

Eco – Pharmaceutical Design of a *Helianthus Annuus* Nanoemulcream: A QbD Optimization Strategy

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Abstract

Purpose

This study aimed to develop an eco-pharmaceutical *Helianthus Annuus* (sunflower) oil nanoemulcream using a Quality-by-Design approach integrated with a Central Composite Design to optimize formulation and process variables for improved stability, skin compatibility, and therapeutic performance.

Method

Sunflower oil was characterized using GC–MS and ATR–FTIR analyses. Nanoemulsions were prepared by high-speed homogenization, and key factors surfactant concentration, homogenization speed, and time were optimized using CCD to minimize particle size. The optimized nanoemulcream was evaluated for physicochemical properties, droplet size, PDI, zeta potential, morphology (SEM), stability, skin irritation, antibacterial activity, in-vitro drug release, and release kinetics.

Results

The developed nanoemulsion exhibited a mean droplet size of 134.7 ± 34.1 nm, Z-average 166.9 nm, PDI 0.409, and zeta potential -30.1 ± 2.3 mV, confirming uniform dispersion and electrostatic stability. The nanoemulcream showed suitable pH (6.2 ± 0.1), viscosity ($28,500 \pm 120$ cP), spreadability (12.5 ± 0.5 g·s⁻¹), high drug content ($94.2 \pm 1.8\%$), and maintained stability over three months. In-vitro release studies (360 min) demonstrated superior drug release from the nanoemulsion (88%), followed by the nanoemulcream (64.8%), cream with sunflower oil (49%), and plain sunflower oil (30%). Kinetic modeling indicated diffusion-controlled and anomalous transport mechanisms, supported by Korsmeyer–Peppas n values between 0.45–0.89. The formulation was non-irritant in skin studies and showed moderate broad-spectrum antibacterial activity (MIC 110–140 µg/mL).

Conclusion

The QbD-guided development enabled a stable, skin-compatible, and therapeutically effective sunflower oil nanoemulcream. Enhanced release and controlled kinetics highlight its potential as a sustainable topical delivery system for natural bioactives.

Introduction

The increasing focus on eco-pharmaceutical innovation drives the development of sustainable drug delivery systems integrating natural bioactives with advanced formulations. Sunflower oil (*Helianthus annuus*), a renewable and biocompatible resource rich in polyunsaturated fatty acids, tocopherols, and phytosterols, possesses broad therapeutic potential due to its antioxidative and anti-inflammatory and emollient properties [1]. However, its direct use is characterized by low aqueous solubility, oxidative instability, and low dermal bioavailability. Nanotechnology enables the development of advanced topical systems with improved performance. Nanoemulsions (20–200 nm) enhance solubility, stability, and dermal penetration of lipophilic actives, while their incorporation into semisolid bases forms nanoemulcream's that combine enhanced permeation with stability and patient acceptability [2].

Following the QbD approach, formulation development begins with defining the QTPP and identifying CQAs such as droplet size, PDI, zeta potential, viscosity, and spreadability. Risk assessment tools like FMEA and Ishikawa diagrams are used to establish CMAs and CPPs [3]. A DoE framework using CCD allows efficient optimization of formulation variables, revealing both linear and quadratic effects. In *H. annuus* nanoemulsions, CCD aids in optimizing oil concentration, S/Cos ratio, and homogenization speed to achieve stability and bioavailability [4]. High-speed homogenization further supports scalability and sustainability by generating nanosized droplets through mechanical shear with minimal solvent and energy use [5].

This study integrates QbD with nanotechnology to establish a sustainable strategy for a sunflower oil-based nanoemulcream. Systematic optimization via CCD produced a formulation with enhanced solubilization, dermal permeation, antimicrobial activity, stability, and patient acceptability, providing a reproducible, scalable, and environmentally conscious platform for next-generation topical formulations [6].

Materials & Methods

Materials

Sunflower oil (*Helianthus annuus*) was procured from a local vendor in India. Tween 80 and Polyethylene Glycol 400 (PEG 400) were obtained from Loba Chemie Pvt. Ltd. (Mumbai, India). Lanolin was purchased from Fisher Scientific, India. The remaining excipients—Stearic acid, Cetyl alcohol, Propylene glycol, and Potassium hydroxide (KOH)—were also supplied by Loba Chemie Pvt. Ltd. HPLC-grade water was supplied by Thermo Fisher Scientific Pvt. Ltd. (Qualigens), India. Analytical-grade chemicals were employed as obtained from the suppliers.

Methods

Characterization Studies

GC–MS Characterization

The compositional profile of sunflower oil was determined using a GC–MS system (Model GCMS-QP2010, Shimadzu, Japan) equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 µm). About 1 µl of oil was injected in splitless mode, with helium served as the carrier gas at a specified flow rate of 1.0 mL/min. The oven temperature was scheduled to ramp from 60°C (2 min) to 280°C at 10°C/min, and maintained for 10 minutes. The injector and detector temperatures were maintained at 250°C and 280°C, respectively. Mass spectra were recorded in electron impact mode (70 eV) over a 50–550 m/z range. Compounds were confirmed through spectral comparison with the NIST reference library. [7].

ATR–FTIR Spectroscopy

Attenuated Total Reflectance–Fourier Transform Infrared (ATR–FTIR) spectroscopy of sunflower oil, Nanoemulsion and the developed nanoemulcream were recorded using a Bruker Alpha II spectrophotometer (Germany). Samples were positioned on the ATR crystal, and spectra were recorded over the range of 4000–400 cm⁻¹ with a resolution of 4 cm⁻¹, averaging 32 scans. [8].

DSC Analysis

Thermal Characterization of crude oil and optimized nanoemulcream was performed using DSC (TA Instruments, USA). Samples (5–10 mg) were sealed in aluminium pans and analyzed under nitrogen (50 mL/min). Heating ranged from 30–300°C at 10°C/min. Thermograms recorded endothermic transitions and degradation peaks to evaluate compatibility and physical stability [9].

Preparation of Nanoemulcream Formulation

The sunflower oil-based nanoemulsion was prepared using high-speed homogenization [10]. Crude *Helianthus annuus* oil was mixed with a surfactant/co-surfactant blend of Tween 80 and PEG 400. Deionized water was added dropwise under continuously stirred to obtain a coarse emulsion, which was subsequently processed using high-speed homogenizer using a Digital Ultra-Turrax® T25 (IKA, Germany) to yield a stable oil-in-water nanoemulsion.

The resulting nanoemulsion was subsequently incorporated into a cream base to form the nanoemulcream [11, 12 & 13]. The oil phase (lanolin, stearic acid, Cetyl alcohol, and the nanoemulsion) and the aqueous phase (propylene glycol, potassium hydroxide, and deionized water) were heated separately to the same appropriate temperature. The aqueous phase was slowly added to the oil phase with continuous stirring followed by homogenization to achieve a uniform cream consistency. The finished formulation was cooled to room temperature and transferred into airtight containers for evaluation.

Quality by Design (QbD) Approach

The QbD framework guided the systematic, risk-based development of the sunflower oil nanoemulcream [3, 14]. The QTPP outlined key formulation characteristics such as dosage form, administration route, strength, pH, appearance, rheological behaviour, and microbiological stability. Based on the QTPP and prior knowledge, CQAs were identified, such as droplet size, zeta potential, pH, viscosity, spreadability, morphology, texture, and washability. Droplet size and zeta potential were emphasized as critical for nanoemulsion stability. Risk assessment utilized an Ishikawa (fishbone) diagram and a REM to categorize and rank formulation and process risks. This process

led to the identification of CMAs and CPPs for optimization, while other less impactful parameters were controlled or fixed, ensuring robust formulation development.

Optimization of Sunflower Oil Nanoemulcream via DoE

DoE-Based Optimization via CCD

A CCD with 16 runs and a three-factor, three-level scheme was executed using JMP software (v17.0.0; SAS Institute Inc., Cary, NC, USA). The design enabled evaluation of quadratic response surfaces and development of second-order models to assess main, interaction, and quadratic effects, incorporating factorial, axial, and central points for robustness [15]. The general polynomial equation generated from the design is expressed as:

$$Y = S_0 + S_1X_1 + S_2X_2 + S_3X_3 + S_4X_1X_2 + S_5X_1X_3 + S_6X_2X_3 + S_7X_1^2 + S_8X_2^2 + S_9X_3^2$$

where Y represents the response, S_0 denotes the intercept (mean of all runs), $S_1 - S_9$ are regression coefficients, X_1 , X_2 , and X_3 represent independent variables, X_1X_2 , X_1X_3 , and X_2X_3 indicate interaction terms, and X_1^2 , X_2^2 , X_3^2 represent quadratic effects.

In the present study, the independent variables were the concentration of Tween 80 and PEG 400 (X_1), homogenization time (X_2), and homogenization speed (X_3), with coded levels of -1 and $+1$ representing low and high values, respectively. The response variable (Y_1) was droplet size of the nanoemulsion, determined for all 16 formulations were incorporated into the design model to assess the effects of the independent factors. Optimization was carried out using the Prediction Profiler tool in JMP to identify the best factor settings for minimizing particle size, thereby ensuring formulation stability. [16]

Evaluation of Sunflower Oil Nanoemulcream

Physicochemical and Performance Evaluation

1. Physicochemical Characterization

The pH of the nanoemulcream was measured at room temperature using a calibrated digital pH meter (Deluxe Model 101, India), standardized with pH 4.01, 7.00, and 10.01 buffers. Approximately 1 g of formulation was dispersed in distilled water, and measurements were performed in triplicate, 24 hours post-preparation. Formulation viscosity was assessed using a Brookfield viscometer (DV II + Pro) with spindle S-64 at 20 rpm and 25°C. Homogeneity and phase separation were evaluated visually and tactilely over 24 hours, while organoleptic properties, including color, odor, and texture, were assessed by observation and application [17, 4].

2. Performance and Application Properties

Spreadability was assessed by placing a fixed cream amount between glass slides and measuring the time for the upper slide to move underweight. Washability was tested by rinsing a controlled amount applied to the skin, and after-feel was evaluated by assessing emollience, residue, and slipperiness post-application [17, 18].

3. Particle Size and Zeta Potential

Droplet size distribution and zeta potential were measured using Dynamic light scattering (Malvern Zetasizer Nano ZS90, UK). Samples were diluted 1:100 (v/v) in deionized water to reduce multiple scattering. Particle size was reported as Z-average diameter, and zeta potential assessed colloidal stability. Analyses were performed in triplicate [19].

4. Safety and Stability Studies

Irritancy testing involved applying the formulation to a 1 cm² dorsal hand area and observing for erythema, edema, or irritation over 24 hours; no reactions were observed [18]. Accelerated stability studies were conducted under room temperature and accelerated conditions (40 ± 2°C / 75 ± 5% RH) for three months, with evaluations at 0, 1, 2, and 3 months, following ICH guidelines [20].

5. Drug Content Determination

A 1 g sample of nanoemulcream was dissolved in 10 mL ethanol, stirred, filtered, and diluted. Absorbance at 273 nm was recorded using a Systronics Double Beam Spectrophotometer (2202) with ethanol as blank, and the drug content was determined. [22].

$$\text{Drug content \%} = \left(\frac{\text{Analyzed content}}{\text{Theoretical content}} \right) \times 100$$

In Vitro Drug Release and Kinetic Analysis

The release of the drug from the cream formulation was analyzed using a USP Type II (paddle) dissolution apparatus at $37 \pm 0.5^\circ\text{C}$ with constant stirring. Aliquots were withdrawn at predetermined intervals, filtered, and replaced with fresh medium to maintain sink conditions. Drug content was quantified using a validated UV–Vis spectrophotometric method at the drug's absorption maximum (λ_{max} determined from 200–400 nm scan). Calibration curves were prepared, and samples were analyzed in triplicate.

Cumulative release data were fitted to kinetic models (zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell) to determine release rate constants and elucidate the pathway of drug release.[23]

Antimicrobial Activity

The antimicrobial efficacy of the sunflower oil nanoemulcream was assessed by determining its MIC against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* using the broth microdilution method in accordance with CLSI guidelines. Two-fold serial dilutions of the formulation (4000–0.125 $\mu\text{g/mL}$) were prepared in Mueller–Hinton broth and inoculated with standardized bacterial suspensions (0.5 McFarland; $\sim 5 \times 10^5$ CFU/mL). Ciprofloxacin and DMSO were used as positive and negative controls, respectively. Plates were incubated at $37 \pm 1^\circ\text{C}$ for 24 h, and MIC was defined as the lowest concentration showing no visible bacterial growth. All tests were conducted in triplicate, and results were expressed as mean $\pm$ SD [24].

Scanning Electron Microscopy Analysis

Surface morphology of the nanoemulsion formulation was examined using scanning electron microscopy. Samples were mounted on stubs, gold-coated to ensure conductivity, and imaged at a 2 μm scale to observe droplet uniformity and structural features [23].

The optimized sunflower oil nanoemulcream exhibited excellent physical stability over three months under accelerated conditions. The pH remained consistent (6.2–6.1), within the skin-compatible range, and viscosity showed negligible variation, indicating preserved semisolid consistency. The formulation maintained uniform homogeneity, smooth texture, and stable organoleptic properties, with no phase separation or visible changes observed, confirming its robustness and suitability for topical application.

Results & Discussion

GC–MS Analysis

GC–MS profiling of *Helianthus annuus* (sunflower) oil identified ten constituents (Rt 0.155–1.220 min), mainly heterocycles, amides, phenolic ketones, and sulphonamides (Fig. 1). Major compounds included 1,3,5-Triazine, hexahydro-1,3,5-trimethyl (4.93%) and N,N-Dimethylethanesulfonamide (4.85%), while Topotecan (0.39%) was least abundant. The presence of bioactive components (Table 1) such as N, N-Dimethylethanesulfonamide and 1,2,5-Oxadiazole supports the antimicrobial, antioxidant, and skin-protective potential of sunflower oil for nanoemulsion-based topical applications.

Table 1
Key bioactive compounds identified in crude sunflower oil by GC-MS.

Peak	Rt (Min)	Compound Name	Molecular Formula	MW (G/Mol)	Peak Area (%)
2	0.155	1,3,5-Triazine, hexahydro-1,3,5-trimethyl-	C ₆ H ₁₅ N ₃	129	4.93
4	0.270	3-tert-Butyl-5-chloro-2-hydroxybenzophenone	C ₁₇ H ₁₇ ClO ₂	288	2.38
6	0.330	Piperidin-2,6-di-carboxylic acid	C ₇ H ₁₁ NO ₄	173	0.63
7	0.395	N, N-Dimethylethanesulfonamide	C ₄ H ₁₁ NO ₂ S	137	4.85
12	0.555	1,2,5-Oxadiazole	C ₂ H ₂ N ₂ O	70	1.76
13	0.620	Propanamide, N-methyl-2-amino-	C ₄ H ₁₀ N ₂ O	102	2.91
18	0.781	3-Pentanone, dimethylhydrazone	C ₇ H ₁₆ N ₂	128	3.47
20	0.900	Topotecan	C ₂₃ H ₂₃ N ₃ O ₅	421	0.39
24	1.085	Propanediamide	C ₃ H ₆ N ₂ O ₂	102	0.79
28	1.220	N'-Isopropylureidoacetic acid	C ₆ H ₁₂ N ₂ O ₃	160	3.44

ATR–FTIR Analysis

The ATR–FTIR spectra (Fig. 2) confirmed the characteristic functional groups of sunflower oil, nanoemulsion, and nanoemulcream. Peaks at 2956–2854 cm⁻¹ (–CH₂/–CH₃ stretching) and 1743–1735 cm⁻¹ (ester C = O) indicated the triglyceride backbone, while C = C stretching near 1636 cm⁻¹ and = C–H vibrations around 3007 cm⁻¹ confirmed the presence of unsaturated fatty acids, particularly oleic and linoleic acids. Broad O–H bands (3972–3266 cm⁻¹) reflected hydrogen bonding among water and surfactant molecules, and C–O stretching (1234–1030 cm⁻¹) (Table 2) verified ester and ether linkages. The nanoemulsion and nanoemulcream exhibited comparable spectral patterns with minor shifts, confirming oil incorporation without chemical modification and overall formulation stability.

Table 2
Functional group profile of sunflower oil as determined by ATR-FTIR

Observed Wavenumber (cm ⁻¹)	Functional Group	Accepted Range (cm ⁻¹)	Probable Compound/Class
3972.39–3748.60	O–H stretching (free/broad)	3700–3600	Alcohols, phenols, water
3319.09–3266.02	O–H/N–H stretch (H-bonded)	3400–3200	Hydroxyls from water/surfactant, amines
3007.72	=C–H stretch (cis-alkene)	3020–3000	Unsaturated fatty acids (oleic, linoleic)
2956.20, 2927.06, 2923.41	–CH ₃ , –CH ₂ stretch	2960–2850	Alkanes, fatty acid chains
2854.76, 2854.17	–CH ₂ symmetric stretch	2855–2840	Saturated fatty acids
1743.79	C = O stretch (ester)	1750–1735	Triglycerides, ester lipids
1648.99, 1636.01	C = C stretch (alkene)	1680–1620	Unsaturated fatty acids
1460.90, 1454.73	CH ₂ bending	1470–1450	Fatty acid chains
1374.06, 1351.22, 1346.24	CH ₃ symmetric bending	1390–1340	Methyl groups in lipids
1234.76–1030.16	C–O stretch (esters)	1300–1000	Glycerides, triglyceride esters
1082.79	C–O stretch (weak)	1100–1000	Possibly surfactant or weakened ester band
963.20–843.27	=C–H out-of-plane bending	1000–650	Alkenes (unsaturation)
720.91	–(CH ₂) _n rocking	750–720	Long alkyl chains
527.95–419.00	Bending vibrations	< 600	Fingerprint region (complex bending)

DSC Analysis

The DSC thermogram (Fig. 3) showed an endothermic peak at 198.36°C for sunflower oil and 103.60°C for the nanoemulcream, indicating good thermal stability of the formulation within the processing range.

QbD-Based Optimization and Risk Assessment of Nanoemulcream

The QbD concept established the QTPP and CQAs for the optimized sunflower oil nanoemulcream (Table 3). Target parameters—pH (5.5–7.0), droplet size (< 200 nm), and zeta potential (– 20 to – 30 mV)—ensured stability and skin compatibility, while rheological properties supported smooth application. Risk analysis using RPN and Ishikawa (Fig. 4) identified the surfactant blend (Tween 80 + PEG 400) as the highest-risk factor, influencing droplet size, zeta potential, viscosity, and stability. Homogenization time and speed affected particle size and texture, whereas sunflower oil, water, and excipients (Table 4) posed moderate risks. This assessment enabled systematic, reproducible, and stable formulation within the QbD framework.

Table 3
QTPP and CQAs under the QbD Framework for Sunflower Oil–Based Nanoemulsioncream.

QTPP	Target	CQAs	Justification
Dosage form	Nanoemul cream	–	Semi-solid emulsion system enhances topical drug delivery
Route of administration	Topical	–	Localized delivery, avoids systemic side effects
Dosage strength	1.0% w/w of active ingredient	–	To ensure therapeutic effect
Dosage design	Oil-in-water nanoemulsion in cream base	–	Enhances solubility, stability, and skin permeation
Appearance	White, smooth, homogeneous cream	–	Impacts patient compliance and product acceptability
Odour	No objectionable Odor	–	Improves user acceptability
Identification	FTIR fingerprint and visual inspection	–	Confirms identity of active and excipients
pH	5.5–7.0	Yes	Should match skin pH to avoid irritation
Droplet size	< 200 nm	Yes	Key for stability, bioavailability, and skin penetration
Zeta potential	–20 mV to – 30 mV (target range)	Yes	Indicator of emulsion stability
Rheological aspects	Appropriate Spreadability and texture (measured via viscosity, etc.)	Yes	Influences application, absorption, and patient experience
Viscosity	Moderate (optimized for Spreadability and retention)	Yes	Affects in-use performance and stability
Spreadability	20–30 g/cm ²	Yes	Ensures easy and uniform application
Texture Profile	Firmness, cohesiveness, adhesiveness, elasticity within acceptable range	Yes	Impacts consumer acceptance and skin feel
Washability	Easily washable with water	Yes	Enhances patient comfort and hygiene
Morphological analysis	Spherical, non-aggregated droplets in nanoemulsion	Yes	Ensures nanoscale dispersion, affects performance
Particle size	Nano-range	Yes	Reflects formulation integrity post-processing

Table 4
RPN Assessment of Formulation and Process Factors Influencing noemulcream Quality Attributes

Process Factor	Viscosity	Spreadability	pH	Texture	Morphology	Particle Size	Zeta Potential	Stability	Action Plan
Sunflower Oil	Medium	Medium	Low	Medium	Medium	Medium	Medium	Medium	verify purity using GC-MS
Surfactant Mixture (Tween 80 + PEG 400)	High	High	Low	High	Medium	High	High	High	Optimize surfactant ratio for stability
Deionized Water	Medium	Medium	Low	Medium	Medium	Medium	Medium	Medium	Use ultrapure water
Homogenization Time	High	High	Low	High	Medium	High	Medium	High	Maintain at 25 minutes; test $\pm$ 5 minutes.
Homogenization Speed	High	High	Low	High	Medium	High	High	High	Maintain 12000 rpm; evaluate stability at $\pm$ 1000 rpm
Cream Base (Lanolin, Cetyl alcohol, Stearic Acid, etc.)	Medium	Medium	Medium	Medium	Medium	Medium	Low	Medium	Balance ingredients for desired texture and stability.

DoE Optimization

CCD (Table 5) was employed to optimize surfactant mix (X_1), Homogenization time (X_2), and Homogenization speed (X_3) for nanoemulsion particle size. The 3D response surface plots (Fig. 5) showed that increasing Smix ratio and speed reduced droplet size, while time had a moderate effect. The prediction profiler (Fig. 6) indicated an optimized particle size of 165.6 nm with a desirability of 0.83, and the interaction profiler (Fig. 7) highlighted synergistic effects of the variables. Statistical validation (Table 6) confirmed the model's significance, good fit, and minimal residual correlation.

Table 5
Responses of experimental trials developed via the CCD approach

Runs	Pattern	Smix (1:1) X1	Time (min) X2	Speed (Rpm) X3	Particle size (nm) Y R1	R2	R2
1	00A	4.5	25	12000	179.9	12000	179.2
2	---	4	20	10000	204.4	10000	204.7
3	++-	5	30	10000	182.8	10000	183.2
4	0a0	4.5	20	11000	194.6	11000	195.1
5	000	4.5	25	11000	200.5	11000	200.8
6	00a	4.5	25	10000	196.4	10000	196.5
7	---+	4	30	12000	174.9	12000	175.1
8	--+	4	30	10000	196.6	10000	196.8
9	+++	5	30	12000	187.3	12000	187.5
10	A00	5	25	11000	176.5	11000	176.8
11	a00	4	25	11000	175.3	11000	176.1
12	--+	4	20	12000	181.2	12000	181
13	++-	5	20	12000	175.2	12000	175.8
14	0A0	4.5	30	11000	209.2	11000	209.5
15	++-	5	20	10000	207	10000	207.4
16	000	4.5	25	11000	200.6	11000	200.3

Table 6
Statistical Summary of Model Fit, ANOVA, and Durbin–Watson Test

Parameter	Statistical Value	Interpretation
R ²	0.7883	Indicates that 78.83% of the variation in the response is explained by the model.
Adjusted R ²	0.7017	Adjusted for degrees of freedom; shows a good model fit.
Root Mean Square Error (RMSE)	6.6188	Represents the standard deviation of residuals; smaller value indicates better precision.
Mean of Response	190.2563	Average response value across all observations.
Observations	32	Total number of experimental runs.
Model Sum of Squares	3588.9361	Variation explained by the regression model.
Error Sum of Squares	963.7826	Unexplained variation (residual error).
F Ratio	9.1026	Indicates model significance.
Prob > F	< 0.0001	Model is statistically significant (p < 0.05).
Durbin–Watson Statistic	2.1204	Suggests no significant autocorrelation (ideal ≈ 2).
Autocorrelation	−0.0784	Very weak negative correlation between residuals.
Prob < DW	0.6720	Confirms independence of residuals.

Optimized Nanoemulsion Formulation

Design of Experiments (DoE) optimization identified the ideal formulation parameters for the sunflower oil nanoemulcream at an Smix (1:1) ratio of 4, homogenization time of 24.26 min, and speed of 12,000 rpm. The developed model predicted a particle size of 165.57 nm (95% CI: 157.37–173.79 nm), demonstrating strong model accuracy and reproducibility. A desirability value of 0.83 confirmed that the formulation satisfied all QTPP and CQA criteria, ensuring stability, uniformity, and effective topical performance.

Optimized Nanoemulsion Characterization

The optimized sunflower oil nanoemulsion exhibited a mean droplet size of 134.7 ± 34.1 nm Z-average 166.9 nm, PDI 0.409 (Fig. 8). The zeta potential of -30.1 ± 2.3 mV (Fig. 9) indicated strong electrostatic stabilization, preventing aggregation and ensuring long-term stability. These characteristics confirm the formation of a stable, nanosized system suitable for topical nanoemulcream formulation.

Physicochemical and Safety Evaluation

The sunflower oil nanoemulcream exhibited desirable physicochemical characteristics, including good spreadability, uniform texture, and excellent stability (Table 7). SEM images (Fig. 10) showed spherical, smooth, and uniformly distributed droplets at a 2 μ m scale, confirming nanoscale uniformity, structural integrity, and consistent morphology of the formulation. Skin irritancy testing further demonstrated the nanoemulcream to be non-irritant (Table 8), indicating its safety and suitability for topical application.

Table 7
Physicochemical characterization of sunflower oil nanoemulcream

Parameter	Observed Result	Acceptable/Standard Range for Creams	Inference
pH	6.2 ± 0.1	4.5–6.5 (skin-friendly range)	Suitable for topical use, non-irritant
Viscosity (cP at 25°C)	$28,500 \pm 120$ cP	20,000–50,000 cP (semisolid creams)	Provides spreadability & stability
Homogeneity	Uniform, smooth, no lumps	Uniform, smooth	Good texture & user acceptability
Phase separation	None observed after 24 h at RT	No separation	Stable emulsion system
Organoleptic properties	White, characteristic Odor, smooth texture	Pleasant colour, Odor, smooth feel	Patient-compliant
Spreadability (gm/s)	12.5 ± 0.5	> 10 gm/s (good spreadability)	Easily spreadable on skin
Washability	Easily washable with tap water	Easily washable	Convenient for user
After-feel	Non-greasy, smooth, emollient	Non-sticky, emollient	Enhances cosmetic acceptability
Particle size (nm)	166.9 ± 34.1 nm	< 200 nm (nano-range)	Nanosized, enhances penetration
Polydispersity index (PDI)	0.409	< 0.5 (moderately narrow distribution)	Homogeneous size distribution
Zeta potential (mV)	-30.1 ± 2.3 mV	> ± 25 mV (stable colloids)	Good electrostatic stability
Drug content (%)	$94.2 \pm 1.8\%$	90–110%	High drug entrapment & uniformity

Table 8
Skin Irritancy Test Results of Sunflower Oil Nanoemulcream

Parameter	Observation (Mean $\pm$ SD, n = 3)	Guideline Range / Scale	Inference
Erythema (Redness)	0 (No redness)	0–4 (OECD 404 / Draize scale)	Non-irritant
Burning / Itching	Absent	—	Safe for skin
Mean Irritation Score (MIS)	0	0.0–0.4 = Non-irritant; 0.5–2.0 = Slight; 2.1–5.0 = Moderate; 5.1–8.0 = Severe	Safe formulation (Non-irritant)

In Vitro Drug Release and Diffusion Kinetics

The in vitro drug release study over 360 minutes revealed notable differences among the formulated systems. The nanoemulsion (NE) exhibited the fastest and most extensive drug release, achieving 88% cumulative release, likely due to its nanoscale droplet size, large interfacial area, and improved drug partitioning into the aqueous phase. The nanoemulcream (NEC) showed a moderately sustained release of 64.8%, reflecting the semisolid cream matrix's mild diffusional barrier, which slows drug migration compared to the freely dispersed nanoemulsion. The conventional cream containing sunflower oil (CMC) displayed a slower release rate (49%), consistent with the restricted drug mobility within the more viscous emulsion base. Plain sunflower oil (SO) showed the lowest release (30%), highlighting the limited diffusion from a hydrophobic, oil-dominant medium (Fig. 11). Overall, these findings demonstrate that while nanoemulsions provide rapid and enhanced drug liberation, incorporation into a cream base allows for a more controlled and sustained drug release, making the nanoemulcream a promising platform for topical drug delivery that balances efficacy with practical handling advantages.

Dissolution Kinetics

The release data were fitted to zero-order, first-order, Higuchi, Korsmeyer–Peppas, and Hixson–Crowell models to elucidate the drug release mechanism. The highest correlation was observed with the Higuchi model ($R^2 = 0.903$), suggesting a diffusion-controlled release. Korsmeyer–Peppas equation/model for drug release ($n = 0.82$, $R^2 = 0.892$) suggested anomalous (non-Fickian) diffusion, involving both diffusion and matrix relaxation (Table 9). Other models showed lower R^2 values, indicating less relevance in describing the release kinetics.

Table 9
Drug Release Kinetic Model Results — Sunflower Oil Nanoemulcream

Kinetic Model	Equation	R ²	Interpretation
Zero-order	$Q_t = Q_0 + k_0t$	0.832	Does not follow constant-rate release (low R ²)
First-order	$\log Q_t = \log Q_0 - k_1t/2.303$	0.846	Release depends on concentration; moderate fit
Higuchi	$Q_t = k_H \sqrt{t}$	0.903	Best fit → diffusion-controlled mechanism
Korsmeyer–Peppas	$M_t/M_\infty \propto kt^n$	0.892	$n = 0.82 \rightarrow$ Anomalous (non-Fickian) diffusion involving diffusion + cream matrix relaxation
Hixson–Crowell	$Q_0^{1/3} - Q_t^{1/3} = kt$	0.811	Weak fit — erosion/dissolution not dominant

Antimicrobial Activity

The sunflower oil nanoemulcream exhibited moderate antimicrobial action against Gram-positive and Gram-negative organisms (Table 10). The MIC values ranged from 110 to 140 µg/mL, with *Bacillus subtilis* (110 ± 4 µg/mL) showing the greatest susceptibility and *Pseudomonas aeruginosa* (140 ± 5 µg/mL) the least. *Staphylococcus aureus* and *Escherichia coli* recorded MICs of 125 ± 5 and 125 ± 6 µg/mL, respectively. The observed activity demonstrates the effective incorporation and retention of the antimicrobial constituents of sunflower oil within the nanoemulcream matrix. Enhanced efficacy can be attributed to the nanoscale droplet size, which increases surface area and facilitates the interaction of bioactive compounds with bacterial cell membranes. The comparable inhibition against both Gram positive and Gram negative strains further confirms the broad-spectrum antimicrobial potential of the optimized formulation for topical applications.

Table 10
Minimum Inhibitory Concentration (MIC) of Sunflower Oil Nanoemulcream

Bacterial strain	Gram	MIC (µg/mL)	Interpretation (Based on standard MIC ranges)
Staphylococcus aureus (ATCC 25923)	Gram +	125 ± 5	Moderate activity (101–500 µg/mL)
<i>Bacillus subtilis</i> (ATCC 6633)	Gram +	110 ± 4	Moderate activity (101–500 µg/mL)
Escherichia coli (ATCC 25922)	Gram −	125 ± 6	Moderate activity (101–500 µg/mL)
<i>Pseudomonas aeruginosa</i> (ATCC 27853)	Gram −	140 ± 5	Moderate activity (101–500 µg/mL)

Accelerated Stability Study

The optimized sunflower oil nanoemulcream exhibited excellent physical stability over three months under accelerated conditions. The pH remained consistent (6.2–6.1), within the skin-compatible range, and viscosity showed negligible variation, indicating preserved semisolid consistency. The formulation maintained uniform homogeneity, smooth texture, and stable organoleptic properties, with no phase separation or visible changes observed, confirming its robustness and suitability for topical application.

Conclusion

This study successfully developed a sunflower oil (*Helianthus annuus*) nanoemulcream using a QbD framework with CCD optimization. The developed nanoemulsion exhibited a mean droplet size of 134.7 ± 34.1 nm, Z-average 166.9 nm, PDI 0.409, and zeta potential -30.1 ± 2.3 mV, confirming uniform nanoscale dispersion and electrostatic stability. Incorporation into a cream base yielded a physicochemically stable and skin-compatible nanoemulcream with pH 6.2 ± 0.1 , viscosity $28,500 \pm 120$ cP, spreadability 12.5 ± 0.5 g·s⁻¹, and drug content $94.2 \pm 1.8\%$. Accelerated stability studies demonstrated consistent droplet size, viscosity, and drug content over three months, while skin irritancy testing verified its non-irritant behavior. In vitro drug release studies showed enhanced and sustained drug release from the nanoemulcream compared to conventional cream and plain sunflower oil. Kinetic modeling further indicated a combination of diffusion-controlled and anomalous (non-Fickian) transport mechanisms, supporting the formulation's controlled-release characteristics. The nanoemulcream also displayed moderate broad-spectrum antibacterial activity (MIC 110–140 µg/mL), highlighting its therapeutic potential. Overall, this work establishes a reproducible, environmentally sustainable, and therapeutically effective platform for topical delivery of natural bioactives. The findings underscore the value of QbD-guided nanoemulsion design for next-generation phytopharmaceutical creams with improved stability and safety. Although in vivo animal safety studies were not conducted in the present investigation, the strong in vitro and stability data provide a solid foundation for future preclinical animal studies to further confirm safety and therapeutic performance.

Abbreviations

ATR
FTIR–Attenuated Total Reflectance–Fourier Transform Infrared Spectroscopy
CCD
Central Composite Design
CMAs
Critical Material Attributes
CPPs
Critical Process Parameters
CQAs
Critical Quality Attributes
DMSO
Dimethyl Sulfoxide
DoE
Design of Experiments
DSC
Differential Scanning Calorimetry
GC
MS–Gas Chromatography–Mass Spectrometry
HPLC
High–Performance Liquid Chromatography
PDI
Polydispersity Index
PEG 400
Polyethylene Glycol 400
pH
Potential of Hydrogen
QbD
Quality by Design
QTPP

Quality Target Product Profile
RPN
Risk Priority Number
MIC
minimum inhibitory concentration
REM
Risk Evaluation Matrix
SEM Scanning Electron
Microscopy.

Declarations

Ethical Compliance

This research did not include human participants or live animals. All experimental procedures, including in vitro analyses, stability studies, and skin irritation assessments, adhered to institutional safety standards and good laboratory practices.

Data and Material Availability

All relevant data generated or analyzed during the study are presented within this article and its supplementary information. Additional datasets can be provided by the corresponding author upon reasonable request.

Funding Information

This work was carried out without any external financial support.

Ethical Approval and Participant Consent

Not applicable, as the study involved neither human nor animal subjects.

Consent for Publication

Not applicable.

Credit Authorship Contribution Statement

(Md Barakathulla): Conceptualization, Methodology, Writing – original draft, Supervision. (Yetukuri Koushik): Methodology, Validation, Writing – review & editing. (Morla Siva Prasad): Data curation, Formal analysis, Writing – review & editing. (Nadendla Rama Rao): Investigation, Visualization, Writing – review & editing.

Declaration of Competing Interests

The authors declare that there are no financial or personal conflicts of interest that could have influenced the work presented in this study.

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Figures

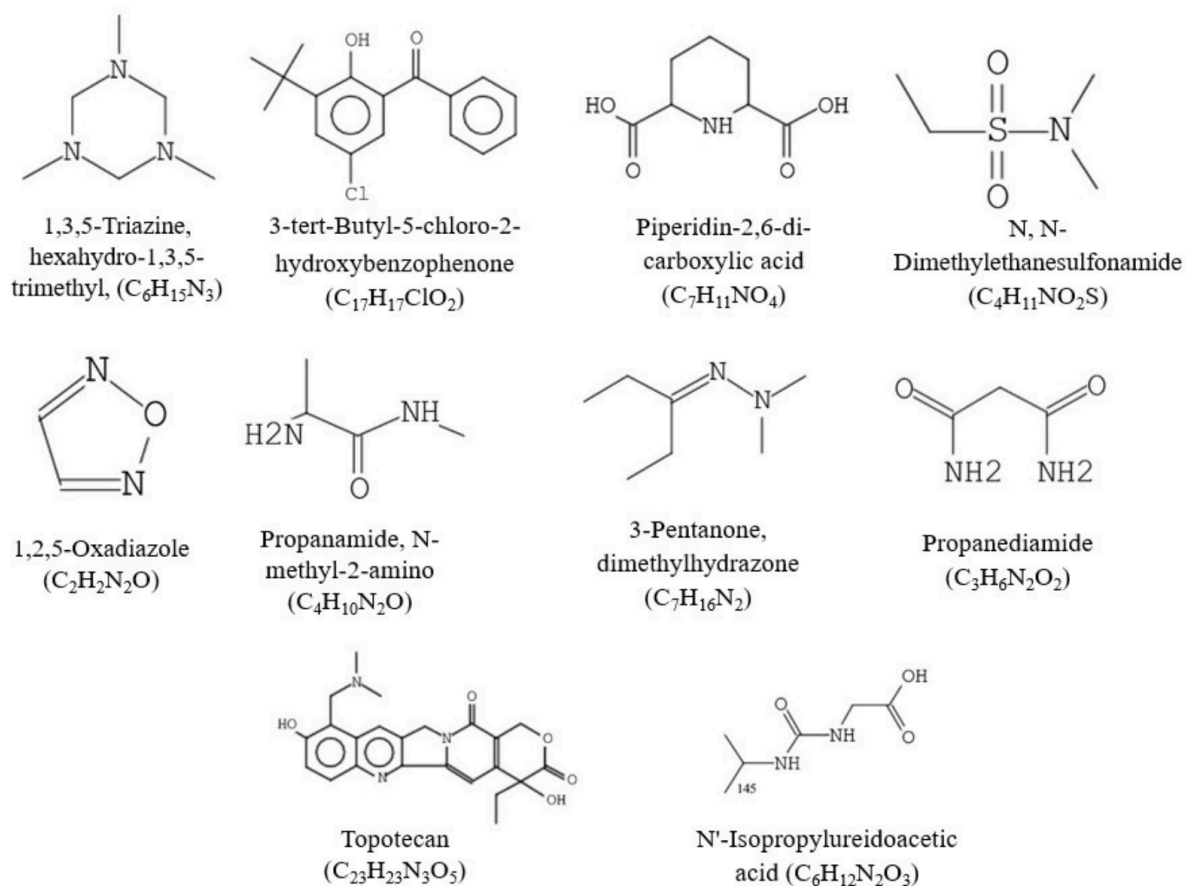


Figure 1

GC–MS Identification of Bioactive Constituents in Sunflower Oil

Figure 1. GC–MS chromatogram of *Helianthus annuus* oil displaying the principal bioactive components with antimicrobial, anti-inflammatory, and antioxidant activities relevant for topical formulations.

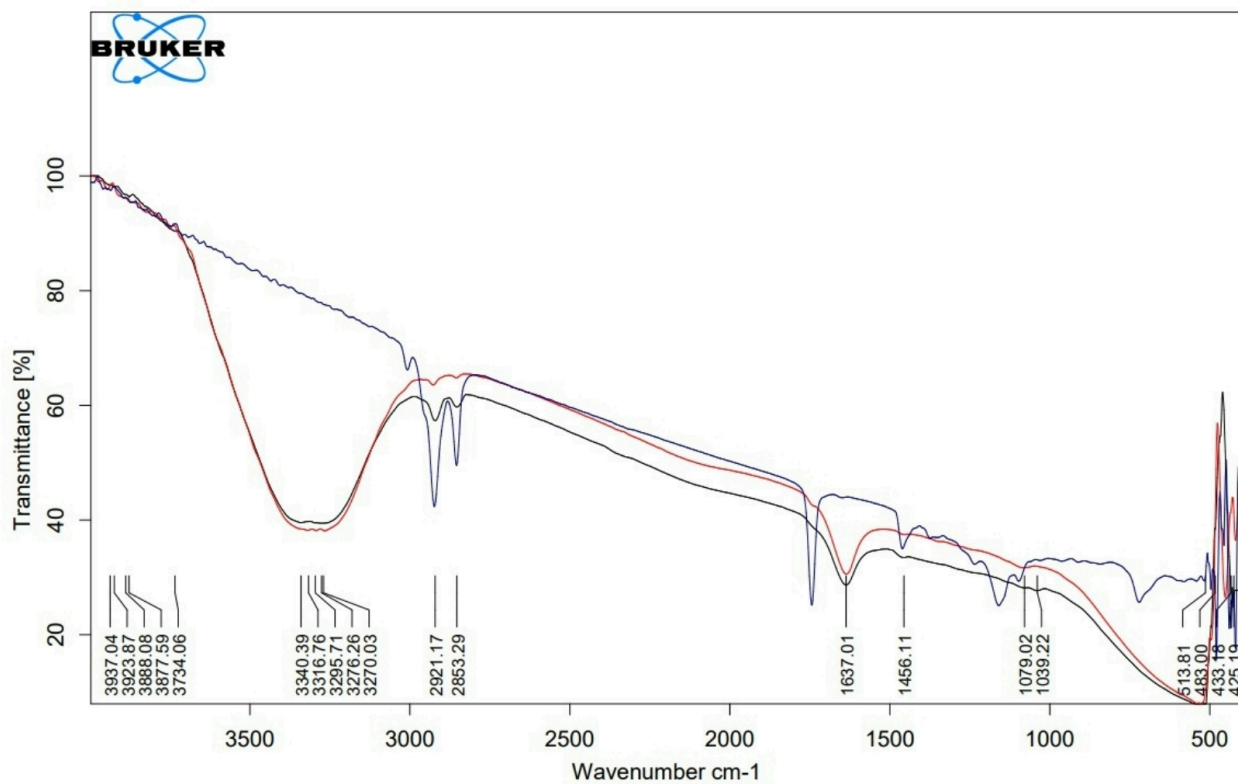


Figure 2

ATR-FTIR of Sunflower Oil, Nanoemulsion and Nanoemulcream

Figure 2. FTIR spectra of sunflower oil (blue), nanoemulsion (red), and nanoemulcream (black) showing characteristic peaks that confirm successful incorporation of the nanoemulsion into the cream base without any significant chemical interaction.

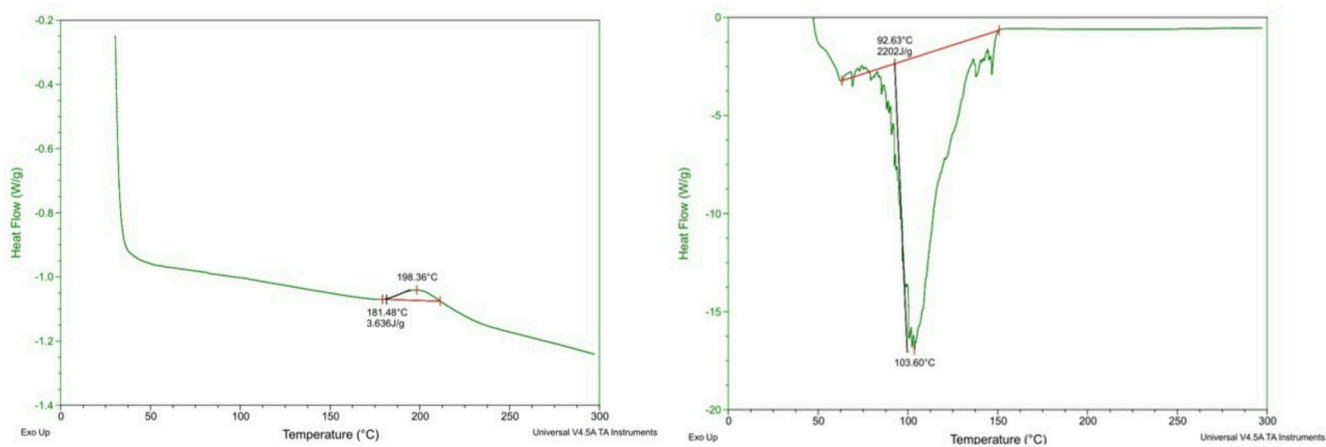


Figure 3

DSC Thermograms of Sunflower Oil and Nanoemulcream

Figure 3. DSC thermograms of *Helianthus annuus* oil and the optimized *Helianthus annuus* nanoemulcream, showing characteristic thermal transitions and stability profiles relevant for topical application.

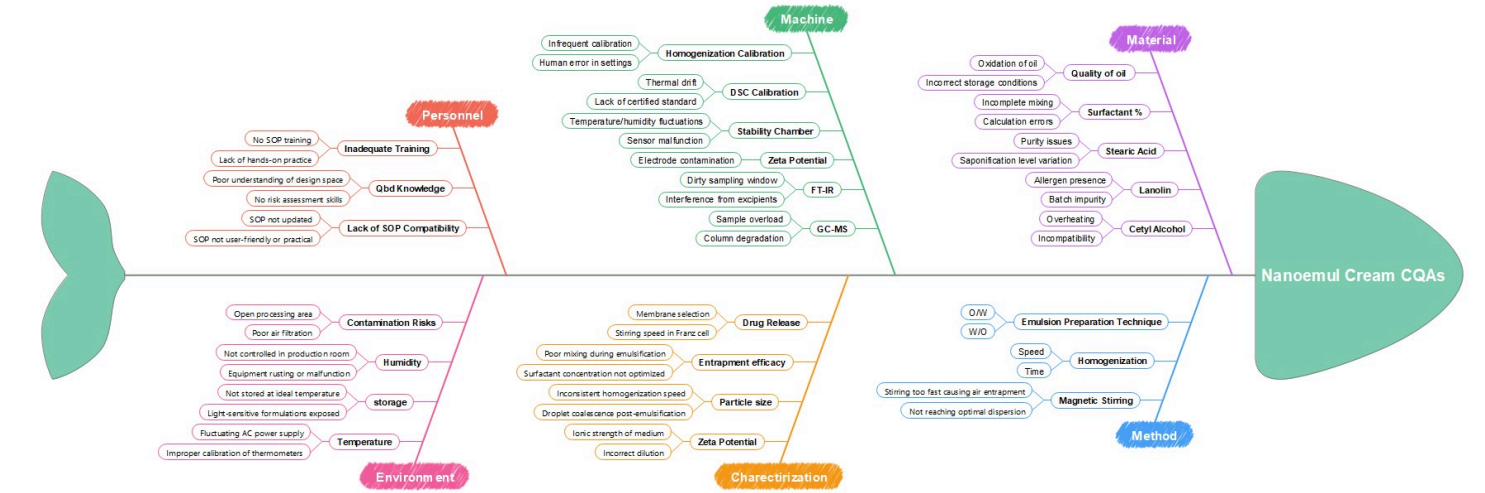


Figure 4

Ishikawa Diagram of Factors Influencing Nanoemulcream CQAs

Figure 4. Ishikawa (fishbone) diagram illustrating the potential Variables impacting the Critical Quality Attributes (CQAs) of the nanoemulcream formulation, including personnel, machine, material, method, characterization, and environmental factors.

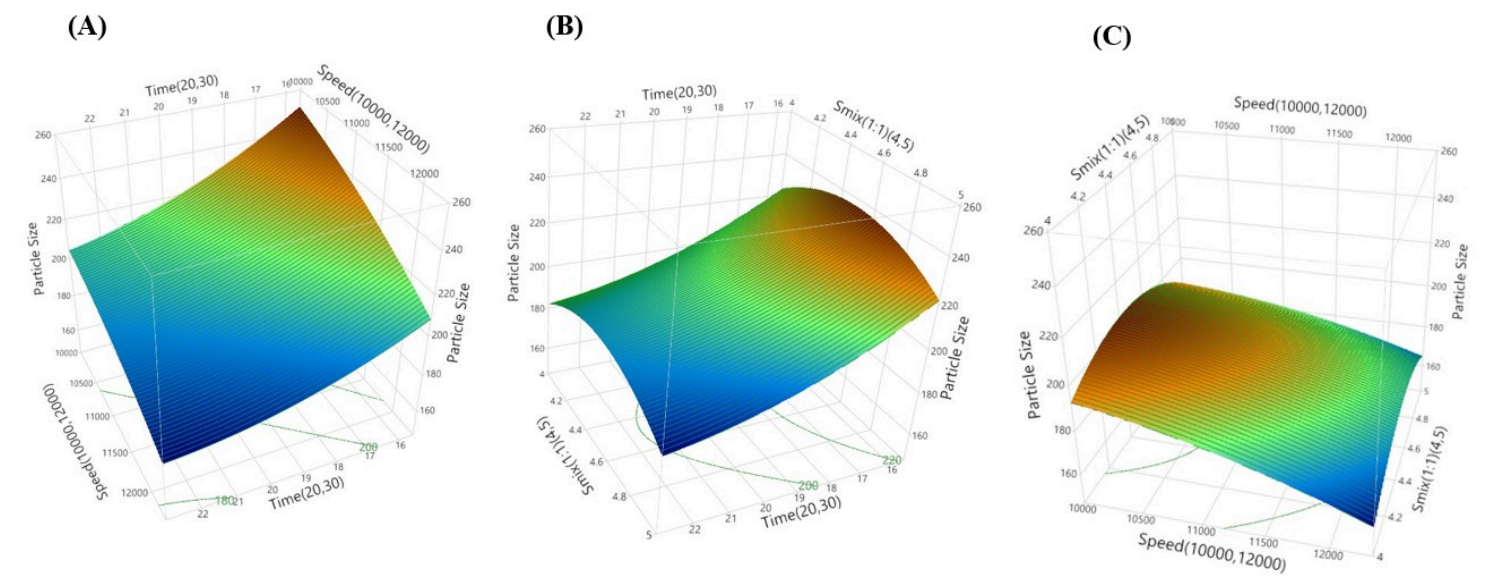


Figure 5

3D Graphs

Figure 5: 3D surface plots illustrating the influence of independent factors on particle size (Y): (A) Time vs. Speed, (B) Time vs. Smix, and (C) Speed vs. Smix.

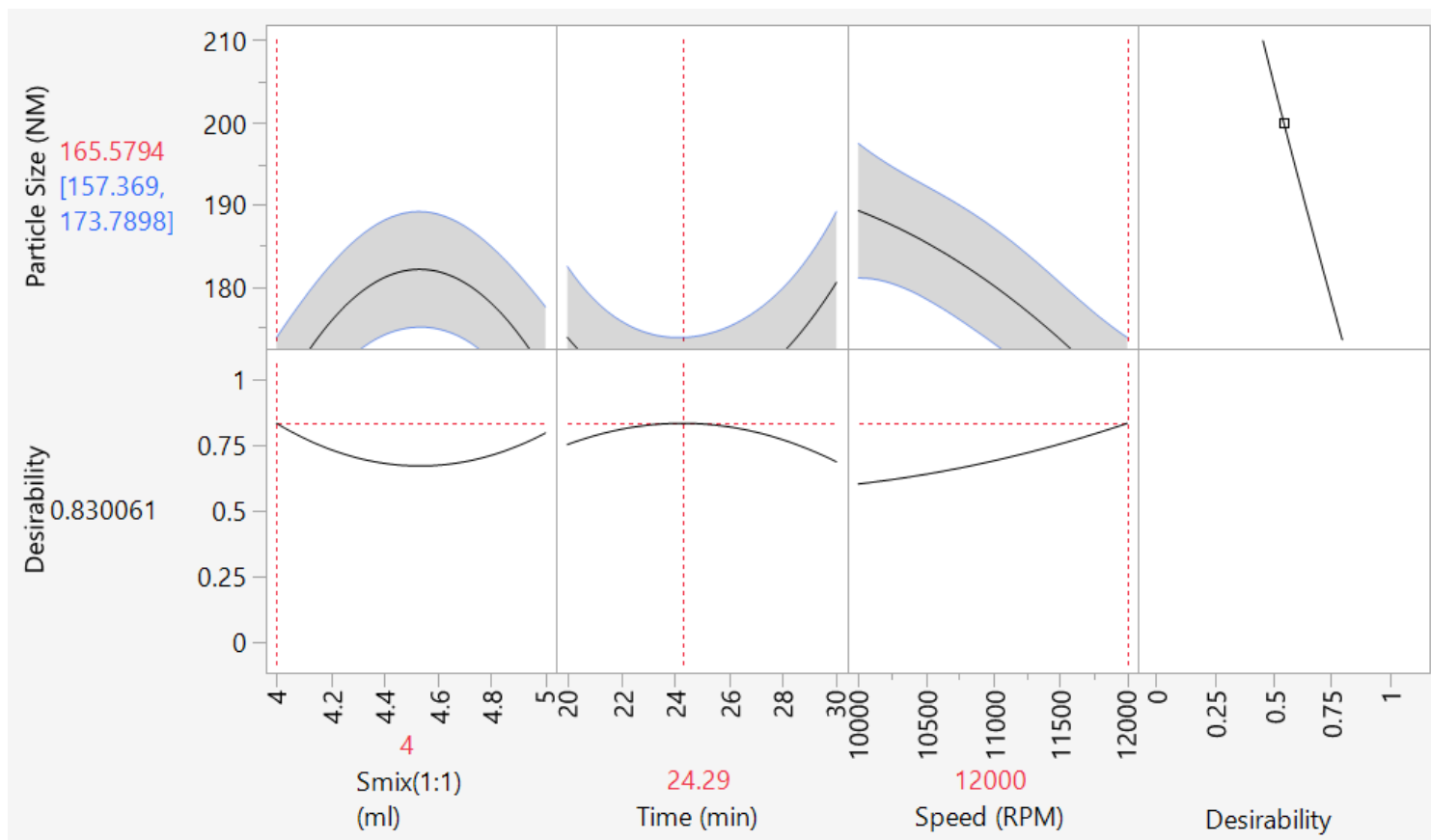


Figure 6

Prediction Profiler of Nanoemulsion

Figure 6: Prediction profiler of the nanoemulsion showing the Impact of the formulation and processing parameters on particle size. The developed conditions predict a droplet dimensions of 165.6 nm with a high desirability of 0.83, indicating good model reliability.

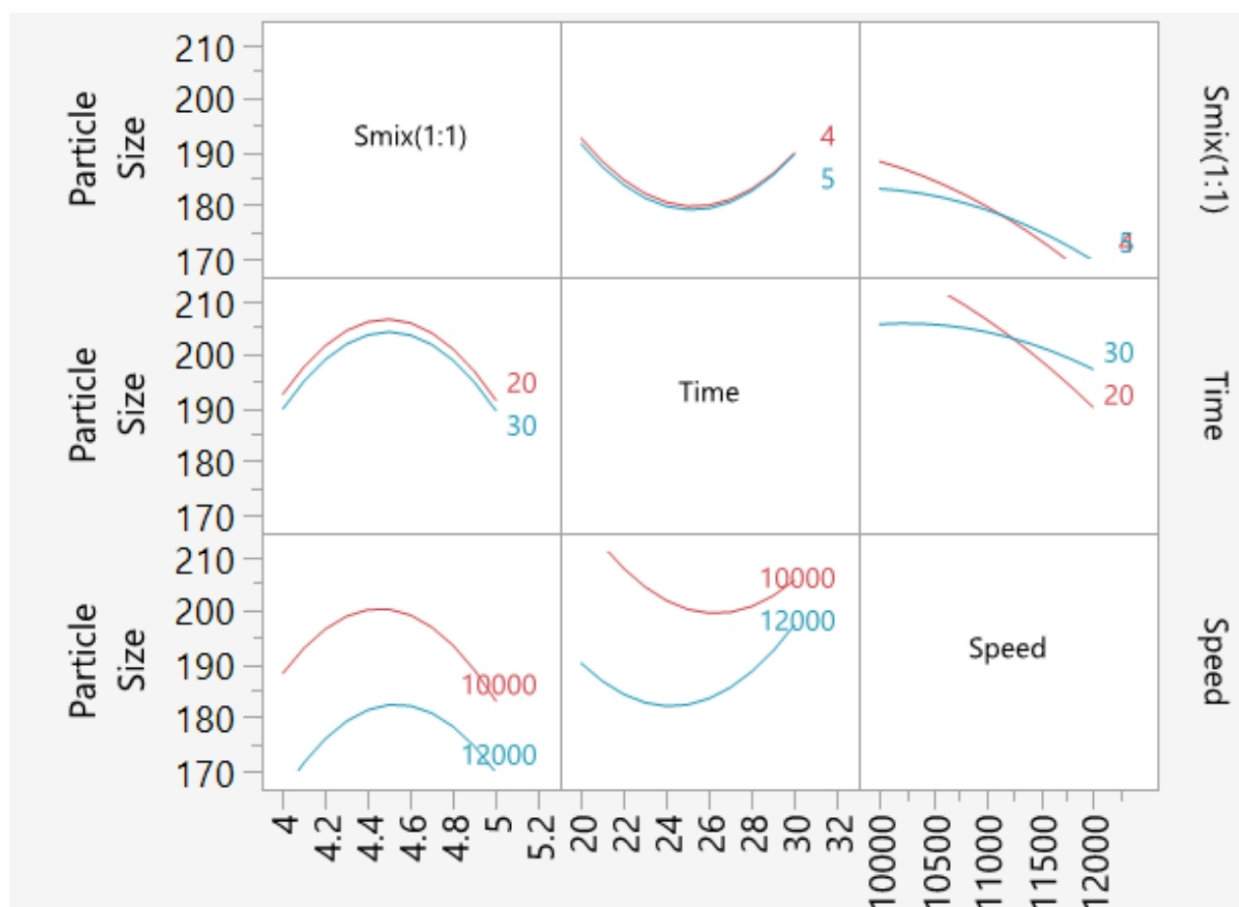


Figure 7

Interaction Profile of optimized Nanoemulsion

Figure 7: Interaction profiler showing the combined effects of Smix ratio, homogenization speed, and emulsification time on nanoemulsion particle size, highlighting key variable interactions influencing droplet size and stability.

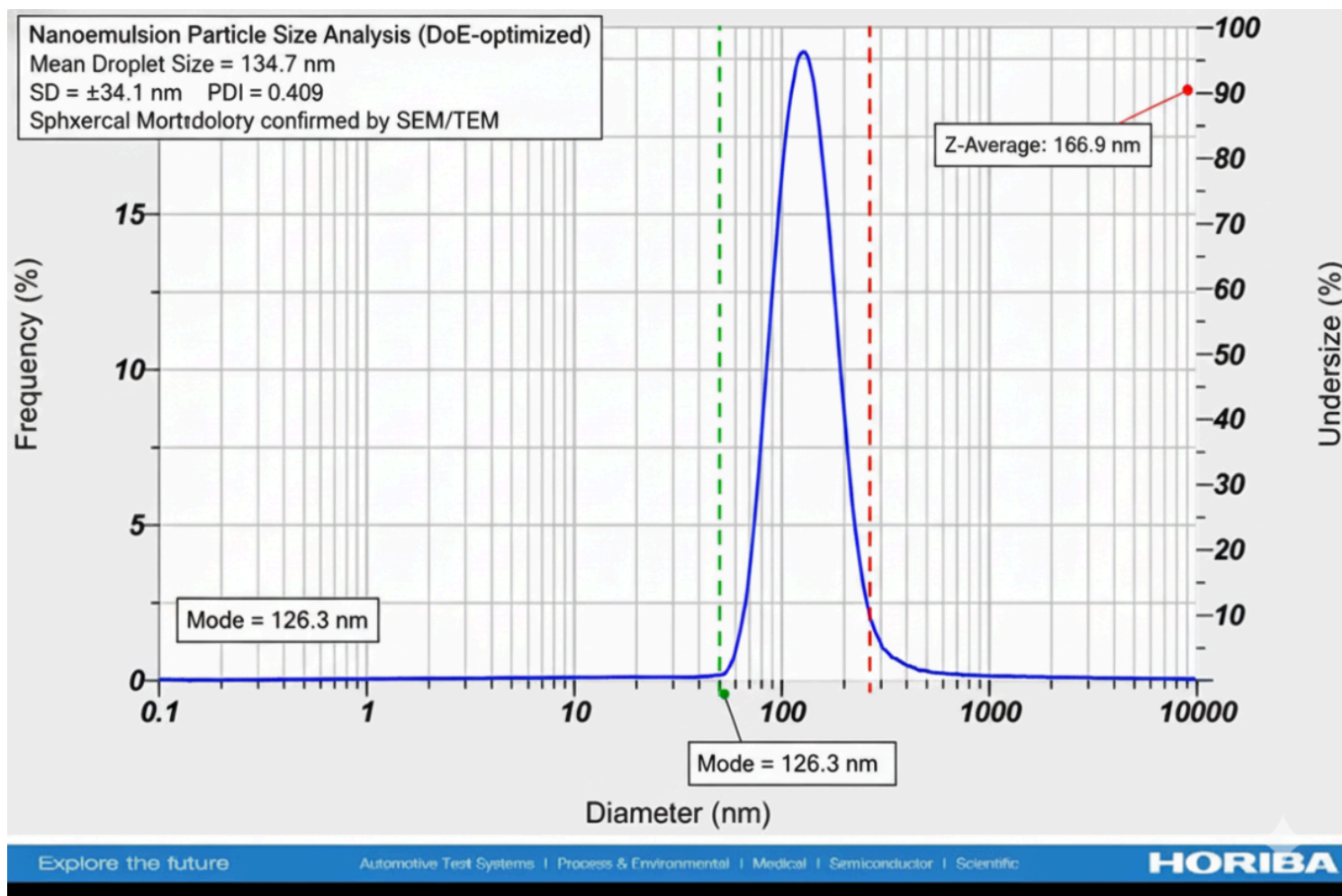


Figure 8

Particle Size Characterization of the Developed Sunflower Oil Nanoemulsion

Figure 8. Particle size profile of the developed sunflower-oil-based nanoemulsion exhibiting uniform droplet dimensions appropriate for topical use.

Zeta Potential Distribution

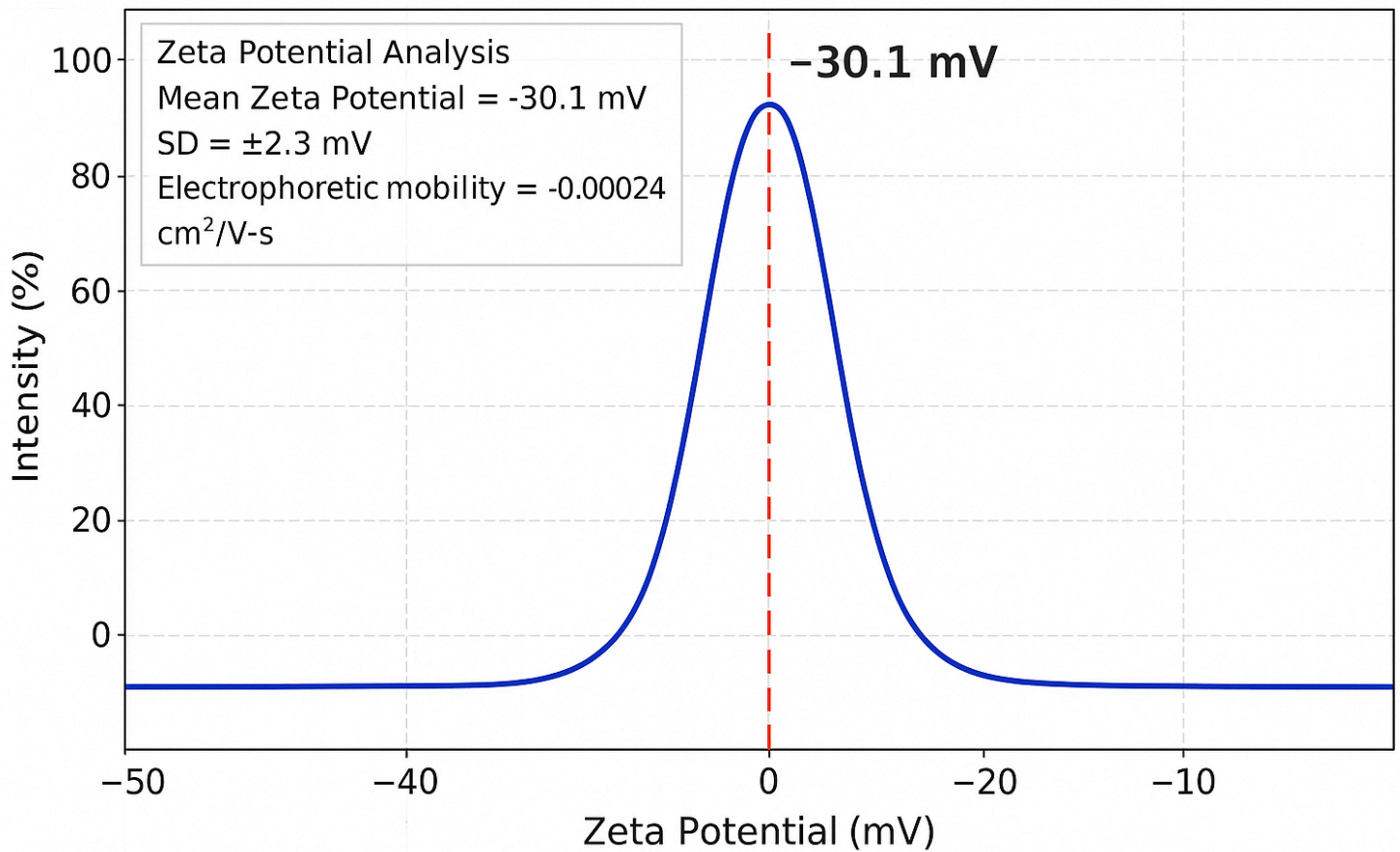


Figure 9

Zeta Potential Characterization of the Optimized Sunflower Oil Nanoemulsion

Figure 9. Zeta-potential profile of the optimized sunflower oil nanoemulsion, demonstrating its stable colloidal behaviour.

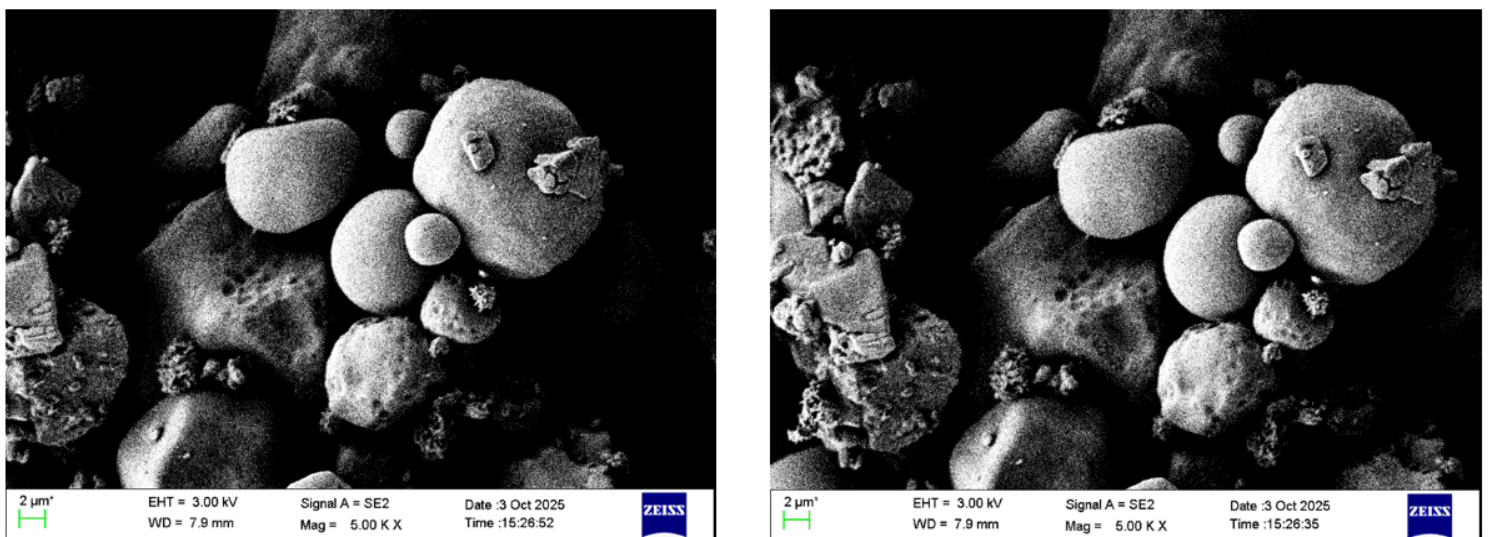


Figure 10

SEM Characterization of Nanoemulsion Surface Morphology

Figure 10. SEM images of the nanoemulsion formulation at 2 μm scale: (A) and (B) showing surface morphology.

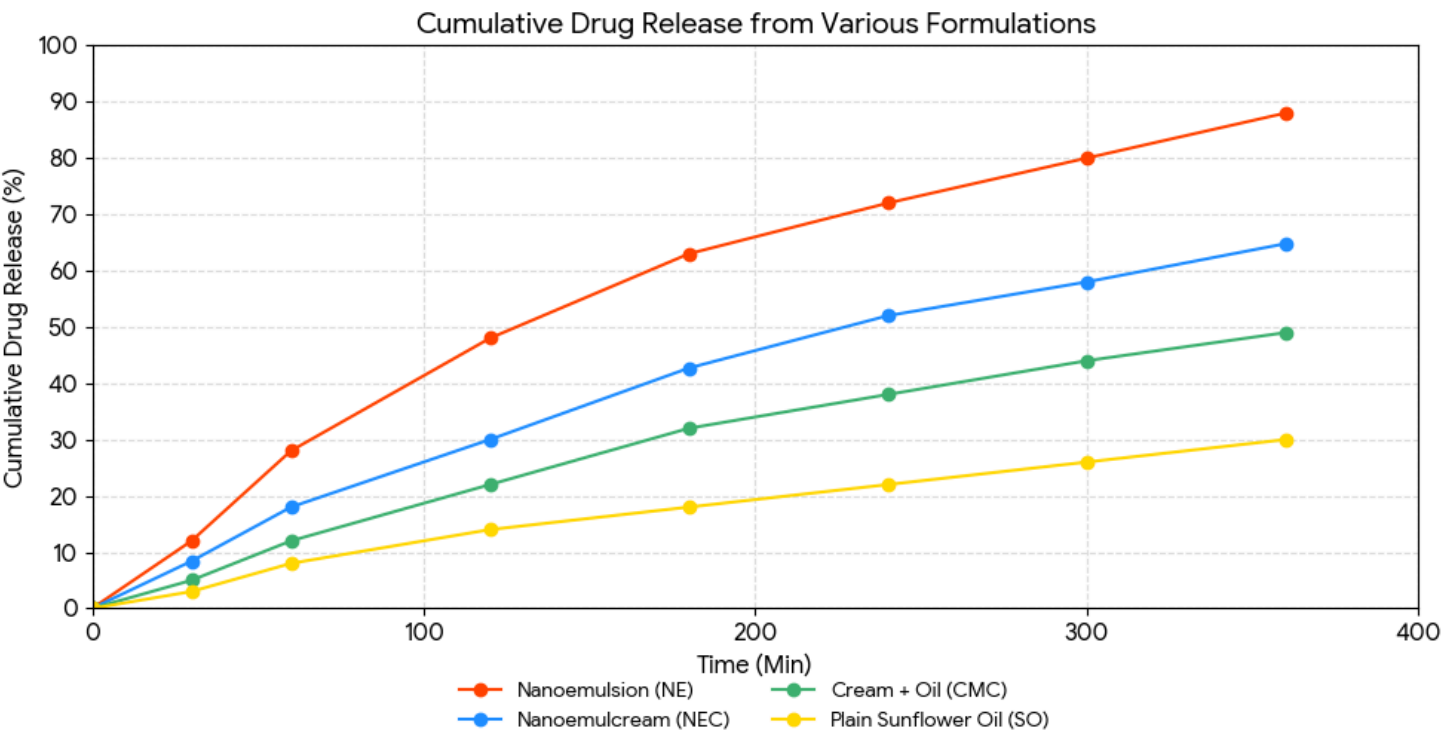


Figure 11

In Vitro Drug Release and Diffusion Kinetics

Figure 11. Cumulative drug release (%) versus time (min) for Nanoemulsion (NE), Nanoemulcream (NEC), Cream + Oil (CMC) and Plain Sunflower Oil (SO). Data represent mean values (n = 3). Error bars indicate standard deviation.