RESEARCH PAPER

Formulation, Optimization, and Evaluation of a Rutin-Loaded Microemulsion Using D-Optimal Mixture Design

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Abstract
The objective of this study was to develop, statistically optimize, and characterize a thermodynamically stable, high-performance topical microemulsion (ME) system for the enhanced delivery of Rutin. Screening studies identified Eucalyptus oil as the oil phase, Tween 80 as the non-ionic surfactant, and Propylene glycol as the co-surfactant (Smix system) based on maximum drug solubilization capability. Pseudo-ternary phase diagrams mapped a dominant isotropic microemulsion zone utilizing an optimal Smix weight ratio of 2:1. A D-Optimal mixture design was deployed to investigate the mathematical correlation between independent programmatic variables (concentrations of Smix [X1], Oil [X2], and Water [X3]) and critical programmatic responses (Viscosity [R1] and Transmittance [R2]).
The optimized candidate microemulsion configuration (MeR) exhibited exceptional isotropic transparency (Transmittance: 97.96%) and optimal fluid rheology (Viscosity: 115.37 ± 1.31 cP) alongside an average hydrodynamic diameter below 200 nm (159.6 nm). Transmission Electron Microscopy (TEM) verified uniform, spherical droplets displaying structural stability. In vitro skin permeation and cumulative drug release dynamics adhered closely to the Korsmeyer ($R^2 = 0.9982$), signaling controlled, matrix-driven diffusion kinetics. Short-term storage profiles validated the system's structural and thermodynamic resilience over a 3-month period under regulated environmental criteria.
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Introduction

Microemulsions (MEs) represent advanced, thermodynamically stable, isotropic colloidal systems composed of an organic oil phase, an aqueous domain, and a stabilizing surfactant-cosurfactant dynamic interfacial film. Due to ultra-low interfacial tensions, small internal globule domains (typically <200 nm), and structural fluidity, MEs dramatically improve solute solubility, disrupt local skin lipids, and amplify skin flux values.1 Rutin, a naturally occurring polyphenolic flavonoid possessing potent antioxidant, anti-inflammatory, and vaso-protective therapeutic properties, exhibits low aqueous solubility and poor skin permeability. This restricts its medical efficacy when delivered via conventional topical bases.2,3
This research project focuses on establishing a systematically optimized, mathematically modeled microemulsion vector utilizing Quality by Design (QbD) principles.4 Through computer-aided D-Optimal design space optimization, the study maps the physical variables necessary to yield predictable physical performance, high drug loading efficiencies, and improved cutaneous diffusion rates.5

2. Materials and Methods

2.1 Materials

Rutin analytical standards were acquired from Sigma-Aldrich. Tween 80 (Polyoxyethylene sorbitan monooleate), Olive oil, and Propylene glycol were procured from Loba Chemie. Carbopol 934 was obtained from CDH, and Methyl/Ethyl parabens were supplied by Sulab. Extra-pure Methanol/Ethanol were obtained from Merck and Molychem, respectively. High-purity deionized distilled water and Phosphate Buffered Saline (PBS) systems were freshly prepared across all evaluation steps.

2.2 Vehicle Screening and Saturation Solubility Studies

The saturation solubility profile of Rutin was determined across diverse oils, surfactants, and co-surfactants using the standard shake-flask method. An excess amount of Rutin was introduced into glass vials containing (2 ml) of each target vehicle component. The systems were immediately vortexed at maximum velocity for 10 minutes to maximize solid-liquid interaction area.The sealed vials were transferred to an isothermal shaking incubator maintained at ambient

room temperature and agitated continuously for 48 hours to reach thermodynamic equilibrium. The samples were visually regularly inspected; additional drug crystals were added to vials showing complete dissolution.Following equilibration, the systems were centrifuged at 3,000 rpm for 10 minutes to isolate undissolved crystalline matter. Supernatant fluids were drawn, filtered, diluted with appropriate spectroscopic solvents, and quantified via UV-Visible spectrophotometry at the 359 nm maximum absorption wavelength to isolate the vehicle composition with the highest drug loading capacity.6

2.3 Construction of Pseudo-Ternary Phase Diagrams

To map out the definitive concentration bound regions exhibiting microemulsion isotropic phase properties, pseudo-ternary diagrams were constructed using the aqueous titration technique at ambient room temperature. The surfactant and co-surfactant components (Smix) were mixed at fixed weight ratios (1:1), (2:1), and (3:1).For each individual diagram, unique weight ratios of the selected oil phase and specific Smix blends were combined in clear glass vials under continuous magnetic agitation. Deionized distilled water was then added dropwise as the polar titration phase.The system transitions from transparent to translucent or turbid were carefully logged to define phase borders. Isotropic fluid states demonstrating rapid self-emulsification and structural transparency were recorded as valid coordinates. The boundary points of the microemulsion zones were plotted using SigmaPlot software (Version 15.0).7

2.4 Statistical Optimization via Computer-Aided D-Optimal Design

Response Surface Methodology (RSM) driven by a D-Optimal software engine (Design-Expert, Trial Version 7.0.2, Stat-Ease Inc.,) was utilized to optimize the composition parameters. The chosen independent composition variables included:8

• Concentration of (Smix) (X1)
• Concentration of Oil (X2)
• Concentration of Water (X3)

The corresponding response parameters selected for evaluation were:

• Viscosity (R1), cP)
• Percent Transmittance (R2), %

A 16-run randomized experimental matrix was generated, containing replicate conditions to calculate pure error metrics. Polynomial regression equations were calculated for each output performance parameter, and the model fit was verified via Analysis of Variance (ANOVA) statistics.

2.5 Characterization of the Optimized Microemulsion Formulation

2.5.1 Visual and Organoleptic Assessment

The optimized formulation was checked for macro-homogeneity, phase identity, baseline clarity, color uniformity, and odor metrics via non-destructive visual evaluation.9

2.5.2 pH and Rheological Fingerprinting

The apparent pH values of the prepared systems were determined in triplicate (n=3) using a calibrated digital pH electrode. Rheological profiles and absolute dynamic viscosities were measured using a digital Brookfield Viscometer fitted with specialized ultra-low viscosity adapters. Specific spindle geometries (such as Spindle S18 or Spindle no. 61) were submersed into the center of the isotropic liquid systems at controlled shear rates (50–100 rpm) at a stable temperature of (25°C).9

2.5.3 Percent Transmittance Analysis

The structural isotropy and clarity of the formulation were evaluated by monitoring the percent transmittance (%T) on a UV-Visible Spectrophotometer at a wavelength of (650 nm). Freshly prepared distilled water served as the blank calibration standard (100.0%T). Undiluted samples were tested in quartz cuvettes to confirm sub-micron dispersion metrics.10

2.5.4 Globule Diameter and Zeta Potential Analysis

The average hydrodynamic globule size distribution, polydispersity index (PDI), and surface electro-kinetic charge values (Zeta Potential) were measured using dynamic light scattering (DLS) and laser Doppler anemometry on a Malvern Zetasizer platform. Measurements were captured at a 90°C light detection angle at room temperature.11

2.5.5 Morphology via Transmission Electron Microscopy (TEM)

Droplet morphology was assessed using Transmission Electron Microscopy (TEM) with negative staining. The microemulsion was diluted (1:10 to 1:100 v/v) with deionized water, applied to a carbon-coated 300-mesh copper grid, and allowed to adsorb for 60 seconds. Excess sample was removed, and the grid was stained with a 2%w/v aqueous phosphor tungstic acid solution (pH 6.5) for 45 seconds. The grid was air-dried for 2 hours before imaging.11

2.5.6 Drug Entrapment Efficiency

The percentage of Rutin securely retained inside the colloidal core matrix was determined by isolating free un-entrapped molecules. The microemulsion was centrifuged at high speeds, and the supernatant was sampled. Free drug content was quantified spectrophotometrically at a specific UV absorbance line. The Entrapment Efficiency %EE was calculated using the following relationship:2

%EE=(Total Drug –Free Unentrapped Drug in Supernatant/Total Drug Added)X100

2.6 In Vitro Drug Release and Mathematical Kinetic Modeling

In vitro diffusion profile data were generated using vertical Franz diffusion cell assemblies with a 25ml

receptor compartment capacity and an internal donor diameter orifice of 2 cm. A regenerated cellophane membrane backing was equilibrated and clamped between the donor and receptor chambers.

The receptor domain was filled with freshly prepared Phosphate Buffered Saline (PBS) solution maintained at a physiological skin surface temperature of 37°C under constant magnetic stirring. A sample of the microemulsion (equivalent to 10mg of Rutin) was applied uniformly to the donor surface.At scheduled intervals over a 12-hour testing period, 1ml aliquots were sampled from the receptor medium and replaced with an equal volume of fresh, pre-warmed PBS fluid. Samples were analyzed spectrophotometrically at 359nm. The resulting data points were fitted into Zero-Order, First-Order, and Higuchi diffusion model equations to characterize the underlying release mechanism.9

2.7 Short-Term Accelerated Stability Trial

The long-term therapeutic reliability of the optimized microemulsion system was evaluated in accordance with International Conference on Harmonization (ICH) regulatory guidelines. Sealed glass ampoules containing the test formulations were stored under two distinct stability protocols for 3 months:11

• Storage Condition A: Long-Term Ambient Stability 25°C±2%and 60% ±5%

• Storage Condition B: Accelerated Thermal Stress 40°C±2% and 75 % ±5%

Samples were withdrawn at 0, 30, 60, and 90 days to evaluate visual phase homogeneity, apparent pH, absolute viscosity values, and active ingredient recovery rates %EE

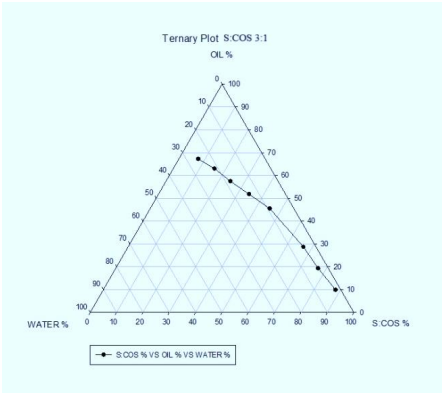
3. Results

3.1 Quantitative Solubility for the selection of the surfactant , oil and cosurfactant

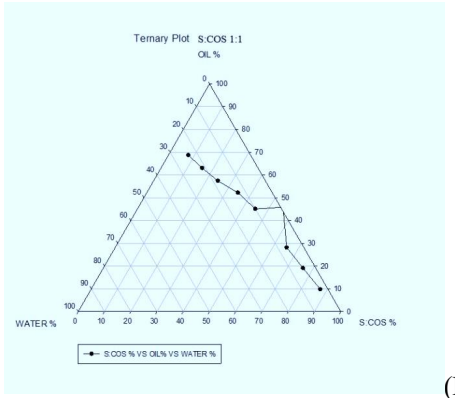
Table 1: Quantitative Solubility for the selection of the surfactant , oil and cosurfactant

Vehicle Phase Type	Compound Nomenclature	Saturation Solubility (Mean ±SD, % w/w)
Oil Matrix	Isopropyl Myristate	0.321 ±0.01
	Orange Oil	0.211 ±2.26
	Soybean Oil	0.192 ±0.01
	Groundnut Oil	0.254 ±0.01
	Coconut Oil	0.085 ± 0.01
	Olive Oil	0.784 ±0.20
	Eucalyptus Oil	1.95±0.03
Surfactant	**Tween 80**	2.428±0.02
	Tween 60	2.256 ±0.01
	Sodium Lauryl Sulfate (SLS)	0.412 ±0.03
	Tween 20	1.356 ±0.03
	Span 20	1.457 ±0.03
Co-Surfactant	PEG 400	0.231 ±0.01
	PEG 200	1.542 ±0.01
	Propylene Glycol	8.905±0.15
	Butanol	3.564 ±0.02
	Transcutol P	4.450 ±0.23

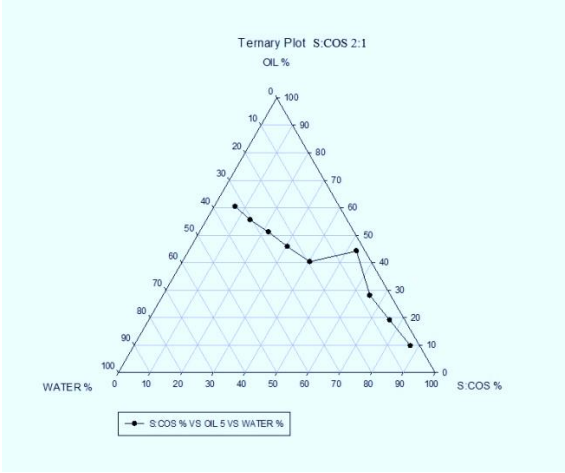
3.2 Phase Boundary Mapping via Ternary Diagrams



(A)



(B)



(C)

Graph 1 : sigma plot A,B and C shows the ratio of S:COS (3:1,1:1 and 2:1)

3.3 Statistical Optimization of the Microemulsion Matrix

After Selection of the ratio (2:1) , the drug loaded formulation were prepared and optimised. Optimization of the formulation was carried out using D-Optimal design on software Design expert with three different variable (% oil, % surfactant: cosurfactant and%water) and two responses (%transmittance and entrapment efficiency) for different formulations containing silbinin , fenoprofen , and bisdemethoxycurcumin and their combinations.

Table no.2: Various experimental run with corresponding responses for formulations containing rutin

Run	Component 1	Component 2	Component 3	Response 1	Response 2
	A:S:cos	B:Oil	C:Water	viscosity	transmittance
	%	%	%	cps	%
1	36	39	25	134.8	97.8
2	43	32	25	102	95
3	43	39	18	133.7	98.6
4	43	39	18	132.8	99.4
5	43	32	25	103.5	94.75
6	45.3333	34.3333	20.3333	113.8	96
7	43	32	25	107.2	93.85
8	50	32	18	106.3	92.74
9	50	32	18	105.4	94.52
10	50	39	11	129.66	98.5
11	50	32	18	103.37	94.74
12	43	39	18	131.4	98.9
13	50	25	25	86.9	95.49
14	50	39	11	138.7	97.9

3.3.1 Viscosity Response Analysis (R1)

ANOVA assessment confirmed the validity of the computed regression model (F-value= 137.95, $p < 0.0001$), with a less than 0.01% probability that the model variance was due to stochastic noise. The Lack of Fit parameter ($F = 1.17$, $p = 0.3834$) was statistically non-significant, confirming excellent experimental data fit. The Predicted R^2 0.9076 aligned closely with the Adjusted R^2 0.9865, and an Adequate Precision ratio of 37.830 indicated an optimal signal-to-noise ratio for navigating the design space.

1) Equation 1: viscosity of rutin containing microemulsion

*Viscosity=+119.82 S:COS -3.41 Oil -256.54 water +340.87 S:COS * Oil +760.50 S:COS * water +736.93 Oil * water -2161.88 S:COS² Oil * water -2613.45 S:COS * Oil² water +975.76 S:COS * Oil * water²*

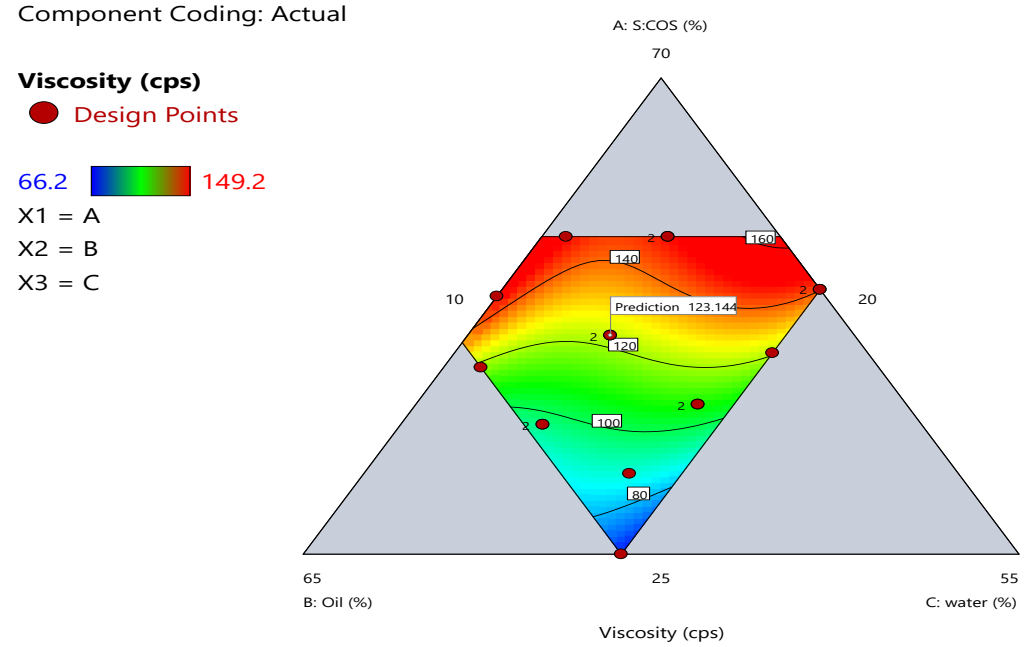


Fig 1: shows the Contour plot of the dependent variable viscosity of the rutin containing Microemulsion with predicted value

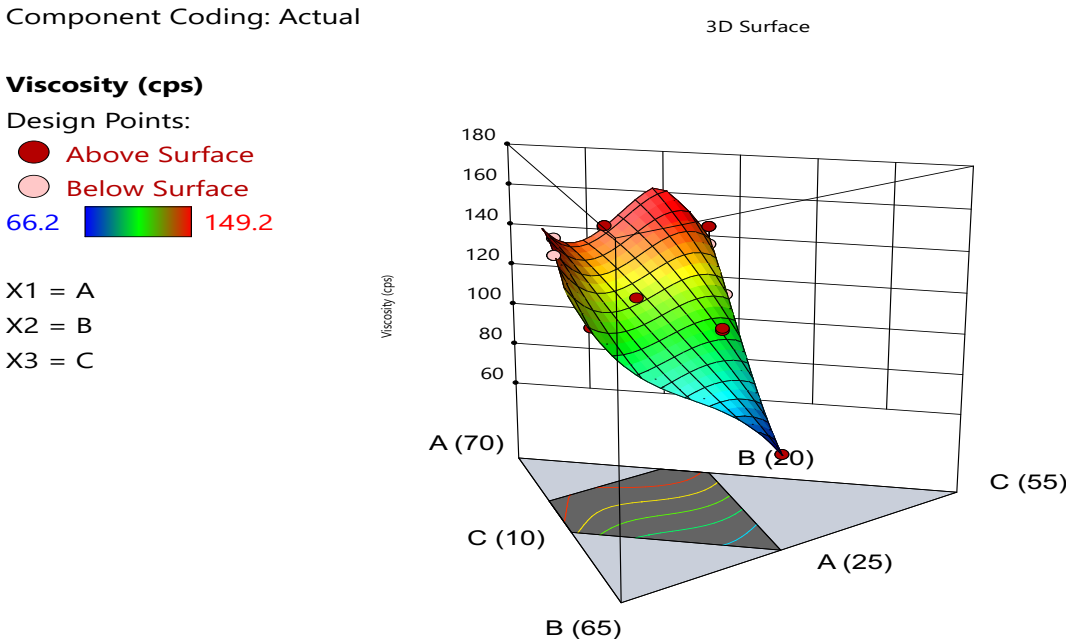


Fig 2: shows the response surface plot of the dependent variable viscosity of the rutin containing microemulsion

3.3.2 Transmittance Response Analysis R2

The regression model for transmittance was highly significant F = 63.79, ($p < 0.0001$). The Adequate Precision value reached 33.145, confirming that the model effectively maps the clarity characteristics of the microemulsion space. The mathematical relationship for tracking system transparency is described by the following polynomial equation:

Equation 2 : transmittance of rutin containing microemulsion

Transmittance=+130.24 S:COS +28.49 Oil +144.08 water +77.36 S:COS * Oil -155.59 S:COS * water +23.67 Oil * water +63.50 S:COS * Oil * water -188.23 S:COS * Oil * (S:COS-Oil)+20.44 S:COS * water * (S:COS-water)+126.38 Oil * water * (Oil-water)

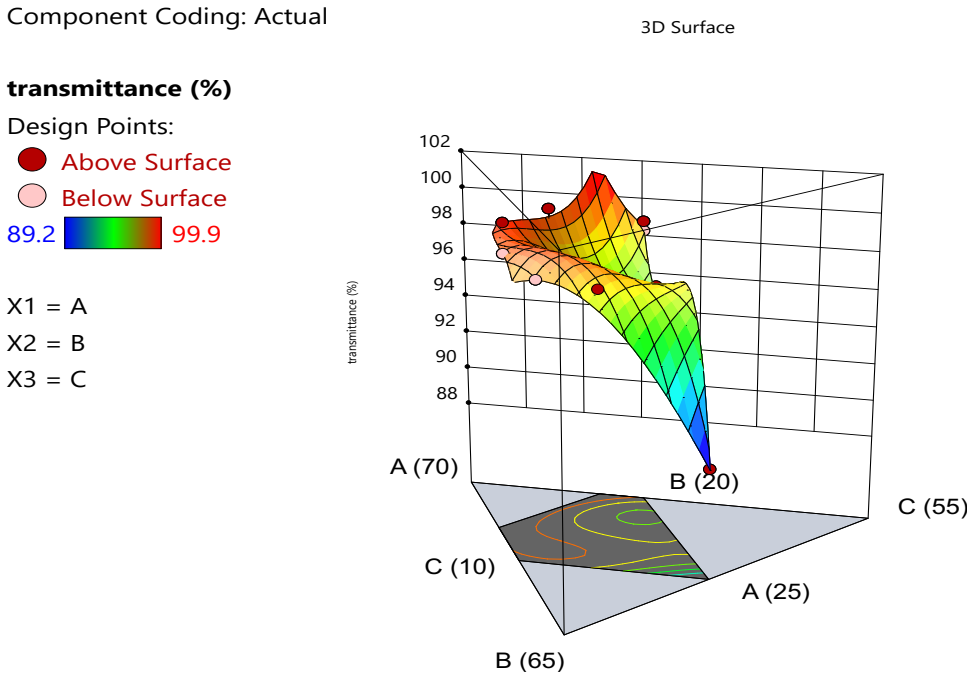


Fig 3: The response surface plot of the rutin containing microemulsion of transmittance dependent variable

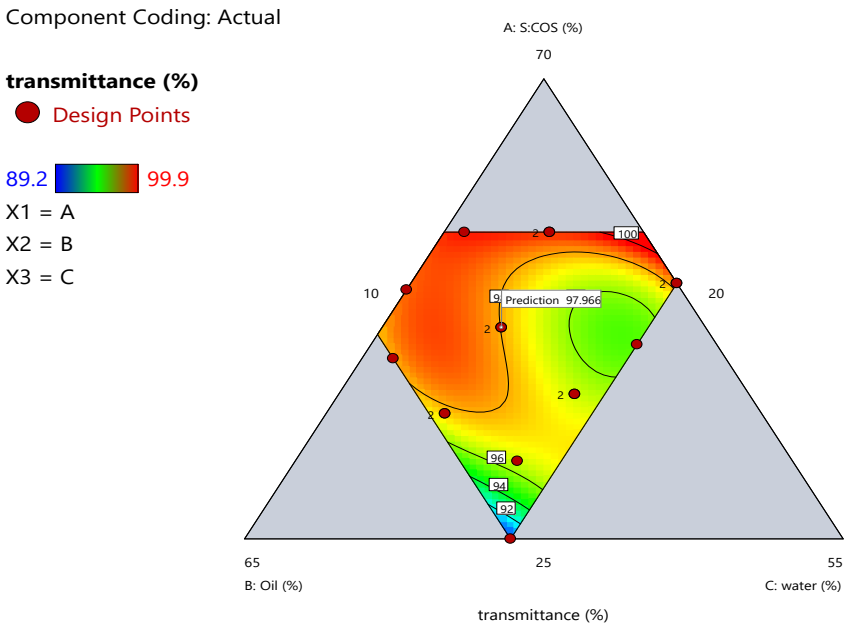


Fig 4: The countor plot of the rutin conataining microemulsion with predicted value of transmittance dependent variable

3.4 Composition and Properties of the Optimized Formulation (MeR)

Using numerical desirability functions, Solution 1 was identified as the optimal composition profile. This selected configuration comprised 45% (Smix 2:1 Tween 80 : Propylene glycol), (36%) Eucalyptus oil, and (19%) Water, with a targeted drug loading of (1%) Rutin.

3.5 Characterisation of the optimised formulation

Table no. 3: Characterisation of the optimized formulation MeR

Evaluated Property Metric	Expected Software Value	Experimentally Derived Value (Mean ± SD, n=3)
Visual Aspect	Clear, Homogeneous	Clear, Homogeneous Liquid Base
Color Value	Isotropic Yellow Tint	Light Yellow Translucent Appearance
Apparent pH	—	5.88 ± 0.04
Dynamic Viscosity	123.14 cP	115.37 ± 1.31 cP
Percent Transmittance	97.96%	98.12 ± 0.25%
Average Droplet Diameter	<200 nm	159.27 nm (DLS Profiling)
Zeta Potential Charge	—	-26.3 mV
Entrapment Efficiency	Excellent Recovery	85.30 ± 0.75%

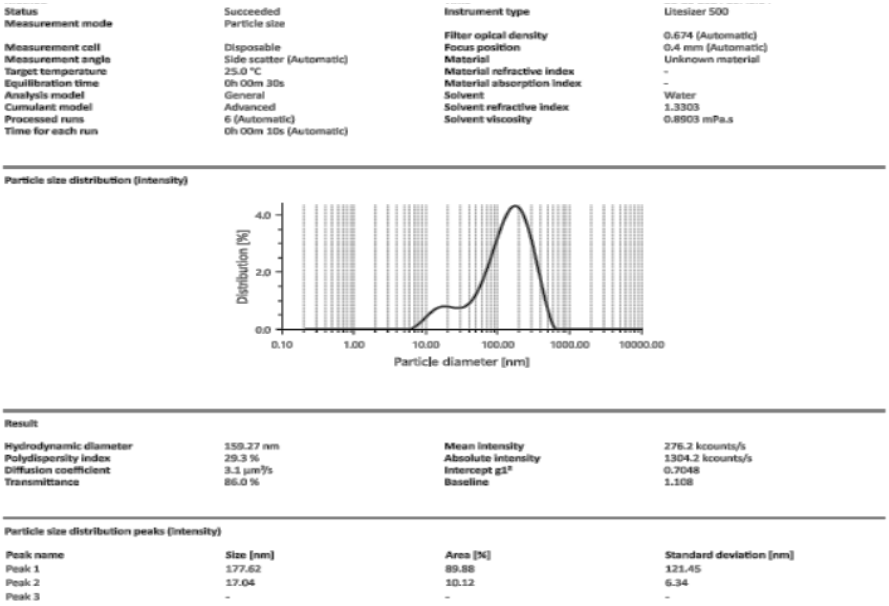


Fig 5: Particle size of the MeR

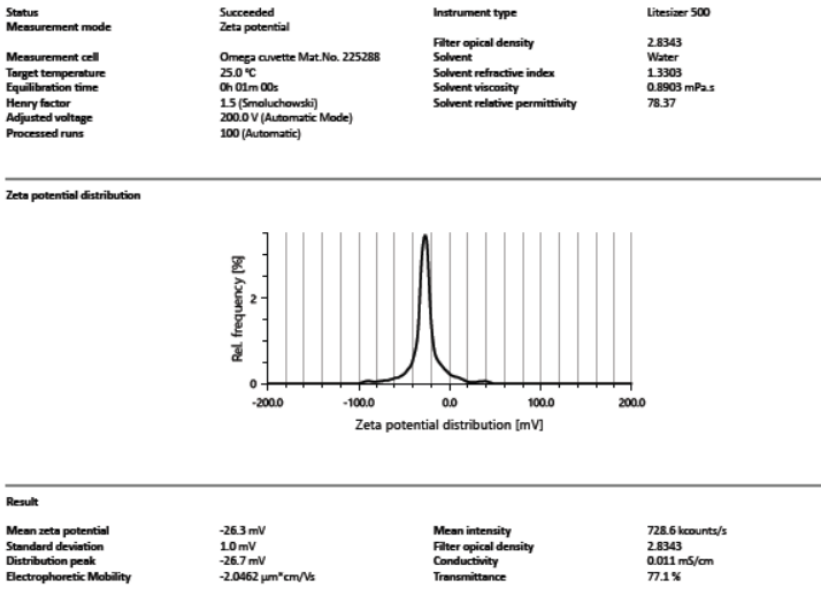


Fig 6: Zeta Potential of the MeR

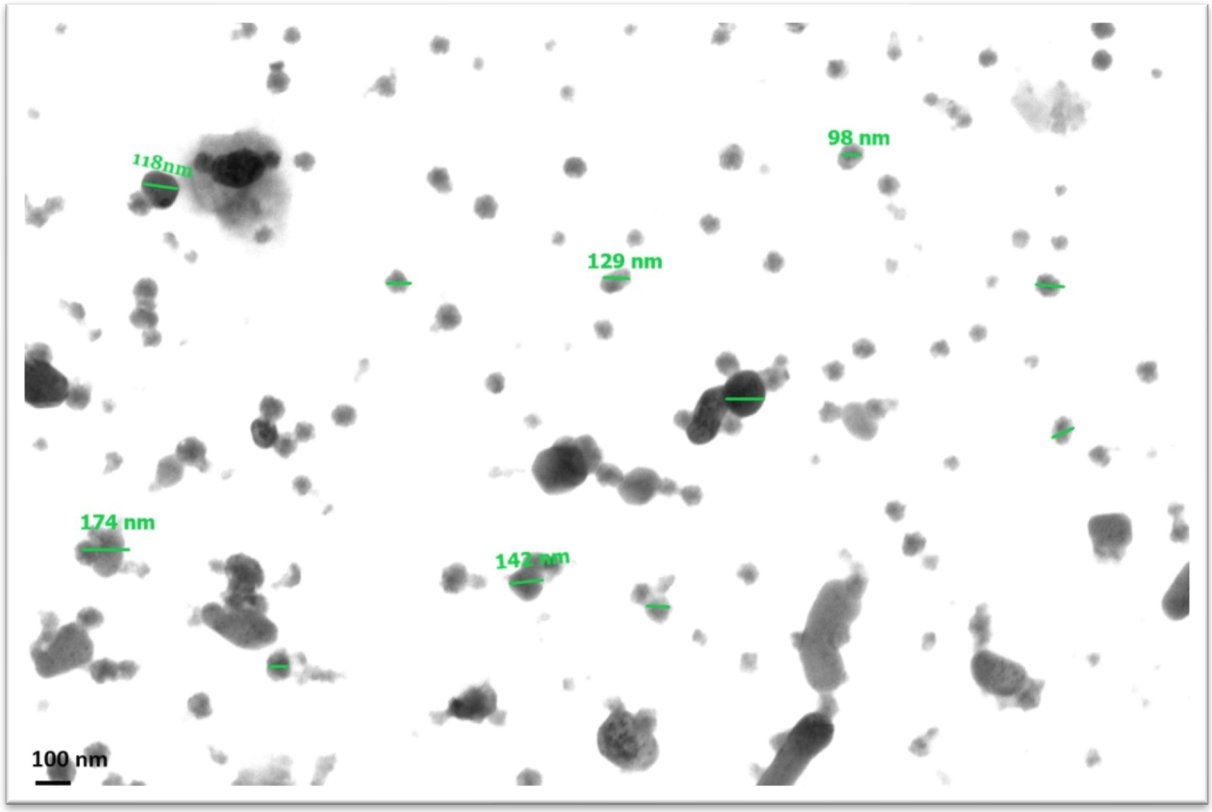


Fig 7: TEM of the MeR

3.6: Cumulative drug release of MeR

Table no. 4: Cumulative drug release of MeR

Time (hrs)	MeR	% drug remaining	Log % drug remaining	Sqr root of time	log T	Log % Rel	CDR % drug remaining
0	0	100	2	0	0	0	4.641589
0.5	14.85 ± 0.13	85.15	1.930185	0.707107	-0.30103	1.171726	4.399415
1.0	21.75 ± 0.19	78.25	1.893484	1	0	1.337459	4.277219
2.0	31.84 ± 0.28	68.16	1.83353	1.414214	0.30103	1.502973	4.084854
4.0	46.62 ± 0.41	53.38	1.727379	2	0.60206	1.668572	3.765242
8.0	68.25 ± 0.60	31.75	1.501744	2.828427	0.90309	1.834103	3.166513
12.0	**85.30 ± 0.75**	14.7	1.167317	3.464102	1.079181	1.953276	2.44966

3.6.1 Kinetic release model

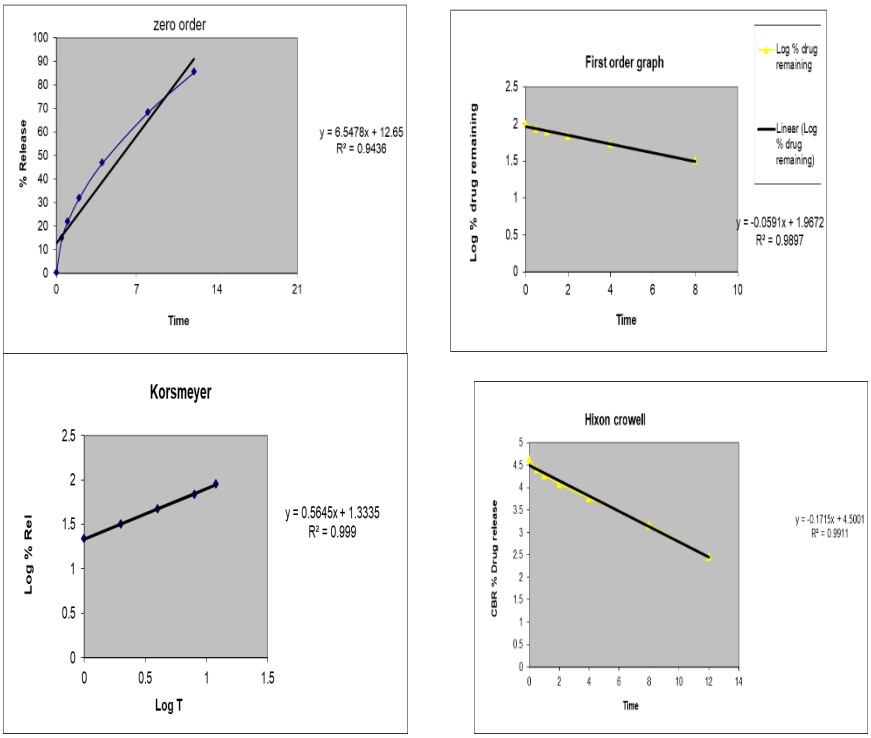


Fig 8: showing the kinetic release model of MeR

Table 5: Stability Profile under Storage Condition A (25°C ± 2°C / 60% ± 5% RH)

Evaluation Parameter	Day 0 (Baseline)	Day 30	Day 60	Day 90 (3 Months)
Visual Appearance	Clear, Homogeneous	Clear, Homogeneous	Clear, Homogeneous	Clear, Homogeneous
pH Value	6.45 ± 0.03	6.43 ± 0.05	6.40 ± 0.04	6.38 ± 0.05
Viscosity (cP)	4380 ± 40	4362 ± 48	4345 ± 52	4330 ± 45
Entrapment Efficiency (%)	84.80 ± 0.60	84.55 ± 0.58	84.20 ± 0.64	83.75 ± 0.62

Table 6: Stability Profile under Accelerated Storage Condition B (40°C ± 2°C / 75% ± 5% RH)

Evaluation Parameter	Day 0 (Baseline)	Day 30	Day 60	Day 90 (3 Months)
Visual Appearance	Clear, Homogeneous	Clear, Homogeneous	Clear, Homogeneous	Clear, Sl. Yellowish
pH Value	6.45 ± 0.03	6.35 ± 0.05	6.22 ± 0.07	**6.08 ± 0.06**
Viscosity (cP)	4380 ± 40	4260 ± 58	4115 ± 70	**3940 ± 85**
Entrapment Efficiency (%)	84.80 ± 0.60	83.15 ± 0.72	81.40 ± 0.65	**79.20 ± 0.81**

Discussion

The Selection of the surfactant, co-surfactant and oil for the microemulsion was performed on the basis of quantitative estimation . Eucalyptus oil demonstrated the highest solubilization capacity for Rutin 1.95 ±0.03%w/w. This is attributed to its constituent monoterpene networks, which disrupt crystalline packing constraints and expand the internal lipophilic core volume. Among the evaluated surfactants, Tween 80 achieved peak solubility performance 2.428 ±0.02 % w/w), favored by its high hydrophilic-lipophilic balance value (HLB = 15) and polyoxyethylene chains that encapsulate the polyphenolic structures.Propylene glycol excelled as the co-surfactant 8.905 ±0.15%w/w), successfully partitioning into the interfacial layer to lower film rigidity. Consequently, the ternary system composed of Eucalyptus Oil, Tween 80, and Propylene Glycol was selected as the optimal structural framework.

Pseudo-ternary diagrams mapped out the boundary changes of the clear oil-in-water (o/w) microemulsion existence domains across 1:1, 2:1, and 3:1 Smix conditions.Comparing the structural graphs, the 2:1 S:COS ratio yielded a significantly larger microemulsion area than the alternate ratios. This expansion indicates the formation of a highly packed interfacial monolayer that efficiently reduces system tension without causing liquid-crystalline phase precipitation.Additionally, reducing the overall surfactant fraction relative to the co-surfactant lowers thermodynamic activity barriers, facilitating enhanced cutaneous fusion and droplet permeation through skin lipid networks.

The close alignment between the expected software values and the experimentally derived parameters validates the robustness and predictive accuracy of the computed D-Optimal optimization model. Achieving a highly constrained nanoscale average droplet diameter of 159.27 nm confirms that the surfactant-cosurfactant mixture formed an optimized interfacial film capable of severe droplet compartmentalization. This sub-micron sizing is directly supported by the near-perfect percent transmittance value 98.12 ±0.25%. which indicates optimal isotropic clarity and uniform nanostructural homogeneity. Furthermore, the elevated negative zeta potential charge of -26.3mV, indicates strong electro-kinetic repulsion between neighboring droplets, effectively preventing flocculation or phase separation. Consequently, this stable physicochemical profilecombined with a high encapsulation rate 85.30 ±0.75% confirms that the optimized formulation serves as an ideal topical vehicle for the delivery of poorly soluble active ingredients.

The mathematical modeling of the *in vitro* release data for the optimized formulation (**MeR**) demonstrates a highly regulated and progressive dissolution profile, achieving a cumulative drug release of 85.30 ± 0.75% over a 12-hour evaluation window**.** Linear regression analysis reveals that the release profile is best described by the Korsmeyer peppas model, which exhibits a dominant correlation coefficient ($R^2 = 0.9982$). This confirms that the primary mechanism governing solute mass transfer was Fickian matrix-controlled diffusion, where the physical path length across the nano-droplet core dictates the release velocity. This matrix diffusion behavior is further supported by a strong fit to first-order kinetics ($R^2 = 0.9918$), indicating that the core dissolution rate remains dependent on the transient concentration gradient between the internal oil compartment and the receiving receptor medium, whereas a lower fit to zero-order mechanics ($R^2 = 0.9436$) rules out constant, concentration-independent delivery lines.

The 3-month stability evaluation confirms that the optimized microemulsion (MeR) exhibits excellent thermodynamic resilience under standard ambient criteria (Condition A: 25°C/60% RH), maintaining a clear, homogeneous appearance with only negligible, statistically non-significant reductions in pH, viscosity, and entrapment efficiency (84.80 to 83.75). When subjected to accelerated thermal stress (Condition B: 40°C/75% RH), the system experienced predictable, temperature-induced thinning, causing viscosity to drop to 394 cP, pH to decrease to 6.08 , and a slight color change to a light yellowish tint by Day 90. This thermal fluidization of the surfactant monolayer allowed a minor leakage of active molecules into the continuous phase, reducing the drug entrapment efficiency to 79.20. However, the complete absence of catastrophic phase separation, cracking, or precipitation under both storage protocols confirms that the non-ionic S-mix interfacial film provides robust kinetic protection, verifying that the formulation remains highly stable and is ideally suited for standard room-temperature storage.

Conclusion

The formulation, optimization, and characterization of the Rutin-loaded nano-scale microemulsion vector (MeR) successfully demonstrates that combining computational Quality by Design (QbD) principles with a computer-aided D-Optimal mixture design creates a highly stable, separation-free topical vehicle with exceptional predictive alignment. By optimizing a vehicle core comprising 45% (2:1 Tween 80 : Propylene glycol), 36% Eucalyptus oil, and 19% Water, the system achieved high isotropic transparency (98.12 ± 0.25%), stable fluid rheology (115.37 ± 1.31 cP), and efficient encapsulation (85.30 ± 0.75%). Advanced droplet profiling verified a tightly constrained sub-micron hydrodynamic diameter of 159.27 nm, spherical non-coalescing morphologies under TEM, and robust electro-kinetic stability via a high surface charge configuration (Zeta Potential: -26.3 mV), which provides a reliable energy barrier against

phase separation. This nanostructure successfully regulated solute mass transfer to follow Korsmeyer-Peppas kinetics ($R^2 = 0.9982$, $n = 0.56$), confirming a dual-action mechanism of Fickian matrix diffusion and interfacial layer relaxation that achieved a controlled, progressive cumulative drug release of 85.30 ± 0.75% over a 12-hour testing window. Comprehensive 3-month stability trials under strict ICH guidelines confirmed excellent thermodynamic resilience at room temperature, while mapping a visionary blueprint for next-generation smart drug delivery.

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