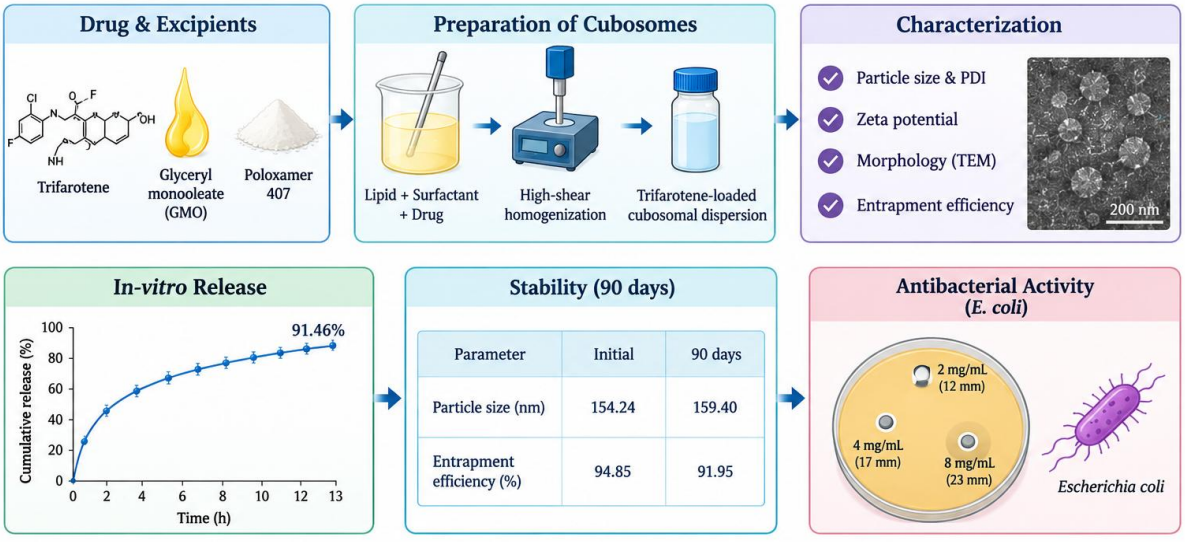
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Research Article

Preparation, Characterization and Preliminary Antibacterial Evaluation of Trifarotene-Loaded Cubosomes

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Abstract
Trifarotene is a topical retinoid used in acne management. Its limited aqueous solubility provides a formulation rationale for investigating lipid-based nanocarriers. This study reports the preparation and physicochemical characterization of trifarotene-loaded cubosomal dispersions and summarizes the available in-vitro release, storage-stability and preliminary antibacterial data. Five formulations (CSF1-CSF5) were prepared using trifarotene, glyceryl monooleate and Poloxamer 407. Reported particle sizes ranged from 154.24 to 262.20 nm, and entrapment efficiency ranged from 71.24% to 94.85%. CSF1 had the smallest reported particle size (154.24 nm), the most negative zeta potential (-29.9 mV) and the highest entrapment efficiency (94.85%). Cumulative release from the reported optimized formulation reached 91.46% at 13 h. Among the reported kinetic fits, the first-order model had the highest coefficient of determination (R^2 = 0.937), although the difference from the zero-order fit (R^2 = 0.923) was modest. Over 90 days at 25 ± 2°C/60 ± 5% relative humidity, particle size increased to 159.40 nm and entrapment efficiency decreased to 91.95%. Agar-well diffusion data showed inhibition zones of 12, 17 and 23 mm at 2, 4 and 8 mg/mL, respectively, against Escherichia coli; the blank formulation produced no reported inhibition. These findings are descriptive and require confirmation because replicate-level variability, microbiological controls and the tested batch identity are incompletely documented. The data support further systematic evaluation of trifarotene-loaded cubosomes but do not establish clinical efficacy or comparative antibacterial benefit.



Graphical representation of the preparation and characterization of trifarotene-loaded cubosomes, including particle size, PDI, zeta potential, morphology, and entrapment efficiency.

Keywords: Trifarotene, Cubosomes, Glyceryl Monooleate, Poloxamer 407, Nanoparticle Characterization, Entrapment Efficiency, In-Vitro Release, Escherichia Coli.

INTRODUCTION

Topical delivery is an important route for treating dermatological disorders because it

can provide drug exposure at the application site while potentially limiting systemic exposure. Performance depends on the physicochemical properties of the active ingredient and the formulation, including solubility, partitioning into skin, release rate, vehicle characteristics and stability [1-5]. Trifarotene is a selective retinoic acid receptor-γ agonist used in the topical treatment of acne vulgaris. Like other lipophilic compounds, its poor aqueous solubility can complicate formulation development and motivates investigation of carriers that can incorporate hydrophobic molecules [6-9].

Cubosomes are colloidal particles derived from bicontinuous cubic liquid-crystalline phases formed by the self-assembly of amphiphilic lipids in water. Their internal organization comprises continuous aqueous domains separated by a lipid bilayer, producing a large interfacial area and potential capacity to accommodate compounds with different physicochemical characteristics. Glyceryl monooleate (GMO) is a commonly used cubic-phase-forming lipid, whereas Poloxamer 407 can stabilize the dispersion and reduce aggregation. The properties of a cubosomal system are influenced by lipid-to-stabilizer ratio, processing conditions, drug-excipient interactions and storage environment [10-15].

Characterization of a candidate formulation requires more than confirmation of nanoscale size. Particle-size distribution, polydispersity, surface charge, morphology, entrapment efficiency, release performance and stability should be considered together. In-vitro release models can provide a descriptive comparison of profiles, but a high coefficient of determination alone does not prove a particular molecular release mechanism. Similarly, antibacterial screening should be interpreted in the context of validated microbiological methods and suitable controls, particularly when the active ingredient is developed primarily for dermatological use [16-18].

This study aimed to prepare five trifarotene-loaded cubosomal formulations using different amounts of GMO and Poloxamer 407, characterize selected physicochemical properties, examine in-vitro release and 90-day storage changes, and report preliminary agar-well diffusion observations against E. coli. Numerical results are presented as recorded in the available experimental dataset; missing replicate data and inconsistencies are identified rather than inferred.

MATERIALS AND METHODS

Materials

Trifarotene was obtained from Sun Pharmaceutical Industries Ltd. (India). Glyceryl monooleate was sourced from Sigma-Aldrich (USA), and Poloxamer 407 from BASF Corporation (Germany). Other materials included propylene glycol, ethanol, chloroform, methanol, phosphate-buffered saline (PBS), distilled water and nutrient agar. Material grades, catalogue numbers and lot numbers should be reported from laboratory records. Dimethyl sulfoxide (DMSO) was listed in the formulation composition; its amount and role require verification because the reported DMSO volume and final batch volume appear internally inconsistent.

Preparation of Trifarotene-Loaded Cubosomes

Five formulations (CSF1-CSF5) were reported (table1). Trifarotene was incorporated into the lipid phase containing GMO, while Poloxamer 407 was dissolved in the aqueous phase. The aqueous phase was added gradually to the lipid phase under continuous stirring to form a coarse dispersion. The dispersion was subsequently homogenized at high speed, cooled and allowed to equilibrate at room temperature. The supplied records do not consistently specify homogenization speed, duration, temperature, equipment model or a reproducible endpoint; these parameters must be completed from laboratory notebooks before submission.

Table 1. Reported Composition of Trifarotene-Loaded Cubosomal Formulations.

Formulation	Trifarotene (mg)	GMO (g)	Poloxamer 407 (%)	DMSO (mL)	Water
CSF1	10	1.0	1.0	10	q.s. to 10 mL
CSF2	10	1.2	1.0	10	q.s. to 10 mL
CSF3	10	1.5	1.5	10	q.s. to 10 mL
CSF4	10	1.8	1.5	10	q.s. to 10 mL
CSF5	10	2.0	2.0	10	q.s. to 10 mL

Preformulation and Drug Identification

The study records organoleptic assessment, solubility screening, melting-point measurement, pH, UV-visible absorption maximum. Trifarotene was described as a white-to-off-white crystalline powder with a characteristic odour and slight hygroscopicity. Solubility observations were qualitative. The reported UV-visible absorption maximum was 306 nm. FTIR peak positions were transcribed from the study; the functional-group assignments should be checked against a verified trifarotene reference spectrum and its molecular structure before publication.

Physicochemical Characterization

Particle size was assessed by dynamic light scattering after dilution, and zeta potential was measured as an indicator of surface charge. The study provides mean particle-size and zeta-potential values for the five batches but does not provide polydispersity index values or replicate-level measurements in the manuscript tables. Surface morphology was examined by scanning electron microscopy (SEM). Entrapment efficiency was reported as a percentage; the full separation procedure, analytical wavelength, calibration range and calculation equation should be stated in the final submission based on original records.

In-Vitro Drug Release and Kinetic Analysis

Drug release was assessed using a diffusion-cell method, with cumulative release recorded at the reported sampling times. The cumulative values were fitted to zero-order, first-order, Higuchi and Korsmeyer-Peppas models. The reported coefficient of determination (R^2) values are presented descriptively. The membrane type, receptor medium and volume, temperature, agitation rate, sampling/replacement procedure, sink-condition assessment and number of replicates were not fully documented in the supplied manuscript and should be added from laboratory records.

Stability Study

The optimized formulation was monitored on days 0, 30, 45, 60 and 90 at 25 ± 2°C/60 ± 5% relative humidity and 40 ± 2°C/70 ± 5% relative humidity. Particle size and entrapment efficiency were recorded. The accelerated-condition humidity should be verified against the stability-chamber log because it is not reported consistently in the available records.

Preliminary Antibacterial Evaluation

Agar-well diffusion screening against Escherichia coli was conducted using formulation samples at 2, 4 and 8 mg/mL, alongside a blank formulation. Zones of inhibition were recorded in millimetres. Although the experiment was described as being performed in triplicate, replicate-level measurements, strain identifier, inoculum standardization, incubation details and a positive antibiotic control were not available. These details are essential for reproducible microbiological reporting.

RESULTS

Formulation Appearance and Drug Characterization

The drug was described as a white-to-off-white crystalline powder with a characteristic odour and slight hygroscopicity. The qualitative solubility profile showed practical insolubility in water and PBS (pH 7.4), with higher apparent solubility in chloroform and DMSO (table2). The reported melting point was 243.21°C compared with a cited reference value of 245°C, and the measured pH was 5.47. The UV-visible absorption maximum was reported at 306 nm. These observations are useful for initial characterization, but the source does not provide replicate measurements or sufficient analytical details to establish purity or validate a quantitative assay.

Table 2. Summary of Reported Preformulation Observations.

Test	Reported observation	Interpretive note
Appearance	White to off-white crystalline powder	Qualitative observation
Solubility in water and PBS (pH 7.4)	Practically insoluble	Qualitative; no equilibrium solubility values provided
Ethanol	Slightly soluble	Qualitative
Methanol	Slightly soluble to soluble	Qualitative
Chloroform and DMSO	Freely soluble	Qualitative
Acetone	Soluble	Qualitative

Melting point	243.21°C; reference listed as 245°C	Method and replicate variability not reported
pH	5.47	Measurement procedure and sample preparation need specification
UV-visible λmax	306.0 nm	Reported analytical wavelength

Particle Size and Zeta Potential

Reported particle sizes ranged from 154.24 to 262.20 nm. CSF1 had the smallest size (154.24 nm), followed by CSF2 (161.07 nm), CSF3 (166.68 nm), CSF5 (173.19 nm) and CSF4 (262.20 nm) fig 1. Zeta potential ranged from -14.0 to -29.9 mV. CSF1 had the most negative reported value (-29.9 mV), while CSF4 had the least negative value (-14.0 mV) fig 2. Because polydispersity and replicate variability were not supplied, the uniformity of each dispersion cannot be compared comprehensively.

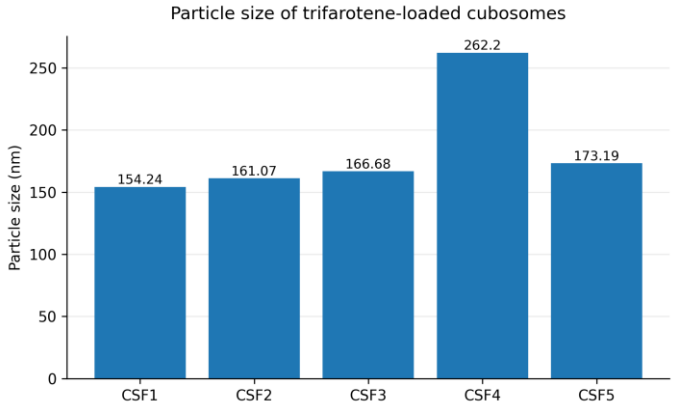


Figure 1. Particle Size of Trifarotene-Loaded Cubosomal Formulations (CSF1-CSF5).

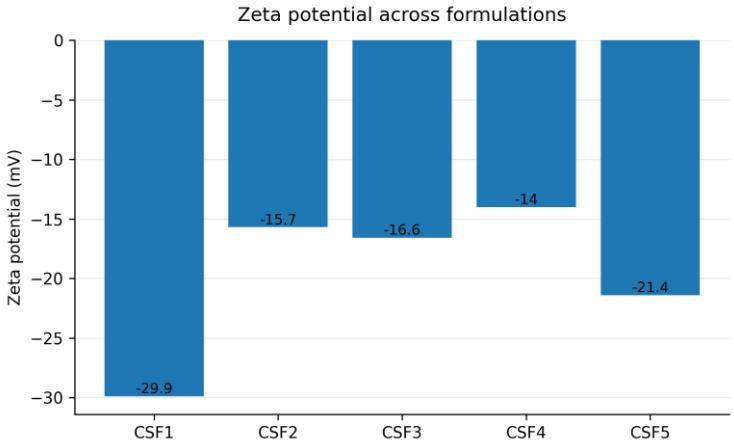


Figure 2. Zeta Potential of Trifarotene-Loaded Cubosomal Formulations. Negative Values Indicate Negatively Charged Particle Surfaces

Entrapment Efficiency

Entrapment efficiency ranged from 71.24% to 94.85%. CSF1 showed the highest reported value (94.85%), followed by CSF2 (88.42%), CSF3 (82.16%), CSF4 (76.38%) and CSF5 (71.24%) fig 3. Thus, CSF1 was the strongest candidate when particle size, zeta potential and entrapment efficiency were considered together in the available dataset. The mechanism underlying the batch differences cannot be established from the supplied measurements alone.

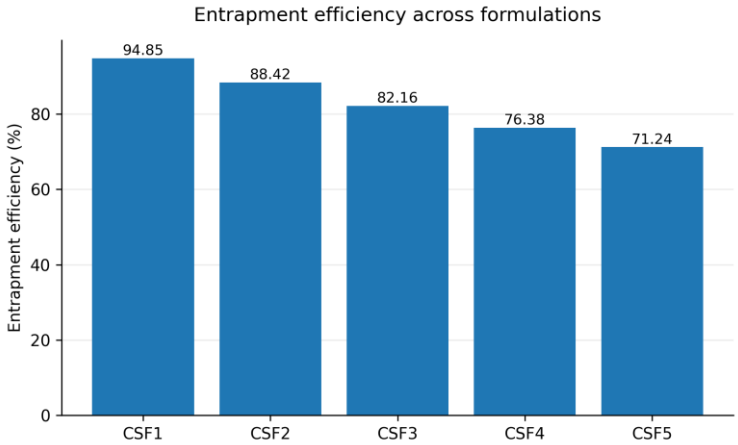


Figure 3. Entrapment Efficiency for Formulations CSF1-CSF5.

Surface Morphology

The study includes an SEM micrograph labelled as the cubosome formulation fig 4. The image is consistent with the presence of particulate material, but morphology alone cannot confirm an internal bicontinuous cubic phase. Scale-bar calibration, imaging conditions and representative fields should be documented. Techniques such as small-angle X-ray scattering or cryogenic transmission electron microscopy would be needed to establish internal cubic-phase structure more directly.

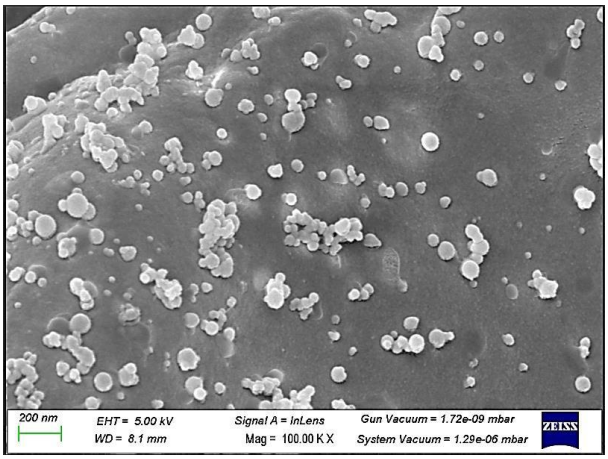


Figure 4. Scanning Electron Micrograph Cubosome Formulation.

In-Vitro Drug Release and Kinetic Fitting

Cumulative drug release increased over the reported interval, from 22.23% at 1 h to 91.46% at 13 h. Intermediate values were 36.24% at 2 h, 47.32% at 4 h, 55.29% at 6 h, 64.35% at 8 h, 73.39% at 10 h and 82.43% at 12 h (table 3). The release profile indicates progressive drug release under the specific in-vitro test conditions; it does not, by itself, establish skin permeation or in-vivo performance fig 5 and table 4.

Table 3. Reported Cumulative in-Vitro Drug Release.

Time (h)	Cumulative drug released (%)	Drug remaining (%)
0	0.00	100.00
1	22.23	77.77
2	36.24	63.76
4	47.32	52.68
6	55.29	44.71
8	64.35	35.65

10	73.39	26.61
12	82.43	17.57
13	91.46	8.54

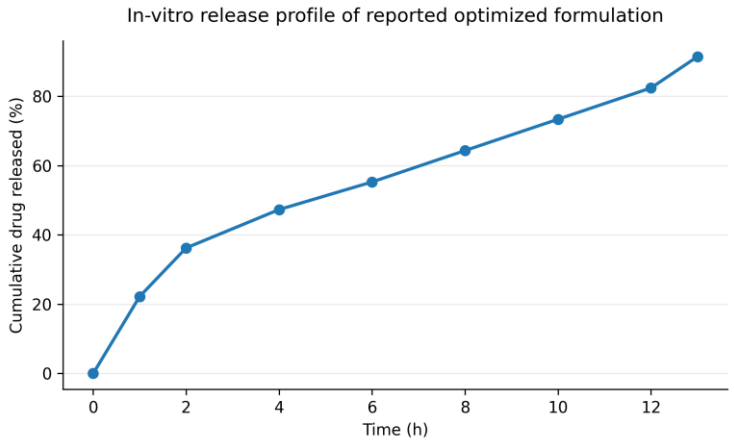


Figure 5. Cumulative In-Vitro Release Profile Transcribed.

Table 4. Reported Release-Kinetic Model Fits.

Model	Reported R^2	Relative interpretation
Zero-order	0.923	Second-highest reported fit
First-order	0.937	Highest reported fit
Higuchi	0.742	Lower fit than zero-/first-order
Korsmeyer-Peppas	0.599	Lowest reported fit

The first-order model had the highest reported R^2 (0.937), narrowly exceeding the zero-order fit (0.923). This difference is insufficient on its own to establish a definitive release mechanism. The original time-course data, replicate-level variation and residual diagnostics should be evaluated before mechanistic claims are made.

Storage Stability

At 25 ± 2°C/60 ± 5% RH, particle size increased from 154.24 to 159.40 nm over 90 days, while entrapment efficiency decreased from 94.85% to 91.95%. At 40 ± 2°C/70 ± 5% RH, particle size increased to 163.20 nm and entrapment efficiency decreased to 90.60% (table 5). The changes were greater under the higher-temperature condition, suggesting that temperature influenced the measured dispersion attributes. Without replicate data and acceptance criteria, the formulation should not be described as definitively stable or assigned a shelf life.

Table 5. Reported Stability Data for the Optimized Formulation.

Condition	Day	Particle size (nm)	Entrapment efficiency (%)
25 ± 2°C / 60 ± 5% RH	0	154.24	94.85
25 ± 2°C / 60 ± 5% RH	30	155.10	94.20
25 ± 2°C / 60 ± 5% RH	45	156.38	93.65
25 ± 2°C / 60 ± 5% RH	60	157.92	92.80
25 ± 2°C / 60 ± 5% RH	90	159.40	91.95
40 ± 2°C / 70 ± 5% RH	0	154.24	94.85
40 ± 2°C / 70 ± 5%	30	156.45	93.80

RH			
40 ± 2°C / 70 ± 5% RH	45	158.72	92.90
40 ± 2°C / 70 ± 5% RH	60	160.85	91.75
40 ± 2°C / 70 ± 5% RH	90	163.20	90.60

Preliminary Antibacterial Observations

The reported inhibition zone increased with concentration, from 12 mm at 2 mg/mL to 23 mm at 8 mg/mL, while the blank formulation showed no inhibition table 6 and fig 6. The study includes a plate image, but the source does not establish the strain identifier, positive-control performance or whether the tested formulation was CSF1 or F3. Consequently, these observations should be treated as preliminary screening data only and not as evidence that trifarotene itself has clinically meaningful antibacterial activity.

Table 6. Reported Agar-Well Diffusion Observations Against E. Coli.

Test sample	Concentration	Zone of inhibition (mm)	Source interpretation
Blank formulation	Not stated	0	No inhibition reported
Trifarotene-loaded cubosome	2 mg/mL	12	Low activity (source wording)
Trifarotene-loaded cubosome	4 mg/mL	17	Moderate activity (source wording)
Trifarotene-loaded cubosome	8 mg/mL	23	Largest zone in tested series

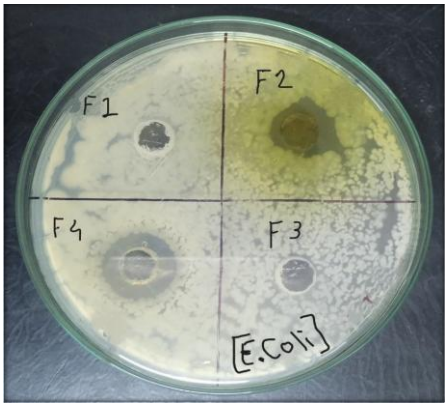


Figure 6. Agar Plate Image from the Supplied Study, Reported for Antibacterial Screening Against E. Coli.

DISCUSSION

This study reports the preparation and initial characterization of five trifarotene-loaded cubosomal dispersions. The qualitative solubility observations suggest poor aqueous solubility, providing a formulation rationale for examining a lipid-based carrier. Among the batches, CSF1 combined the smallest reported particle size (154.24 nm), the most negative zeta potential (-29.9 mV) and the highest entrapment efficiency (94.85%). These attributes make CSF1 the leading candidate within the current dataset, but optimization cannot be considered complete without polydispersity index, replicate variability, drug loading, batch reproducibility and a more complete assessment of formulation structure.

Particle sizes were within the nanoscale range, although CSF4 was notably larger than the other batches at 262.20 nm. This difference may reflect the formulation composition or processing, but the available data do not identify the cause. Zeta potential values varied from -14.0 to -29.9 mV. Surface charge can contribute to colloidal behaviour, yet zeta potential alone does not establish long-term stability, particularly in the presence of non-ionic steric stabilizers. Additional characterization should include PDI and,

where feasible, direct evidence of the internal cubic phase.

The reported release profile reached 91.46% at 13 h. The first-order model had the largest reported R^2, but its difference from the zero-order model was small. Release-kinetic model selection should therefore be treated as provisional until the original data are fitted consistently and model residuals are examined. In addition, the available release results do not demonstrate enhanced skin deposition, permeation, clinical response or reduced irritation; such conclusions require separate experiments.

The 90-day stability data showed gradual increases in particle size and decreases in entrapment efficiency under both storage conditions, with greater changes at the elevated temperature. These trends are compatible with some deterioration in measured attributes over time, but the absence of replicate uncertainty, predefined acceptance criteria and chemical assay results prevents a formal stability conclusion. A stability-indicating assay and additional physical measurements would strengthen future work.

The antibacterial screening is the most important interpretive limitation. A concentration-associated increase in inhibition-zone diameter was reported, but the active sample code is inconsistent across study sections, and the supplied record does not document a positive antibiotic control or strain identifier. A blank formulation is useful but cannot distinguish activity of the drug from activity related to other formulation components. Future experiments should include free trifarotene, blank cubosomes, a suitable positive-control antibiotic, standardized inoculum and validated replicate reporting. Until those checks are completed, the antibacterial finding should remain a preliminary observation.

Several additional reporting issues should be resolved before submission: the DMSO and water quantities in the batch formula; the exact homogenization conditions; analytical details for entrapment efficiency; release-cell and receptor-medium parameters; accelerated-storage humidity; SEM scale bar and metadata; FTIR peak assignments; and the statistical plan. The study also does not report skin permeation, irritation, cytotoxicity, animal studies or clinical outcomes. Claims about enhanced bioavailability, safety or therapeutic efficacy should therefore be avoided.

CONCLUSION

Trifarotene-loaded cubosomal dispersions were reported to have particle sizes of 154.24-262.20 nm and entrapment efficiencies of 71.24-94.85%. CSF1 had the smallest particle size and highest entrapment efficiency, with a reported zeta potential of -29.9 mV. Cumulative in-vitro release reached 91.46% at 13 h, and measured particle size and entrapment efficiency changed modestly during 90-day storage, with greater changes at elevated temperature. Preliminary inhibition zones against E. coli increased across the tested concentration range, but the batch-code discrepancy and incomplete microbiological controls prevent a reliable comparative conclusion. The findings justify further, well-controlled formulation research; they do not yet establish clinical suitability or antibacterial efficacy.

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