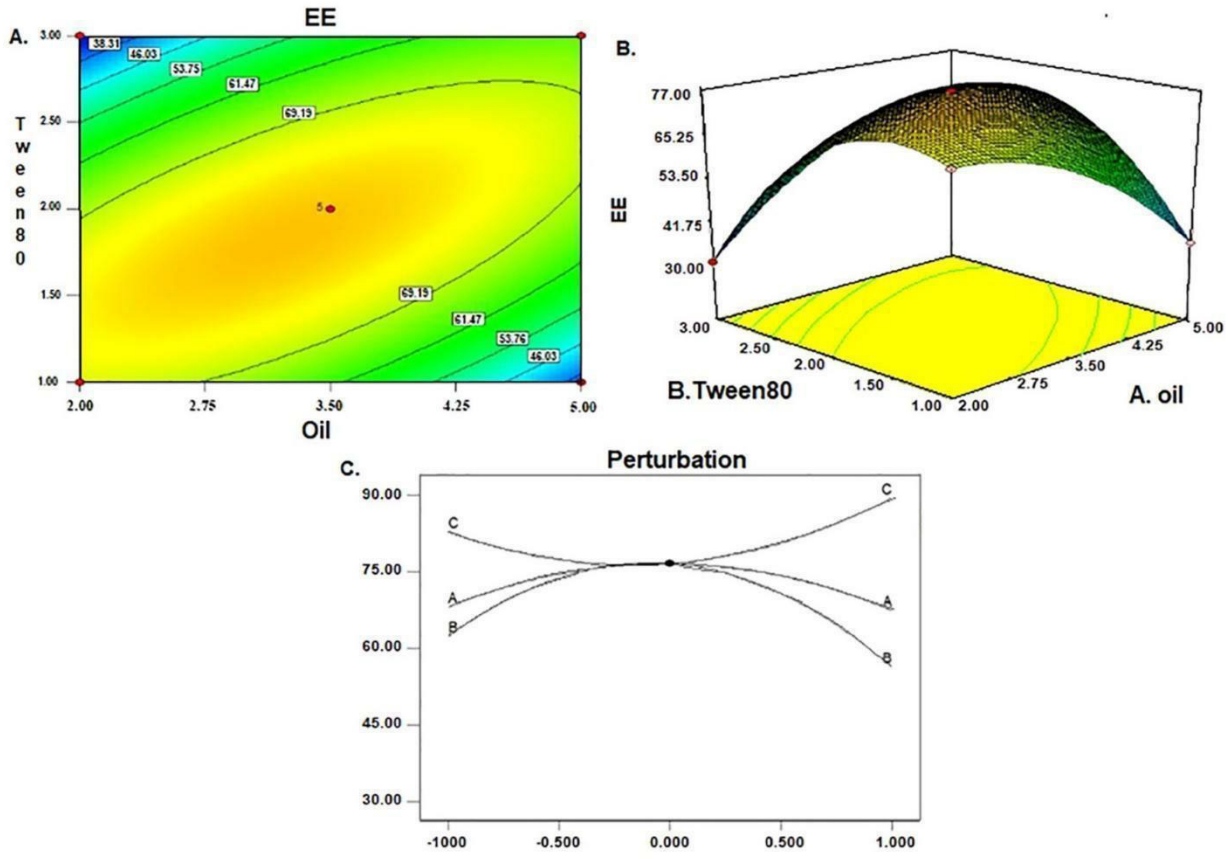

Fig 8: Optimization of EE, A. contour plot, B. 3D response surface graph, C. Perturbation graph

Figure shows the various response graphs of QC-NG based on Entrapment efficiency (EE). A. shows the EE in the 3D response curve, and the colour distribution in the graph indicates the highest and lowest levels of the response. The green color indicates the maximum % of EE upon decreasing the amount of oil and increasing the amount of Tween 80 (surfactant). Similarly, the dark blue color has the lowest % of EE upon decreasing the amount of Tween and coconut oil. It is the same with the color distribution of the contour pot of EE, which indicates the optimum point is inside the experimental design.

4.4 Optimization of Nanogel Formulation and its Evaluation

Based on obtaining the ideal particle size, PDI, the optimal Quercetin-loaded nanogel was chosen to utilize the point prediction approach of an experimental design program. The Nanogel was found to have 5 ml oil and 2 ml Tween 80 (surfactant), which satisfied the parameters for an ideal formulation (Run 5) after analyzing the various reaction factors and assessing the environment. The optimized Quercetin-loaded nanogel consequently showed a particle size of 29.68 nm and a PDI of 0.208. In addition, a quantitative linear link between experimental value responses and all of the dependent variables' anticipated values was seen.

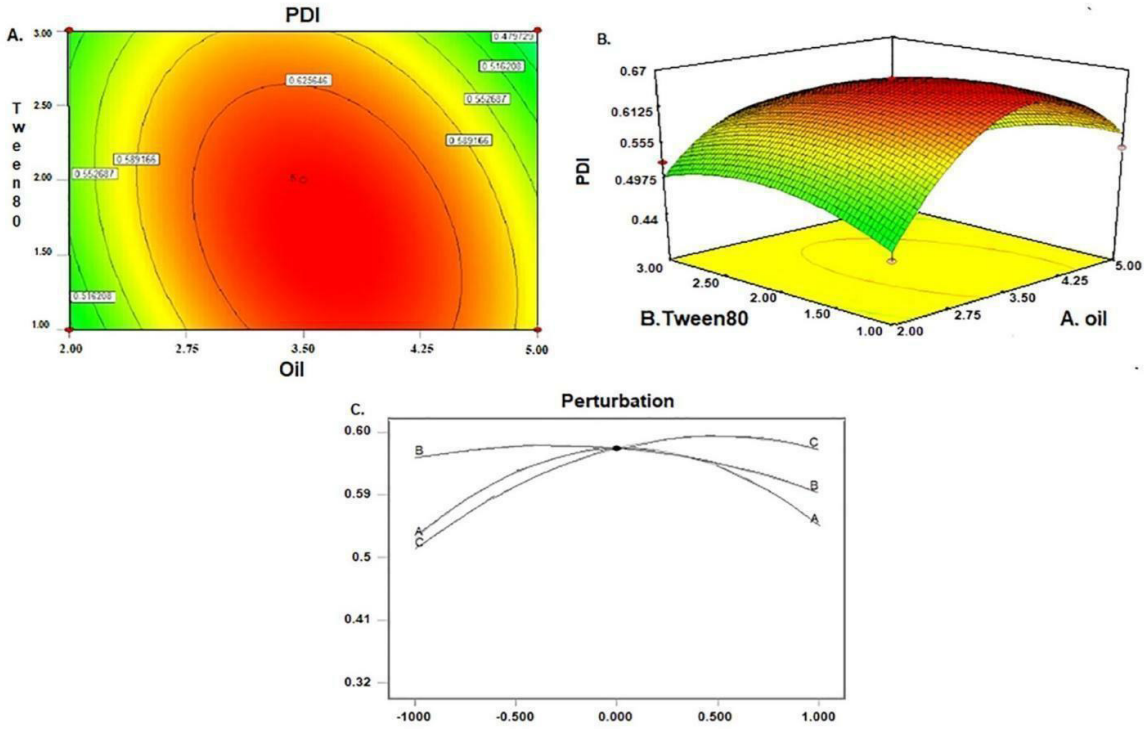

Fig 9: Optimization of PDI, A. contour plot, B. 3D response surface graph, C. Perturbation graph

Figure shows the various response graphs of QC-NG based on PDI A. Shows the PDI in the 3D response curve; the color distribution in the graph indicates the highest and lowest levels of the response. The green indicates the maximum PDI upon increasing the sonication time and decreasing the Tween 80 (surfactant) amount. Similarly, the green color has the lowest PDI upon decreasing the amount of Tween and sonication time. It is the same with the color distribution of the contour pot of PDI, which indicates the optimum point is inside the experimental design.

6. DISCUSSION

According to this study, The Nanogel was fabricated and optimized for the topical application of quercetin to treat psoriasis. At first, the preformulation study of Drugs and Excipients was done by doing an FTIR study to check the compatibility of the drugs and the excipients used in preparing the Nanogel[33]. The result of the FTIR study found that the drug and excipients are perfectly compatible with each other[1]. The quercetin-loaded nanogel was prepared by using emulsification followed by an ultra-sonication technique[34,35]. The prepared Nanogels are characterized by testing for homogeneity, pH, Spreadability, and extrudability. The formulations are homogeneous, and the pH of the formulation was relevant to the skin pH. The result of the extrudability test was positive for all formulations[28,37]. The formulation was optimized by a statistical design using BBD (Box-Behnken Design). The formulation was optimized based on three response parameters: particle size, PDI, and entrapment efficiency[2]. ANOVA was used to identify any non-significant components, and the data were assessed using Design-Expert Software (7.1.5.)[38]. According to the data, the results showed that every dependent variable had a p-value of less than 0.05 (p0.05). The PS, PDI, and EE% Models prove the model's significance[39]. The percentage of the dependent variable's variance that the independent variables can collectively explain is shown by R-Squared, a measure of the goodness-of-fit for linear regression models[40].The optimized batch had an entrapment efficiency of 90%, which was in line with the anticipated result[41,42]. The values obtained for the optimized batch fell within a satisfactory range, according to statistical analysis, which had a 95% degree of confidence in its results. The projected values for particle size and entrapment efficiency were also achieved. The particle size of the optimized product came into the nano range and was respectively smaller than other formulations[43,44]. The optimized formulation was more homogeneous than other formulations, so the polydispersity index of the optimized formulation was also smaller than other formulations[45,46]. Then, the optimized product was tested for morphological analysis, such as SEM analysis and dynamic light scattering (DLS), for further modification of the optimized formulation. However, the study lacked an Ex-vivo permeation study and an Invitro Drug Diffusion study of the optimized Nanogel formulation to make it more modified and suitable for the application. Thus, the study proves that the quercetin-loaded nanogel is potentially active for the topical application of quercetin to treat psoriasis.

7. CONCLUSION

The study employed a Box-Behnken design comprising 17 runs, with 4 runs repeated and subsequently excluded from the analysis. Utilizing Design Expert software, the investigation centered on the formulations established through a comprehensive 43-complete Box-Behnken design, facilitating the simultaneous assessment of two key formulation variables and their interactions. Two independent variables, namely the concentrations of oil and surfactant, were selected based on prior research, and their effects were explored across three levels (High, Medium, and Low). The impact of these variables on three dependent variables, namely Particle Size (R1), P.D.I (R2) and %EE (R3) were evaluated using a polynomial equation. Through surface response methods, optimization was performed to achieve an ideal dosage form for Quercetin-loaded Nanogel, focusing on Particle Size and entrapment efficiency. The comparison of actual and expected responses demonstrated the efficacy of this approach. In nanoparticle development, optimization represents a sophisticated

experimental process frequently utilized to establish reliable preparation methods and yield products with desired attributes. The preferred method for conducting such optimization studies, determining dominant components, and identifying optimal levels of variables for a desirable Dosage Form is the employment of BBD (Box-Behnken design).

8. ACKNOWLEDGMENT

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9. AUTHORS CONTRIBUTION STATEMENT

The Corresponding author, Dr. Gopa Roy Biswas, designed the research work. Pakhi Chakraborty and Swagata Das conducted the experimental work; all the co-authors collected data, interpreted data, and prepared manuscripts. Dr. Gopa Roy Biswas made the final checking of the manuscript.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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