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# DESIGN AND STATISTICAL OPTIMIZATION OF QUERCETIN NANOEMULGEL USING PSEUDO-TERNARY PHASE DIAGRAM AND FACTORIAL DESIGN APPROACH FOR ENHANCED TOPICAL ACNE THERAPY

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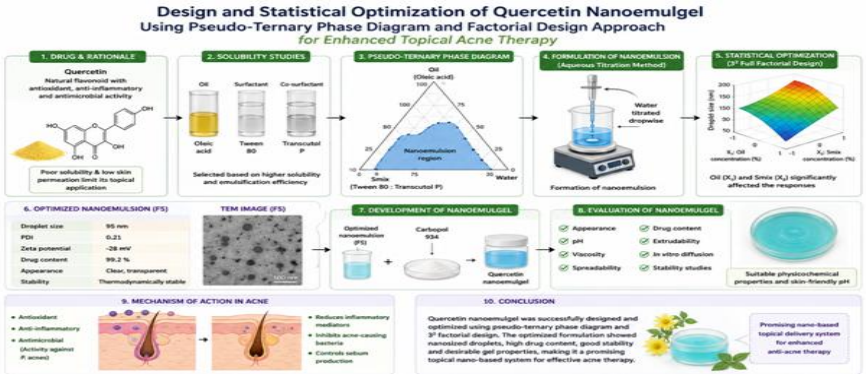
**ABSTRACT:**
**Objective:** In order to increase quercetin's solubility, skin penetration, stability, and therapeutic efficacy, the current study developed and optimized a quercetin-loaded nanoemulgel for topical anti-acne therapy.
**Method:** Solubility studies were conducted in order to select suitable formulation components. Oleic acid was selected as the oil phase, Tween 80 as the surfactant, and Transcutol P as the co-surfactant based on maximum solubility and emulsification efficiency. Using different Smix ratios, pseudo-ternary phase diagrams were made in order to calculate the nanoemulsion area. Oil concentration and Smix concentration were employed as independent variables in a $3^2$ full factorial design for optimization, while droplet size, polydispersity index (PDI), and zeta potential were selected as dependent responses. The improved nanoemulsion was combined with Carbopol 934 gel to generate nanoemulgel, which was subsequently evaluated.
**Result:** The enhanced formulation (F5) showed exceptional transparency, homogeneity, and stability despite a droplet size of 95 nm, PDI of 0.21, and zeta potential of −28 mV. Effective drug integration was demonstrated by the discovery of a 99.2% drug content. Thermodynamic stability investigations showed no evidence of phase separation, creaming, or precipitation. TEM analysis revealed that the nano-sized droplets were uniformly distributed and spherical. The enhanced nanoemulgel demonstrated suitable pH, viscosity, spreadability, homogeneity, and extrudability for topical application. Statistical study and the response surface approach confirmed the significant influence of oil and Smix concentration on formulation properties.
**Conclusion:** Thequercetin nanoemulgel demonstrated superior stability, enhanced physicochemical qualities, and qualities appropriate for topical anti-acne administration.

**KEYWORDS:** Acne vulgaris; Nanoemulgel; Quercetin; Response Surface Methodology; Topical Drug Delivery.




## INTRODUCTION

One of the most prevalent chronic inflammatory skin conditions affecting teenagers and young adults globally is acne vulgaris. Excessive sebum production, follicular hyperkeratinization, microbial colonization, and pilosebaceous unit inflammation are its hallmarks. Antibiotics, retinoids, and benzoyl peroxide are examples of conventional topical treatments that have a number of drawbacks, such as poor skin penetration, irritation, dryness, low patient compliance, and the emergence of microbial resistance. Thus, there has been a great deal of interest in the development of sophisticated topical medication delivery systems that are more effective and have fewer side effects1,2.Quercetin is a naturally occurring flavonoid with potent antioxidant, anti-inflammatory, antibacterial, and anti-acne properties. It is a member of the polyphenolic class of chemicals. Quercetin's weak water solubility, low permeability, and insufficient bioavailability restrict its clinical use despite its significant therapeutic potential3. By enhancing drug solubilization, stability, skin penetration, and therapeutic effectiveness, nanoemulsion-based drug delivery systems have become a

viable way to get around these restrictions. Oil, surfactant, co-surfactant, and aqueous phase make up nanoemulsions, which are thermodynamically or kinetically stable colloidal systems with droplet sizes typically in the nanoscale range. Nanoemulsions improve topical administration and increase drug absorption because of their huge surface area and small droplet size4. Viscosity, spreadability, retention time, and patient acceptability are all further enhanced when nanoemulsion is incorporated into a gel foundation, creating nanoemulgel systems that are appropriate for topical treatment5.

The current study used oleic acid as the oil phase, Tween 80 as the surfactant, and Transcutol P as the co-surfactant to design and optimize quercetin-loaded nanoemulsion and nanoemulgel formulations. An optimized and stable formulation for improved topical anti-acne therapy was developed using pseudo-ternary phase diagram investigations, factorial design optimization, physicochemical characterization, thermodynamic stability research, TEM analysis, and response surface methods6,7.

## 2. MATERIALS AND METHODS

### 2.1 Material

Analytical and pharmaceutical-grade materials were utilized without additional purification in the creation and assessment of quercetin nanoemulgel. Based on their solubilization efficiency, compatibility, stability, and appropriateness for topical nanoemulgel formulation, the medication, oils, surfactants, co-surfactants, gelling agents, preservatives, antioxidants, and other excipients were chosen8. Quercetin have limited bioavailability and poor water solubility, It is naturally occurring flavonoid with strong antioxidant, anti-inflammatory, antibacterial, and anti-acne properties, was chosen as the model medication for nanoemulsion and nanoemulgel development. In order to choose an appropriate oil phase, a number of oils were screened, including oleic acid, clove oil, eucalyptus oil, cinnamon oil, lemongrass oil, peppermint oil, anise oil, neem oil, arachis oil, and coconut oil9. Of these, oleic acid showed the highest solubility and the best penetration-enhancing qualities, so it was chosen as the optimized oil phase. Based on emulsification efficiency and HLB value, surfactants including Tween 80, Tween 20, Span 80, and Span 20 were assessed; Tween 80 had the best stability and capacity to create nanoemulsions.Propylene glycol, ethanol, and Transcutol P were among the co-surfactants whose solubilization and stabilization qualities were examined; Transcutol P was chosen because of its superior solubilizing ability and penetration-enhancing effect10. To enhance gel consistency, stability, preservation, moisturization, and overall formulation performance, additional excipients like Carbopol 934, Triethanolamine, methyl paraben, propyl paraben, BHT, glycerin, and purified water were added.

Ethical approval and consent - Animal experimentation, human participants, or human biological materials were not used in this investigation. Therefore, informed permission and ethical approval were not required for this research to be conducted.

### 2.2 Method

#### 2.2.1 Preformulation Studies

Preformulation studies were carried out to evaluate the compatibility and physicochemical properties of quercetin before the formulation was created. Organoleptic characterization, which includes color, odor, appearance, and powder nature, was done to confirm the drug's identity and physical characteristics. Solubility experiments were performed in a range of solvents, oils, surfactants, and co-surfactants to identify suitable formulation components based on maximum drug solubility. The validity and purity of the medication sample were confirmed by measuring the melting point of quercetin using the capillary tube method11. UV-visible spectroscopy analysis was done to find λmax and develop the standard calibration curve for quantitative quercetin measurement. Fourier Transform Infrared Spectroscopy (FTIR) was utilized to identify unique functional groups and confirm the drug's compatibility and purity.

#### 2.2.2 Selection of Component for Nanoemulsion

The components of the formulation were chosen based on their solubility, emulsification efficiency, and capacity to generate nanoemulsions. The solubility of quercetin in ethanol, Transcutol P, and DMSO was used to select the solvent. To determine the best oil phase, various oils were evaluated according to the solubility of quercetin. To choose a suitable surfactant capable of creating stable nanoemulsions, solubilization and emulsification efficiency experiments were used to assess surfactants12. Co-surfactants were also evaluated according to drug solubility and their capacity to improve the stability and production of nanoemulsions. For additional formulation development, the component with the highest solubilization and superior emulsification properties was chosen.

#### 2.2.3 Drug Excipient compatibly study

Drug-excipient compatibility tests were used to evaluate the physical and chemical compatibility of quercetin with particular formulation excipients. For the physical compatibility study, drug-excipient combinations were kept at accelerated circumstances, and any color, turbidity, precipitation, or phase separation changes were routinely observed. FTIR compatibility tests were conducted using pure quercetin and drug-excipient mixtures to identify unique functional groups and search for possible chemical interactions13. The absence of significant changes in characteristic peaks confirmed that quercetin was compatible with the selected excipients.

#### 2.2.4 Construction of Pseudo- ternary Phase Diagram

The aqueous titration approach was used to create pseudo-ternary phase diagrams in order to determine the nanoemulsion area and optimize the ratio of water, oil, surfactant, and co-surfactant. Different ratios of surfactant to co-surfactant (1:1, 1:2, 2:1, 3:1, 4:1, and 5:1) were used to create Smix. At room temperature, different combinations of oil and Smix were gradually titrated with filtered water while being continuously stirred. The clarity, transparency, homogeneity, turbidity, and phase separation of the final systems were assessed visually14. The systems were divided into anisotropic (turbid or phase-separated region) and isotropic (clear and stable nanoemulsion region) regions based on

visual observations. The ideal composition range for stable nanoemulsion formulation was found using the phase diagrams.

**2.2.5 Optimization of Nanoemulsion by Factorial Design**

A $3^2$ complete factorial design was used to optimize the nanoemulsion formulation in order to assess how formulation factors affected the nanoemulsion's physicochemical properties. Droplet size ($Y_1$), polydispersity index ($Y_2$), and zeta potential ($Y_3$) were the responses assessed, and two independent variables, oil concentration ($X_1$) and Smix concentration ($X_2$), were chosen at various levels15. The experimental approach was used to determine the optimal formulation with desired properties and to investigate the impact of formulation variables on nanoemulsion stability.

**2.2.6 Preparation of Nanoemulsion**

Nanoemulsion formulations were made using the aqueous titration method. Smix containing surfactant and co-surfactant in the proper ratio was added after quercetin was dissolved in the selected oil to generate the oil phase. The mixture was continuously stirred in order to produce a homogenous system16. To achieve a pure, transparent, and homogeneous nanoemulsion, purified water was then added dropwise while being continuously agitated magnetically. The generated formulations' stability and physicochemical characteristics were further evaluated.

**2.2.7 Evaluation of Nanoemulsion**

Visual appearance, droplet size, zeta potential, viscosity, pH, drug content, thermodynamic stability, and polydispersity index (PDI) were among the physicochemical parameters that were assessed for the generated nanoemulsion formulations. To evaluate the formulations' homogeneity, clarity, and transparency, visual inspection was done. Zeta potential analysis was used to assess formulation stability, and the dynamic light scattering method was used to quantify droplet size and PDI17. Standard analytical techniques were used to quantify pH and viscosity in order to assess topical application appropriateness. To assess the consistency of medication distribution within the formulations, drug content estimation was done. To evaluate the physical stability of the nanoemulsion and spot any indications of phase separation, creaming, or instability, thermodynamic stability tests using centrifugation, heating–cooling cycles, and freeze–thaw cycles were carried out18.

**2.2.8 Transmission Electron Microscopy Analysis**

The morphological properties of the improved nanoemulsion formulations were assessed using Transmission Electron Microscopy (TEM) analysis. The nanoemulsion droplets' size, distribution pattern, surface morphology, and shape were all determined using TEM19. A diluted nanoemulsion sample was put on a copper grid coated with carbon, properly stained, and examined under a transmission electron microscope. The creation of uniformly dispersed, spherical, nano scale droplets with smooth surfaces was verified by the study.

**2.2.9 Optimization using Response Surface Methodology**

The nanoemulsion formulation was optimized and the impact of formulation variables on the physicochemical responses was assessed using Response Surface Methodology (RSM). Analysis of variance (ANOVA) was used to statistically analyze the experimental data, and polynomial equations were created to determine the relationship between independent and dependent variables20. In order to determine the optimal formulation with desired properties and enhanced stability, response surface plots were created to examine the impact of formulation factors on droplet size, polydispersity index (PDI), and zeta potential.

**2.2.10 Selection of Optimized Nanoemulsion**

All produced nanoemulsion formulations were compared using physicochemical criteria as droplet size, drug content, zeta potential, polydispersity index (PDI), and thermodynamic stability. Topical delivery was deemed more stable and appropriate for formulations with smaller droplet sizes, lower PDI, higher absolute zeta potential, homogeneous drug distribution, and no phase separation21. Even though formulations F5 and F8 all shown remarkable attributes, formulation F5 was selected as the ideal nanoemulsion due to its smallest droplet size, low PDI, good zeta potential, excellent stability profile, and increased drug concentration. Nanoemulgel was then created using the improved formulation.

**2.2.11 Preparation of Nanoemulgel**

**Preparation of gel base**

Carbopol 934 was used as the gelling agent to create the gel basis. To create a homogenous gel dispersion, precisely weighed carbopol 934 was dissolved in filtered water while being continuously stirred and allowed to fully hydrate. Glycerin was added as a humectant, while preservatives including methyl and propyl paraben were used to enhance microbiological stability. Triethanolamine was used to modify the dispersion's pH, which produced a clear, stable gel basis with the right viscosity for topical application.

**Incorporation of Nanoemulsion into gel**

For homogenous nanoemulgel system, the optimum nanoemulsion formulation was progressively added to the prepared gel foundation while being continuously stirred. To guarantee that the nanoemulsion droplets were evenly distributed throughout the gel matrix, the formulation was further homogenized22. Prior to additional characterization research, the generated nanoemulgel was assessed visually for homogeneity, smoothness, consistency, and lack of phase separation.

**2.2.12 Evalution of Nanoemulgel**

For the assessment of the generated nanoemulgel compositions' appropriateness for topical application, a number of physicochemical properties were assessed. The formulations' color, smoothness, homogeneity, consistency, grittiness, and phase separation were all visually assessed. To guarantee compatibility with skin pH and reduce the risk of irritation, the pH of the nanoemulgel was evaluated using a calibrated digital pH meter.

To evaluate the rheological behavior and suitability of the nanoemulgel, the viscosity of the formulations was measured using a Brookfield viscometer at a certain rotating speed and temperature. The slip and drag method was used in a spreadability research to assess the formulation's ease of application across the skin's surface. To determine the drug

content, a known amount of nanoemulgel was dissolved in an appropriate solvent. This was followed by UV-visible spectrophotometric examination to guarantee that quercetin was distributed uniformly throughout the formulation23.
Collapsible tubes were used in an extrudability testing to find out how easily the nanoemulgel could be extruded under pressure. Visual inspection was used to assess the formulations' homogeneity in order to verify that the nanoemulsion was distributed uniformly throughout the gel matrix and that there were no clumps or lumps. To assess the nanoemulgel's physical stability, phase separation, consistency, and appearance over a predetermined amount of time, stability studies were conducted under particular storage circumstances24.

**2.3 Statistical Analysis**

All experimental results were statistically examined and given as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used to assess the impact of formulation variables on the results. Response surface methodology (RSM) based on a $3^2$ complete factorial design was used to optimize the nanoemulsion formulation25. The impact of independent factors on droplet size, polydispersity index (PDI), and zeta potential was investigated using polynomial equations, response surface plots, and contour plots. Design-Expert software was used for statistical analysis and formulation optimization26.

## 3. RESULT

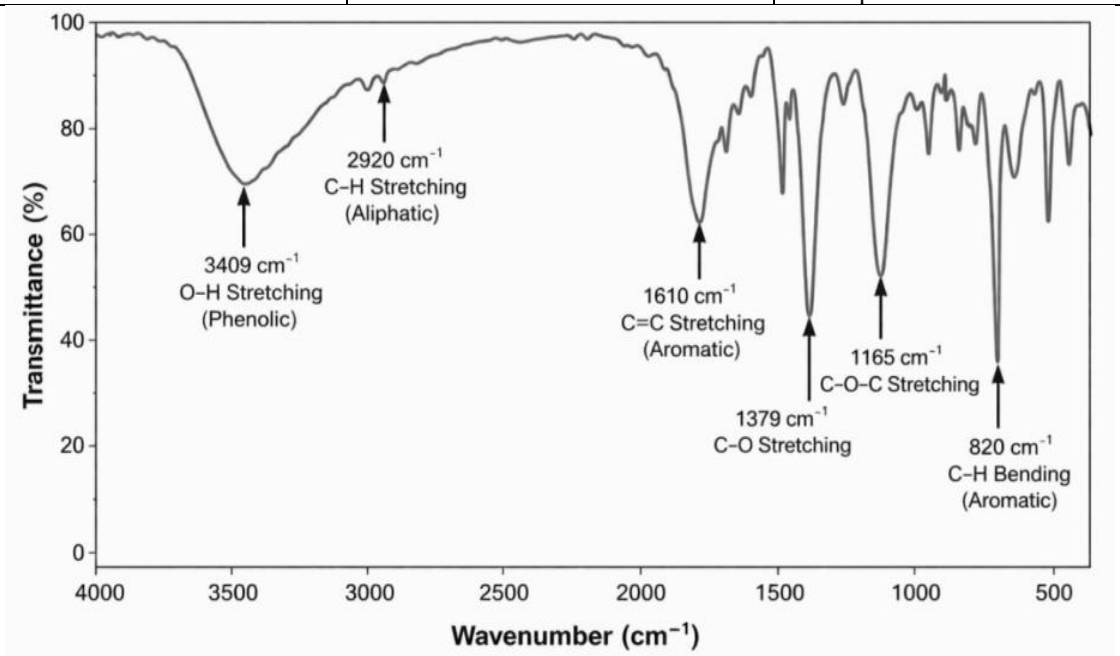
### 3.1 Preformulation Study

#### 3.1.1 Authentication of Drug

Through organoleptic evaluation, solubility investigations, melting point measurement, UV–visible spectroscopy , and FTIR analysis (Table no – 1), quercetin's identity and purity were verified. Quercetin was found to be a free-flowing, yellow, odorless crystalline powder. According to solubility tests, quercetin was most soluble in oleic acid among oils and shown good solubilization in Tween 80 and Transcutol P, suggesting that these oils might be used to formulate nanoemulsions. Quercetin's melting point was determined to be between 316 and 320°C, which was consistent with the stated standard value and verified the medication's purity.

**Table 1. Preformulation Studies and Selection of Formulation Components**

| Parameter | Observation/Result | Conclusion |
|---|---|---|
| FTIR Peaks of Quercetin | O–H (3400–3250 $cm^{-1}$), C=O (1665–1672 $cm^{-1}$), Aromatic C=C (1610–1510 $cm^{-1}$), C–O (1250–1160 $cm^{-1}$) | Confirmed identity and purity of quercetin |
| Solubility in Solvents | Transcutol P > DMSO > Ethanol | Transcutol P selected |
| Solubility in Oils | Oleic acid showed maximum solubility | Selected as oil phase |
| Surfactant Screening | Tween 80 showed highest emulsification efficiency | Selected as surfactant |
| Co-surfactant Screening | Transcutol P showed superior nanoemulsion formation | Selected as co-surfactant |
| Optimized Smix Ratio | Tween 80 : Transcutol P (3:1) | Selected for formulation development |



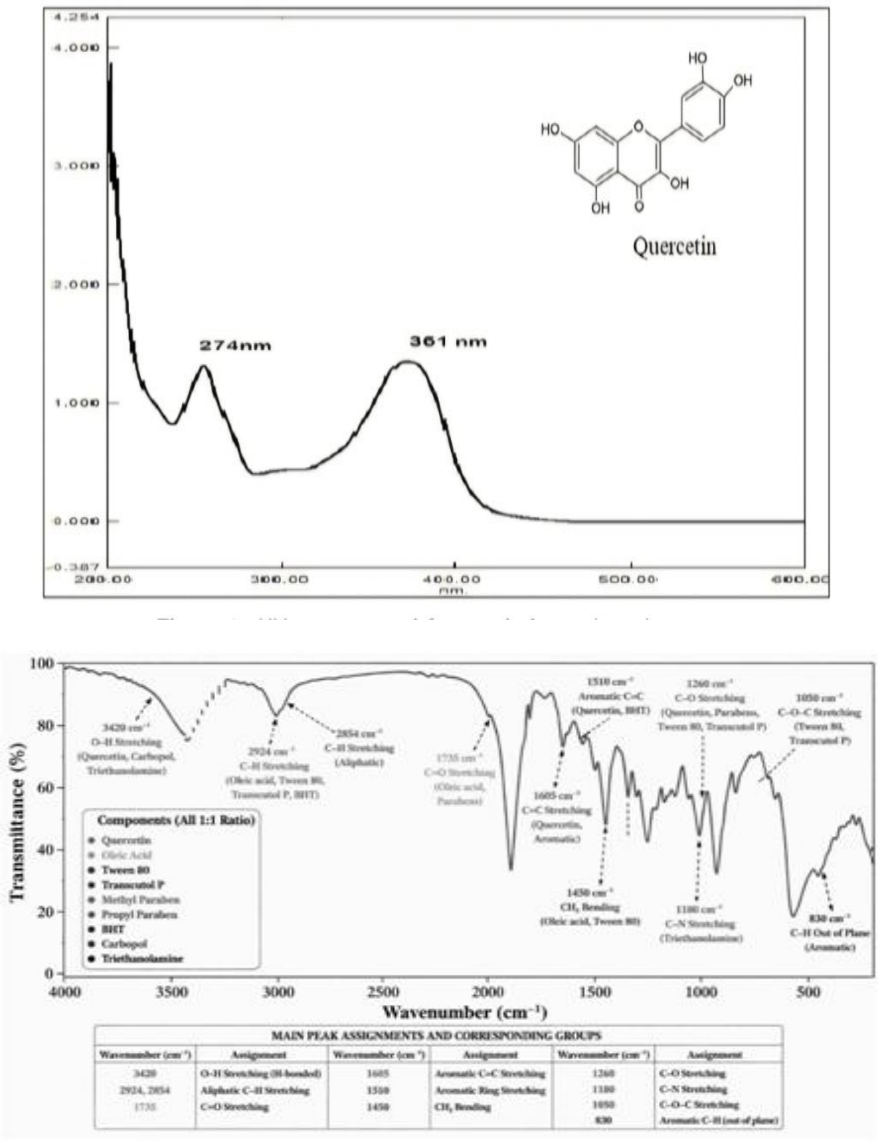



**Fig. No. – 1 A. UV Spectrum of Quercetin B. FTIR Spectrum of Quercetin C. FTIR of Quercetin + All Excipient Mixture**

A distinctive absorption maximum (λmax) at 370 nm in ethanol was revealed by UV–visible spectroscopic analysis, which was then utilized for quantitative analysis and calibration curve construction. Quercetin's chemical identity and purity were confirmed by FTIR spectroscopy (Fig - 1), which showed distinctive peaks corresponding to phenolic –OH, carbonyl (C=O), aromatic C=C, and C–O functional groups. There was no indication of impurity or degradation.

**3.2.2 Selection of Component for Nanoemulsion**

Drug solubility, emulsification efficiency, system stability, and clarity were taken into consideration when choosing appropriate components for nanoemulsion formulation. To determine the best excipients for formulation development, solubility tests were conducted in a variety of solvents, oils, surfactants, and co-surfactants. Transcutol P showed the highest solubility for quercetin among the studied solvents, followed by ethanol and DMSO, resulting in stable and transparent solutions.

To choose the oil phase, a variety of oils were evaluated, including oleic acid, clove oil, eucalyptus oil, cinnamon oil, lemongrass oil, peppermint oil, anise oil, neem oil, arachis oil, and coconut oil. Oleic acid was chosen as the optimal oil phase because it demonstrated the greatest solubilizing capacity of all the assessed oils and created a transparent and stable system.

The clarity of the final emulsion system, HLB value, and emulsification efficiency were taken into consideration when choosing a surfactant. Because of its high HLB value and higher solubilizing ability, Tween 80 showed superior emulsification efficiency with oleic acid, resulting in transparent and stable nanoemulsions. While Span 80 and Span 20 created relatively turbid solutions because of their lower HLB values, Tween 20 (Table no – 1) demonstrated a modest emulsification efficiency.

Drug solubility, nanoemulsion-forming capacity, and formulation stability were used to assess co-surfactants. When combined with Tween 80 and oleic acid, Transcutol P demonstrated exceptional solubilization and produced clear,

transparent, and stable nanoemulsion systems. Because ethanol is volatile, it created less stable systems, but propylene glycol performed moderately. Transcutol P (Table no – 1) was therefore chosen as the ideal co-surfactant for the creation of nanoemulsions.

**3.2.3 Drug- Excipient Compatibility Study**

The physical and chemical compatibility of quercetin with particular formulation excipients used in the manufacturing of nanoemulsion and nanoemulgel was assessed using drug-excipient compatibility (Table no – 1) experiments. The drug and drug-excipient mixes were stored under accelerated stability conditions of 40 ± 2°C/75% RH and 25 ± 2°C/60% RH for 28 days in order to conduct physical compatibility testing. At prearranged intervals, the samples were visually inspected for any indications of phase separation, turbidity, precipitation, color change, or physical instability.

Throughout the course of the investigation, no appreciable physical alterations were seen in any of the samples, suggesting that quercetin was stable and compatible with the chosen excipients.

For any potential chemical interactions between quercetin and formulation excipients, additional FTIR compatibility experiments were conducted. Phenolic –OH stretching, carbonyl (C=O) stretching, aromatic C=C stretching, and C–O stretching vibrations were all represented by distinctive peaks in the FTIR spectrum of pure quercetin. There was no discernible shift, disappearance, or broadening of the distinctive peaks in the spectra of drug–excipient mixes, indicating that there was no chemical interaction between quercetin and the chosen excipients. These results showed that all of the chosen excipients were compatible with quercetin and appropriate for the production of nanoemulsion and nanoemulgel formulations.

**3.2.4 Construction and Evaluation of Pseudo-ternary Phase diagram**

Using the aqueous titration approach, pseudo-ternary phase diagrams were created to determine the stable nanoemulsion area and optimal Smix ratio. Transparency, clarity, emulsification efficiency, and phase behavior were used to evaluate various Smix ratios of Tween 80 and Transcutol P (1:1, 1:2, 2:1, 3:1, 4:1, and 5:1). Phase separation, turbidity, and isotropic behavior were visually assessed while oil and Smix solutions were titrated with filtered water while being continuously stirred. Smix 3:1 had the greatest transparency, increased homogeneity, and the biggest nanoemulsion region across all assessed ratios, suggesting effective interfacial tension reduction and enhanced stabilization of nano-sized droplets. Systems with lower Smix concentrations were somewhat turbid, whereas systems with greater surfactant concentrations exhibited mild instability. In order to identify stable nanoemulsion locations, various oil-to-Smix ratios (Fig. – 2) were further assessed. Among all the formulations, F5 and F8 showed the best clarity, transparency, and homogeneity, indicating that the oil-to-Smix composition was appropriate for the generation of stable nanoemulsions. Overall, the phase behavior investigation demonstrated that oil content and Smix ratio had a substantial impact on the production and stability of nanoemulsions, and Smix 3:1 was chosen as the ideal ratio for additional formulation development.

**3.2.5 Optimization of Nanoemulsion by factorial Design**

The nanoemulsion formulation was optimized using a $3^2$ complete factorial design, which assessed the impact of two independent factors on the physicochemical properties of the nanoemulsion: oil concentration ($X_1$) and Smix concentration ($X_2$). Droplet size ($Y_1$), polydispersity index (PDI) ($Y_2$), and zeta potential ($Y_3$) were the dependent responses that were examined. Nine experimental formulations (F1–F9) in all were created and assessed. The results showed that the concentration of both oil and Smix had a substantial impact on the properties of the nanoemulsion. Because of effective emulsification and stabilization of the droplets, an increase in Smix concentration led to a decrease in PDI and droplet size (Table no – 2), while an increase in oil concentration resulted in somewhat larger droplet size and decreased stability.

**Table 2. Factorial Design and Composition of Nanoemulsion Formulations**

| Formulation | Oil (%) | Smix (%) | Water (%) | Droplet Size (nm) | PDI | Zeta Potential (mV) |
|---|---|---|---|---|---|---|
| F1 | 30 | 50 | 20 | 168 | 0.412 | -18.6 |
| F2 | 30 | 55 | 15 | 142 | 0.365 | -20.4 |
| F3 | 30 | 60 | 10 | 126 | 0.321 | -22.1 |
| F4 | 35 | 50 | 15 | 119 | 0.282 | -24.8 |
| F5 | 35 | 55 | 10 | 92 | 0.218 | -31.6 |
| F6 | 35 | 60 | 5 | 106 | 0.247 | -28.9 |
| F7 | 40 | 50 | 10 | 134 | 0.338 | -23.5 |
| F8 | 40 | 55 | 5 | 97 | 0.226 | -30.2 |
| F9 | 40 | 50 | 0 | — | — | _ |

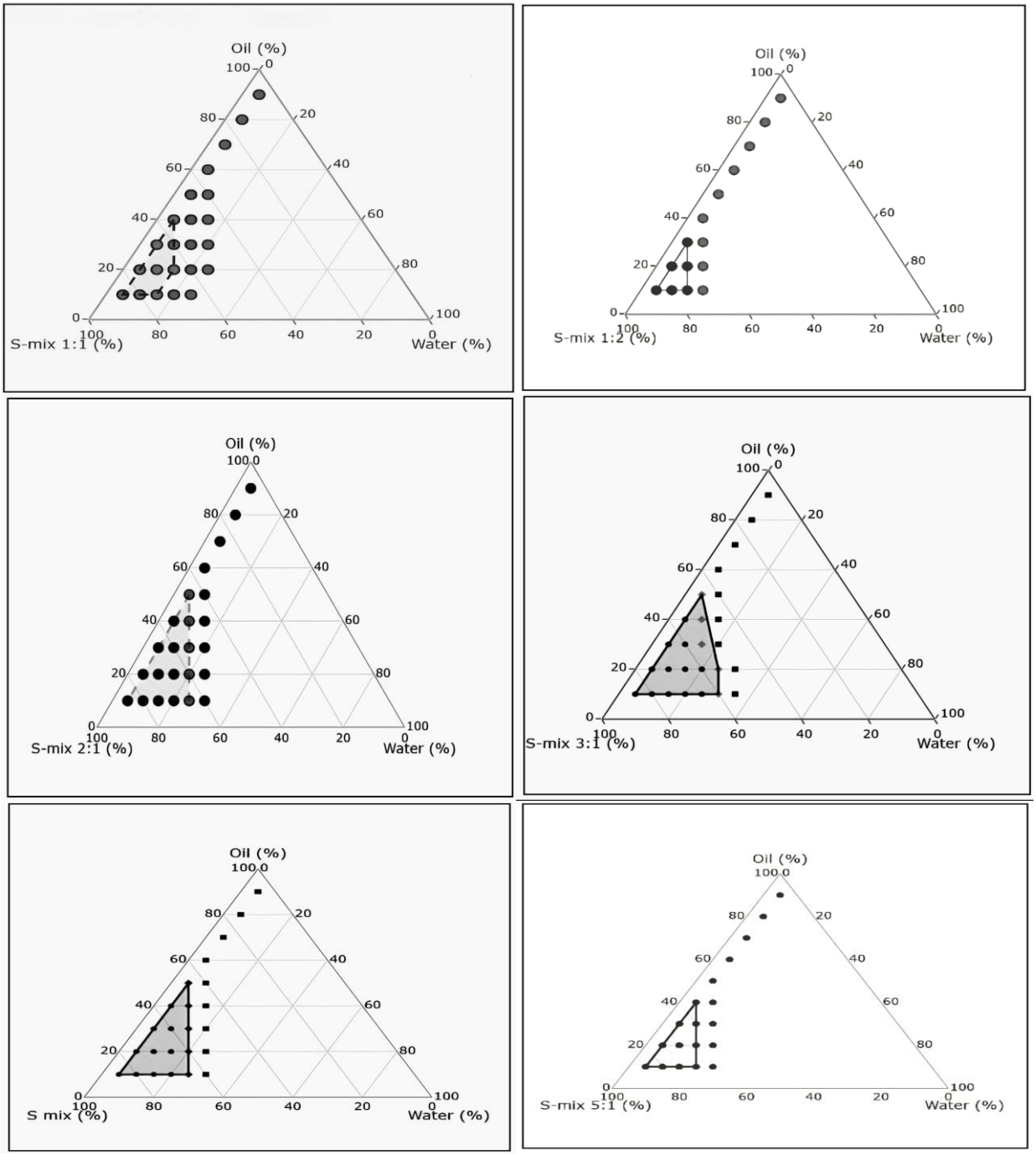



**Fig. No. 2 : TPD of nanoemulsion consisting of oleic acid (oil) and Smix (Tween 80: Transcutol P) at Smix Ratio: A- 1:1; B- 1:2; C- 2:1; D- 3:1; E- 4:1; F-5:1**

Formulations had droplet sizes between 95 and 165 nm, PDI values between 0.21 and 0.45, and zeta potentials between −16.5 and −28 mV. With smaller droplet sizes, lower PDI, and better zeta potential values, F5 and F8 showed the best physicochemical characteristics of all the formulations, suggesting increased homogeneity and stability. Formulation F8 shown similar properties with droplet size of 100 nm, PDI of 0.24, and zeta potential of −26.5 mV, but Formulation F5 displayed the smallest droplet size (95 nm), lowest PDI (0.21), and maximum stability with zeta potential of −28 mV. While F8 was regarded as a secondary optimized batch for comparative analysis, F5 was chosen as the optimum formulation based on overall evaluation and stability qualities.

**3.2.6 Preparation of Nanoemulsion**

The aqueous titration approach was effectively used to create nanoemulsion formulations (F1–F9) using oleic acid as the oil phase, Tween 80 as the surfactant, and Transcutol P as the co-surfactant (Table no – 2). Quercetin was first dissolved in oleic acid to create the oil phase, and then optimized Smix was added in varying amounts in accordance with the factorial design. After that, purified water was added drop wise while being continuously stirred magnetically to create translucent, uniform nanoemulsions. Depending on the percentage of oil and Smix, all formulations displayed differing levels of clarity, transparency, and stability. Due to effective interfacial tension reduction and appropriate droplet stabilization, formulations with lower oil concentrations and greater Smix concentrations resulted in transparent and stable nanoemulsions.

Formulations with a lower Smix concentration and a greater oil concentration, on the other hand, appeared more turbid and were less stable. F5 and F8 showed the best transparency, homogeneity, and lack of phase separation of all the formulations, suggesting that the nanoemulsion was successfully formed. The best overall look was shown by Formulation F5, which confirmed optimal emulsification and nanosized droplet formation with clear, transparent, and stable properties.

### 3.2.7 Evaluation of Prepared Nanoemulsion

Visual appearance, droplet size, polydispersity index (PDI), zeta potential, viscosity, pH, drug concentration, and thermodynamic stability were all assessed for the generated nanoemulsion (Table no – 3) formulations (F1–F9). With differing levels of stability and clarity, every formulation displayed acceptable physicochemical properties. greater oil concentrations resulted in rather turbid systems, while formulations with greater Smix concentrations showed improved clarity and homogeneity. F5 and F8 had the best physical stability, clarity, and transparency of all the formulations without phase separation.The formulas' droplet sizes varied from 95 to 165 nm; F5 had the smallest droplet size (95 nm), followed by F8 (100 nm). While F5 (0.21) and F8 (0.24) showed improved homogeneity, PDI values varied from 0.21 to 0.45, indicating an appropriate droplet size distribution. Zeta potential values (Fig. – 3) ranged from −16.5 to −28 mV, indicating that the nanoemulsions were sufficiently stable. The highest zeta potential was found in F5 (−28 mV), followed by F8 (−26.5 mV), indicating better droplet stabilization.

**Table 3. Evaluation of Nanoemulsion Formulations**

| Formulation | Droplet Size (nm) | PDI | Zeta Potential (mV) | Viscosity (cP) | pH | Drug Content (%) | Stability |
|---|---|---|---|---|---|---|---|
| F1 | 140 | 0.42 | -18.2 | 120 | 5.8 | 96.5 | Pass |
| F2 | 165 | 0.45 | -16.5 | 135 | 5.6 | 95.8 | Pass |
| F3 | 110 | 0.31 | -22.4 | 95 | 6.0 | 97.2 | Pass |
| F4 | 130 | 0.35 | -20.3 | 110 | 5.9 | 98.0 | Pass |
| F5 | 95 | 0.21 | -28.0 | 85 | 6.2 | 99.2 | Pass |
| F6 | 120 | 0.33 | -23.5 | 100 | 6.1 | 97.8 | Pass |
| F7 | 155 | 0.41 | -18.5 | 130 | 5.7 | 96.2 | Pass |
| F8 | 100 | 0.24 | -26.5 | 90 | 6.1 | 98.6 | Pass |

*Stability includes centrifugation, heating–cooling, and freeze–thaw cycle studies.

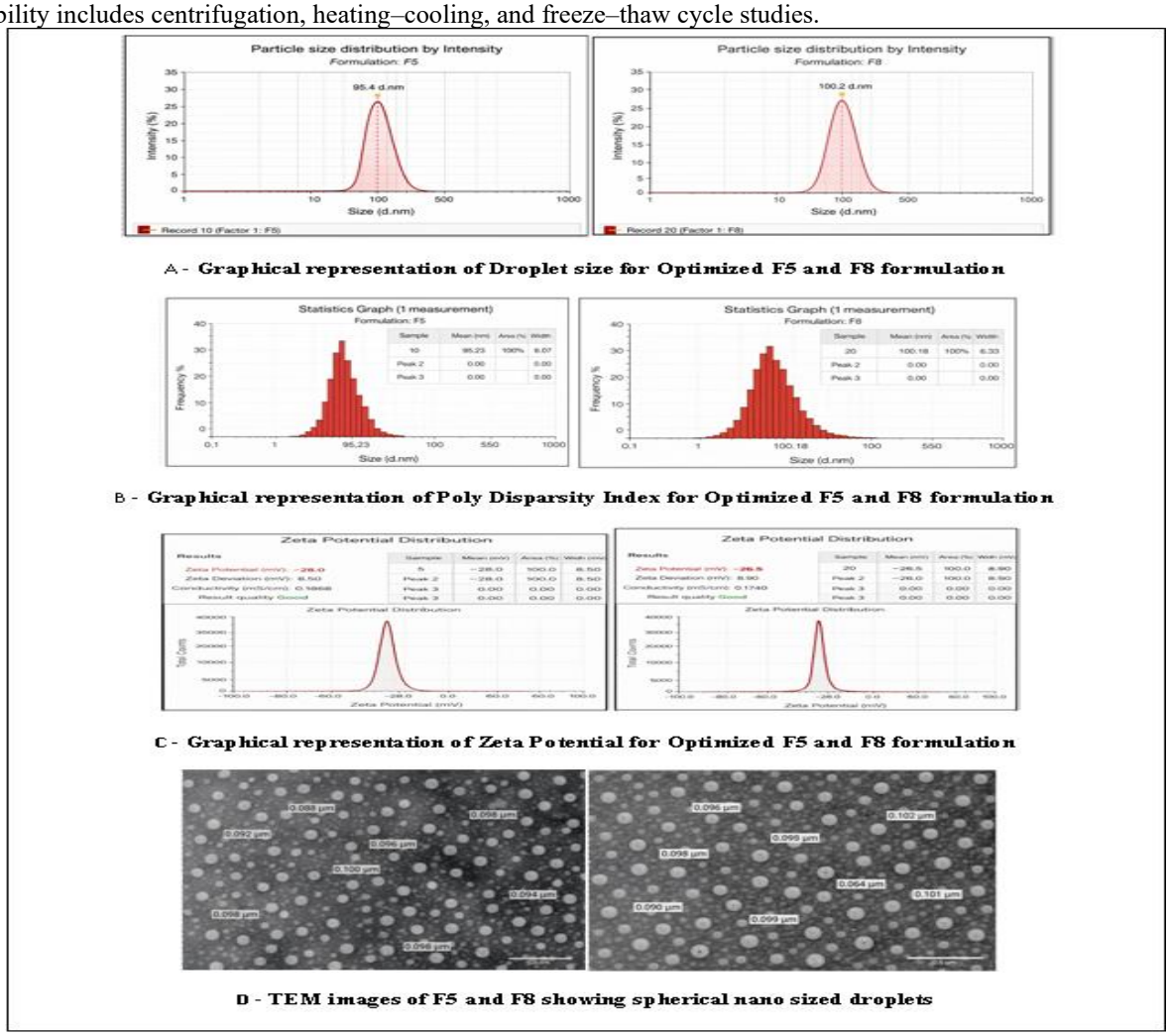



**Fig. No. – 3 Graphical Representation of Droplet size, PDI, Zeta Potential and TEM Image**

The range of viscosity values, which showed appropriate flow characteristics for nanoemulsion systems, was 85–135 cP. All products' pH values were within the permissible topical range (5.6–6.2), indicating skin compatibility. The range of drug content was 95.8–99.2%, with F5 exhibiting the highest drug level (99.2%). Phase separation and instability in improved formulations were not detected by thermodynamic stability tests, such as centrifugation, heating-cooling, and freeze-thaw cycles. F5 was chosen as the optimum nanoemulsion formulation based on the overall evaluation, while F8 was regarded as a secondary optimized batch.

**3.2.8 Transmission Electron Microscopy (TEM) Analysis**

The morphological properties, droplet shape, surface morphology, size distribution, and aggregation behavior of improved nanoemulsion formulations F5 and F8 were assessed by Transmission Electron Microscopy (TEM) analysis. Successful nanoemulsion generation was confirmed by TEM images, which showed that both formulations were composed of spherical, distinct, and evenly distributed nano-sized droplets with little aggregation or coalescence.

The equally dispersed droplets in Formulation F5 had a smooth surface morphology and a rather narrow size distribution, with droplet sizes roughly ranging from 88 to 100 nm. Better homogeneity and physical stability of the formulation were demonstrated by the uniform droplet distribution and lack of agglomeration. Formulation F8, on the other hand, likewise displayed spherical nano-sized droplets with a size range of roughly 64–102 nm; nevertheless, a somewhat different droplet homogeneity and a relatively wider size distribution were noted.

Comparative TEM investigation revealed that formulation F5 was more stable, had a better dispersion pattern, and had greater droplet homogeneity than formulation F8. The improved properties of formulation F5 were further confirmed by the TEM results, which were in good agreement with droplet size and PDI values obtained during physicochemical evaluation.

**3.2.9 Optimization using response surface methodology**

In order to maximize the nanoemulsion formulation and assess the impact of independent variables, specifically oil concentration ($X_1$) and Smix concentration ($X_2$), on droplet size ($Y_1$), polydispersity index ($Y_2$), and zeta potential ($Y_3$), Response Surface Methodology (RSM) utilizing a $3^2$ full factorial design was utilized. ANOVA (Table no – 4) was used for statistical analysis after the experimental data from formulations F1–F9 were fitted into a quadratic polynomial model.

**Table 4. ANOVA Results and Evaluation of Optimized Nanoemulgel**

| **A. ANOVA Summary** | | | | | |
|---|---|---|---|---|---|
| Response | F-value | p-value | Adjusted $R^2$ | Predicted $R^2$ | Adequate Precision |
| Droplet Size ($Y_1$) | 48.76 | <0.0001 | 0.978 | 0.952 | 18.4 |
| PDI ($Y_2$) | 36.55 | <0.0001 | 0.965 | 0.941 | 16.2 |
| Zeta Potential ($Y_3$) | 42.88 | <0.0001 | 0.972 | 0.948 | 17.6 |

| B. Evaluation of Optimized Nanoemulgel | | |
|---|---|---|
| Parameter | F5 Nanoemulgel | F8 Nanoemulgel |
| Physical Appearance | Smooth and Homogeneous | Smooth and Homogeneous |
| pH | 6.1 ± 0.1 | 6.0 ± 0.1 |
| Viscosity (cP) | 18,500 ± 120 | 17,200 ± 110 |
| Spreadability (g·cm/s) | 6.8 ± 0.3 | 7.1 ± 0.2 |
| Drug Content (%) | 98.7 ± 0.5 | 97.9 ± 0.4 |
| Extrudability | Excellent | Excellent |

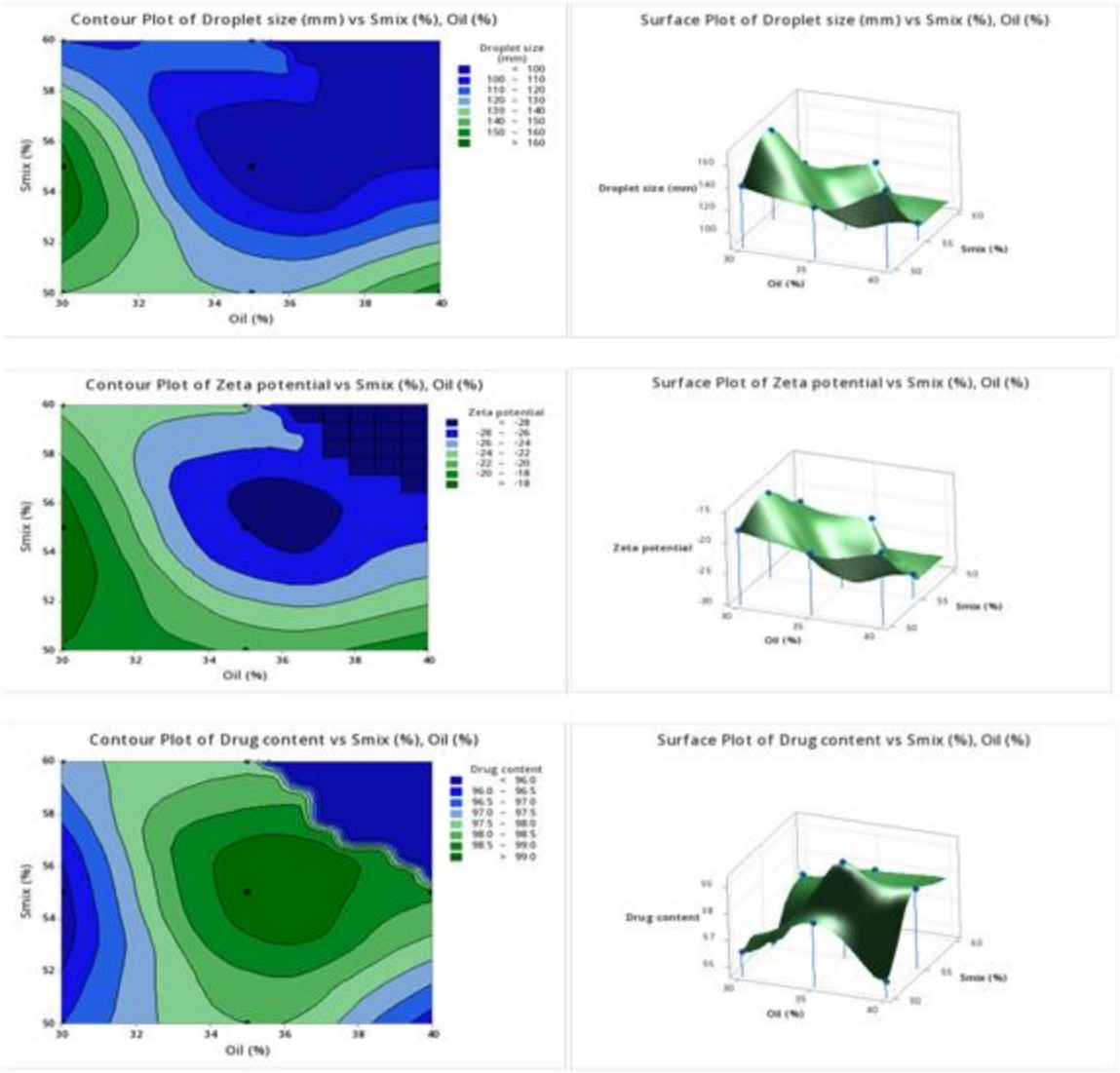



**Fig. No. 4 : Response surface plots (3D) showing the effect of the amounts of oil, Smix (Tween 80 and Transcutol P 3:1) and water on particle size, PDI and zeta potential of nanoemulsion.**

Using p-values < 0.05, the constructed models demonstrated substantial impacts of formulation variables on all answers, demonstrating good model fitting and dependability. The most significant factor influencing droplet size, PDI, and zeta potential was found to be Smix concentration. Due to effective interfacial tension reduction and enhanced droplet stabilization, increasing Smix concentration decreased droplet size and PDI (Table no – 4). On the other hand, due to increased viscosity and inadequate surfactant coverage, increasing oil content resulted is increased droplet size and PDI. Formulations F5 and F8 showed superior zeta potential values, minimal PDI, and optimal responses with smaller droplet sizes (Fig. – 4).

The substantial impact of formulation variables and their interaction terms on nanoemulsion properties was validated by the quadratic polynomial equations produced for responses. Response surface plots also showed that within the chosen design space, stable and uniform nanoemulsions were formed by optimal combinations of oil and Smix concentration. With high F-values, p-values < 0.0001, non-significant lack of fit, and strong agreement between adjusted and predicted R2 values, the ANOVA findings validated the suitability of the constructed models. Sufficient signal-to-noise ratios and model fit for nanoemulsion formulation optimization were indicated by adequate precision values larger than 4.

**3.2.10 Selection of Optimized Nanoemulsion-**

Critical physicochemical parameters such as droplet size, drug content, zeta potential, polydispersity index (PDI), and thermodynamic stability were used to compare all developed nanoemulsion formulations. Out of all the formulations (Table no – 4), F5 and F8 showed greater qualities than the other batches in terms of clarity, transparency, homogeneity, and stability.

With the smallest droplet size (95 nm), lowest PDI (0.21), largest negative zeta potential (−28 mV), and maximum drug content (99.2%), Formulation F5 demonstrated superior emulsification efficiency, a narrow droplet size distribution, enhanced stability, and effective drug integration. With a droplet size of 100 nm, PDI of 0.24, zeta potential of −26.5 mV, and drug content of 98.6%, formulation F8 likewise showed acceptable qualities. Without exhibiting any symptoms of phase separation, creaming, or precipitation, both formulations passed thermodynamic stability tests. Formulation F8 was chosen as a secondary optimized formulation for comparative studies, while formulation F5 was chosen as the optimal nanoemulsion formulation based on total comparative evaluation because of its superior physicochemical qualities and improved stability.

### 3.2.11 Preparation of Nanoemulgel

The refined nanoemulsion formulations (F5 and F8) were effectively incorporated into a Carbopol 934 gel foundation to create the nanoemulgel formulations. In order to create a homogenous gel system, Carbopol 934 was dispersed in purified water while being continuously stirred. A transparent and stable gel network was created by adding methyl and propyl paraben as preservatives and neutralizing and adjusting the pH with Triethanolamine.

For the homogenous nanoemulgel formulation, the tailored nanoemulsion was progressively added to the prepared gel foundation while being continuously stirred and homogenized. The successfully incorporated nanoemulsion into the gel matrix was demonstrated by the generated nanoemulgels' smooth texture, uniform appearance, good consistency, and lack of grittiness or phase separation. Additionally, the formulations demonstrated adequate spreadability and viscosity for topical administration. The nanoemulgel containing formulation F5 outperformed formulation F8 in terms of consistency and stability among the created formulations, demonstrating effective gel formation and the nanoemulsion system's compatibility with the gel basis.

### 3.2.12 Evaluation of Nanoemulgel

A number of physicochemical characteristics, such as physical appearance, pH, viscosity, spreadability, drug content, extrudability, homogeneity, and stability, were assessed for the generated nanoemulgel formulations comprising optimized nanoemulsions F5 and F8. The successful integration of nanoemulsion into the gel matrix was demonstrated by the smooth texture, consistent consistency, and homogeneous appearance of both formulations, which showed no indications of grittiness, phase separation, or lump formation. The pH of the nanoemulgels was determined to be within the acceptable topical range (5.0–6.5), indicating minimal irritation risk and good skin compatibility. The pH values for Formulation F5 and F8 were 6.1 ± 0.1 and 6.0 ± 0.1, respectively (Table no – 4). Both formulations had adequate consistency for topical application, according to viscosity measurements. F5 had a slightly higher viscosity (18,500 ± 120 cP) than F8 (17,200 ± 110 cP), which would improve retention at the application site. Both formulations exhibited satisfactory spreading behavior, according to spreadability experiments. Because of its somewhat lower viscosity, formulation F8 exhibited slightly stronger spreadability (7.1 ± 0.2 g·cm/sec) than F5 (6.8 ± 0.3 g·cm/sec). With drug content values of 98.7 ± 0.5% for F5 and 97.9 ± 0.4% for F8, drug content analysis verified the homogeneous distribution of quercetin inside the nanoemulgel formulations, suggesting effective drug incorporation and little drug loss during formulation. Excellent extrusion capabilities for both formulations were demonstrated by extrudability testing, indicating patient compliance and simplicity of application. The homogeneity study verified that the formulation components were evenly distributed throughout the gel matrix and that there were no aggregates. Throughout the study time, neither formulation's appearance, pH, viscosity, or phase separation significantly changed, according to stability studies. Formulation F5 was deemed the optimal nanoemulgel formulation due to its overall superior viscosity, medication content, and stability.

### 3.3 Statistical Analysis

The mean ± standard deviation (Mean ± SD) of three independent measurements was used to express all experimental data collected during evaluation trials. Design-Expert® software was used to statistically analyze the nanoemulsion formulations in order to assess the impact of independent formulation variables on the chosen responses. The nanoemulsion system was optimized using Response Surface Methodology (RSM) with a $3^2$ complete factorial design. Droplet size ($Y_1$), polydispersity index ($Y_2$), and zeta potential ($Y_3$) were regarded as dependent responses; oil concentration ($X_1$) and Smix concentration ($X_2$) were chosen as independent variables. Analysis of variance (Table no – 4) was used to assess the derived models' significance after the experimental data was fitted into quadratic polynomial equations. All created models were statistically significant, with p-values $< 0.05$, according to the ANOVA results. Good agreement between the experimental and projected responses was confirmed by high F-values and non-significant lack of fit values. The models' high predictive performance within the chosen design space was indicated by the satisfactory correlation between the adjusted R2 and anticipated R2 values. Sufficient signal-to-noise ratio and model fidelity were proven by adequate precision values larger than 4. The substantial impact of oil and Smix content on droplet size, PDI, and zeta potential was amply illustrated by response surface plots and polynomial equations produced by Design-Expert® software. The software's anticipated optimal formulation matched the actual findings, demonstrating the statistical design's applicability and validity for optimizing quercetin nanoemulsion and nanoemulgel formulations.

## 4. DISCUSSION

A quercetin-loaded nanoemulgel for topical anti-acne delivery was effectively created and optimized by the study. Oleic acid, Tween 80, and Transcutol P were found to be the best formulation ingredients based on solubility studies. Studies using pseudo-ternary phase diagrams showed that the biggest nanoemulsion zone was created at a Smix ratio of 3:1. The substantial impact of oil and Smix concentration on droplet size, PDI, and zeta potential was shown by factorial design optimization. With a droplet size of 95 nm, PDI of 0.21, zeta potential of −28 mV, and drug content of 99.2%, the improved formulation (F5) demonstrated exceptional stability and homogeneity. Thermodynamic investigations demonstrated formulation stability, while TEM analysis indicated spherical nano-sized droplets. A nanoemulgel with appropriate physicochemical characteristics for topical application was created by effectively incorporating the tailored nanoemulsion into a Carbopol 934 gel base. These results show how nanoemulgel technology can improve quercetin's solubility, stability, and topical distribution for the treatment of acne.

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## 6. CONCLUSION

A quercetin-loaded nanoemulsion and nanoemulgel formulation was effectively created and refined for topical anti-acne delivery in the current investigation. Quercetin's purity, compatibility, and suitability for formulation development were validated by preformulation tests. Oleic acid, Tween 80, and Transcutol P were found to be the best oil phase, surfactant, and co-surfactant, respectively, in solubility and screening investigations.

Studies using pseudo-ternary phase diagrams verified that a wider and more stable nanoemulsion region was formed by the Smix ratio of Tween 80:Transcutol P (3:1). The physicochemical parameters of nanoemulsion formulations made using the aqueous titration method were good. F5 exhibited the best zeta potential, the largest drug concentration, the smallest droplet size, the lowest PDI, and the best thermodynamic stability of all the formulations. The adequacy of the optimized formulation was confirmed by response surface methods and ANOVA analysis, which showed significant effects of formulation factors on nanoemulsion properties.

The development of spherical, evenly distributed nanoscale droplets was verified by TEM examination. A stable nanoemulgel with sufficient pH, viscosity, spreadability, homogeneity, extrudability, and drug content for topical application was created by successfully incorporating the tailored nanoemulsion into the Carbopol 934 gel foundation. Because of its superior physicochemical characteristics, stability, and drug incorporation efficiency, formulation F5 was determined to be the optimal nanoemulgel formulation overall. This suggests that it may be a useful topical delivery system for quercetin in the treatment of acne.

## 7. Abbreviation

ANOVA - Analysis of Variance; BHT - Butylated Hydroxytoluene; DLS-Dynamic Light Scattering; DMSO- Dimethyl Sulfoxide; DOE- Design of Experiments; FTIR- Fourier Transform Infrared Spectroscopy; HLB- Hydrophilic–Lipophilic Balance; HPMC- Hydroxypropyl Methylcellulose; NE- Nanoemulsion; NEG-Nanoemulgel; O/W- Oil-in-Water; PDI- Polydispersity Index; QbD- Quality by Design; RSM-Response Surface Methodology; SD- Standard Deviation; Smix- Surfactant–Co-surfactant Mixture; TEA- Triethanolamine; TEM- Transmission Electron Microscopy; UV- Ultraviolet Spectroscopy; λmax- Maximum Absorption Wavelength.

## 8. Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this research work.


## 9. Financial Support and sponsorship

Nil.


## 10. Author Contribution

Author 1 performed conceptualization, formulation development, experimental studies, data analysis, statistical evaluation, interpretation of result and manuscript preparation. Author 2 contributed to manuscript review, supervision and final approval of the manuscript.

## 10. Summary

Using factorial design and pseudo-ternary phase diagram techniques, a quercetin-loaded nanoemulgel was effectively created and optimized. Excellent physicochemical characteristics, stability, and nano-sized droplet distribution were displayed by the improved formulation, indicating its promise as a topical delivery system for the treatment of acne.

## REFERENCE


1. Patel JK, Patel RP, Thakore SD. Development and optimization of quercetin-loaded nanoemulsion for topical delivery. Int J Biol Macromol. 2019;125:546–555.
2. Sharma N, Bansal M, Visht S. Formulation and evaluation of nanoemulgel for enhanced topical drug delivery. J Drug Deliv Sci Technol. 2019;52:370–381.
3. Gupta A, Eral HB, Hatton TA, Doyle PS. Nanoemulsions: formation, properties and applications. Soft Matter. 2019;15(35):6830–6841.
4. Kumar R, Sinha VR. Preparation and characterization of quercetin nanoemulsion for dermal application. Colloids Surf B Biointerfaces. 2020;189:110852.
5. Singh V, Hassan M, Sharma K. Nanoemulgel: a promising platform for topical drug delivery. Int J Pharm Investig. 2020;10(2):129–140.
6. Eid AM, Elmarzugi NA, Jaradat N. Topical nanoemulsion-based gels: formulation strategies and therapeutic applications. Pharmaceutics. 2020;12(9):879.
7. Ahmad J, Kohli K, Mir SR. Design and optimization of quercetin nanoemulsion using response surface methodology. Drug Dev Ind Pharm. 2020;46(11):1818–1828.
8. Kotta S, Khan AW, Ansari SH. Self nanoemulsifying drug delivery systems of quercetin: formulation and evaluation. J Mol Liq. 2020;304:112760.
9. Algahtani MS, Ahmad MZ, Ahmad J. Nanoemulgel for topical delivery of bioactive compounds: recent advances. Gels. 2021;7(4):181.
10. Chavda VP, Vora LK, Paudel A. Nanoemulsion-based drug delivery systems for improved therapeutic efficacy. Pharmaceutics. 2021;13(9):1384.
11. Bhattacharya S, Prajapati BG. Formulation and characterization of quercetin-loaded topical nanoemulgel. Int J Appl Pharm. 2021;13(5):120–127.
12. Kalam MA, Alshamsan A. Polyphenol-loaded nanoemulsion systems for topical therapy. Drug Dev Ind Pharm. 2021;47(8):1250–1260.
13. Sharma P, Denny WA, Garg S. Effect of surfactant concentration on nanoemulsion stability and droplet size distribution. AAPS PharmSciTech. 2021;22(6):198.

14. Gupta R, Rai VK, Mishra N. Pseudo-ternary phase diagram approach in nanoemulsion formulation development. J Mol Struct. 2021;1231:129914.
15. Soni K, Rizvi SMD, Kohli K. Statistical optimization of nanoemulsion systems using factorial design. J Drug Deliv Sci Technol. 2022;69:103170.
16. Basha SK, Kumari VS, Doble M. Nanoemulsion-based hydrogel systems for topical drug delivery. Colloids Surf A Physicochem Eng Asp. 2022;648:129281.
17. Kaur G, Harikumar SL. Evaluation of nanoemulgel formulations using thermodynamic stability studies. Int J Pharm Sci Res. 2022;13(3):1001–1010.
18. Choudhury H, Gorain B, Pandey M. Nanoemulsion systems for dermal and transdermal applications. Pharmaceutics. 2022;14(5):1028.
19. Raza K, Shareef MA, Singal P. Recent advances in quercetin-loaded nanocarriers for skin disorders. Drug Deliv Transl Res. 2023;13(2):457–472.
20. Yadav P, Verma A, Amin S. Carbopol-based nanoemulgel formulations: preparation and evaluation. J Pharm Innov. 2023;18(1):95–107.
21. Sharma D, Ali AA, Singh SK. Application of response surface methodology in nanoemulsion optimization. Heliyon. 2023;9(4):e15129.
22. Elsheikh MA, Ahmed OA, El-Say KM. Transmission electron microscopy analysis of nanoemulsion systems for topical delivery. Pharmaceutics. 2023;15(3):911.
23. Rathod S, Mehta DP, Parikh RK. Development of quercetin nanoemulgel for anti-acne activity. Int J Biol Macromol. 2024;254:127812.
24. Khan I, Aqil M, Imam SS. Nanoemulsion and nanoemulgel systems for enhanced dermal delivery of flavonoids. AAPS PharmSciTech. 2024;25(1):14.
25. Patel H, Desai K, Shah D. Optimization and characterization of quercetin-loaded topical nanoemulgel using Design-Expert software. J Mol Liq. 2025;401:124622.
26. Verma S, Chandra A, Gupta PK. Advanced nanoemulsion-based topical delivery systems for anti-acne therapy: formulation, optimization and evaluation. Braz J Pharm Sci. 2026;62:e24123.