

Formulation, Optimization and Evaluation of Annona squamosa Seed Oil–Loaded Nanoemulsion and Nanostructured Lipid Carrier–Based Topical Gels for the Management of Psoriasis

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ABSTRACT

Psoriasis is a chronic, immune-mediated inflammatory skin disorder characterised by hyperproliferation of keratinocytes; long-term management with topical corticosteroids is limited by cutaneous atrophy, tachyphylaxis and poor patient compliance, prompting interest in safer, plant-derived alternatives. The present study aimed to formulate, optimize, characterize and evaluate a herbal nanoformulation of *Annona squamosa* seed oil (ASO) for topical application in psoriasis. ASO was extracted from *Annona squamosa* seeds by Soxhlet extraction and standardized for physicochemical parameters. A marker-based reverse-phase HPLC method using linoleic acid (LA) as marker was developed and validated (linearity $r^2 = 0.9998$ over 4–100 $\mu\text{g/mL}$; % RSD < 2 for intra- and inter-day precision; % recovery 98.8–99.1%). ASO nanoemulsion (ANE) and nanostructured lipid carrier (ANLC, using candelilla wax) were prepared by high-pressure homogenization and optimized for surfactant concentration and homogenization pressure using a 3^2 factorial design, yielding particle sizes of 33.8 nm (ANE) and 83.7 nm (ANLC) with narrow polydispersity (PI < 0.20) and zeta potentials of –25 to –33 mV. Both dispersions were incorporated into carbopol-974P gels (nanoemulgel, ANE gel; nanolipogel, ANLC gel) that showed acceptable spreadability (8.5–9.8 s/g), pseudoplastic rheology and drug content within $\pm 5\%$ of label claim. In vitro release through cellulose-nitrate membranes showed sustained release of LA from ANE gel (~98% over 8 h) and ANLC gel (~96% over 10 h) compared with rapid release of plain ASO (~100% within 30 min). In an in vitro sulforhodamine-B (SRB) anti-proliferative assay on HaCaT keratinocytes, ASO inhibited keratinocyte proliferation more potently (GI₅₀ = 32.4 $\mu\text{g/mL}$) than the reference corticosteroid clobetasol propionate (GI₅₀ = 75.9 $\mu\text{g/mL}$), while exhibiting comparably low cytotoxicity (LC₅₀ > 80 $\mu\text{g/mL}$ for both). Twelve-month accelerated and long-term stability studies confirmed that ANLC and ANLC gel were stable across all storage conditions, whereas ANE and ANE gel showed measurable changes only at $40 \pm 2^\circ\text{C}/75 \pm 5\%$ RH. These findings indicate that ASO-loaded nanocarriers, particularly the candelilla-wax based ANLC, are promising, corticosteroid-sparing platforms for the topical management of psoriasis, combining anti-proliferative efficacy, sustained cutaneous delivery and long-term physical stability.

Keywords: Psoriasis; *Annona squamosa* seed oil; Nanoemulsion; Nanostructured lipid carrier; HaCaT keratinocytes; Linoleic acid; Topical drug delivery; Herbal nanotechnology

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INTRODUCTION

The skin is the largest organ of the human integumentary system, covering a surface area of approximately 2 m² with a thickness ranging from 0.5 to 4 mm, and functions as the primary barrier against microbial invasion, chemical and physical insult, and dehydration, while also contributing to thermoregulation and vitamin D synthesis [1]. Disruption of normal keratinocyte turnover underlies a range of chronic inflammatory dermatoses, of which

psoriasis is among the most prevalent and best characterised.

Psoriasis is a chronic, immune-mediated inflammatory skin disease characterised clinically by well-demarcated, erythematous plaques covered with silvery scales, most commonly affecting the elbows, knees, scalp and lower back [2,3]. Its worldwide prevalence is estimated at around 2%, with marked geographic variation; the condition is less common in Asian and African populations than in Caucasian and

Scandinavian populations, where prevalence approaches 11% [4,5]. Population surveys nonetheless indicate that self-reported "sensitive" skin affects roughly one-third of the Indian population, with sensitive-skin subjects two- to four-times more likely to report psoriasis, acne, atopic dermatitis or vitiligo [6,7]. The disease is driven by dysregulated crosstalk between keratinocytes and the innate and adaptive immune system: activated dermal dendritic cells produce TNF- α and IL-23, which stimulate CD4+ and CD8+ T-cell activation and proliferation; the resulting T-cell infiltration and cytokine milieu drive keratinocyte hyperproliferation, shortening epidermal turnover from the normal 3–4 weeks to 3–7 days and producing the characteristic scaly plaques [8–11]. Genetic susceptibility loci (e.g., the LCE gene cluster, HLA-C*06 and MHC-linked loci) and environmental triggers such as trauma, infection, certain drugs, alcohol and stress modulate disease onset and flares [12–14].

Topical corticosteroids remain first-line therapy for mild-to-moderate plaque psoriasis because of their potent anti-inflammatory, anti-proliferative and vasoconstrictive actions [15]. However, prolonged use is limited by cutaneous atrophy, telangiectasia, striae, tachyphylaxis and, on withdrawal, disease rebound, which together compromise long-term patient compliance [16,17]. This has motivated growing interest in plant-derived actives and in nanocarrier-based topical delivery systems that can improve skin targeting, reduce systemic exposure and permit sustained release of the active at the site of action [18]. Among lipid-based nanocarriers, nanoemulsions (NE) and nanostructured lipid carriers (NLC) are particularly attractive for dermal delivery. Nanoemulsions are kinetically stable oil-in-water dispersions (droplet size typically < 200 nm) that enhance percutaneous penetration of lipophilic actives, while NLC — composed of a blend of solid and liquid lipids — combine the occlusive, film-forming properties of solid-lipid nanoparticles with a higher loading capacity and reduced drug expulsion on storage [19–23]. Both platforms have been explored for topical delivery of anti-inflammatory and anti-psoriatic actives, including corticosteroids and other synthetic and natural drugs [24–26].

Annona squamosa L. (custard apple) seeds are a rich source of polyunsaturated fatty acids, principally linoleic acid, along with tocopherols and other bioactives, and the seed oil (ASO) has documented antitumour, antioxidant, insecticidal and spermicidal activities [27–30]. Linoleic acid itself is known to modulate epidermal barrier function and to down-regulate NF- κ B-driven inflammatory signalling, providing a plausible mechanistic basis for anti-psoriatic activity [31,32]. Despite these leads, the therapeutic potential of ASO in psoriasis, and its suitability for incorporation into modern nanocarrier-based topical delivery systems, has not been systematically investigated.

The present study was therefore designed to (i) extract and standardize ASO and develop a validated marker-based HPLC method for its principal fatty-acid marker, linoleic acid; (ii) formulate and optimize an ASO-loaded nanoemulsion (ANE) and an ASO-loaded nanostructured lipid carrier (ANLC) using a factorial design; (iii) incorporate the optimized dispersions into carbopol-974P gels for ease of topical application; (iv) characterize the formulations for physicochemical properties, particle size, zeta potential, morphology, rheology and in vitro release; (v) evaluate anti-proliferative activity against HaCaT keratinocytes relative to the reference corticosteroid clobetasol propionate; and (vi) assess the short- and long-term stability of the developed systems, with the overall goal of establishing ASO-loaded nanocarriers as a corticosteroid-sparing option for the topical management of psoriasis.

Materials and Methods

Materials

Seeds of *Annona squamosa* were procured from Durva Herbal Centre and authenticated by Dr. P. Santhan, Chennai, Tamil Nadu. Tween 80 (polysorbate 80, HLB 15) and Span 80 (sorbitan monooleate, HLB 4.3) were used as hydrophilic and lipophilic surfactants respectively for the nanoemulsion; Myrj S40 and Span 80 were used as the surfactant system for the nanostructured lipid carrier, with candelilla wax as the solid lipid matrix. Carbopol 974P was used as the gelling agent and triethanolamine as the neutralizing agent. The human immortalized keratinocyte cell line HaCaT was procured from the National Centre for Cell Science (NCCS); clobetasol propionate (CP) was used as the reference standard/positive control. All other reagents used were of analytical or HPLC grade.

Extraction and standardization of *Annona squamosa* seed oil (ASO)

Fruit pulp and seeds were manually separated, washed and air-dried, and the dried seeds were powdered using an electric blender. Fifty grams of powdered seed was extracted with 100 mL petroleum ether at room temperature for 24 h using Soxhlet apparatus. The extract was filtered through Whatman filter paper and the residual solvent removed in a fume cupboard over 72 h; the resulting oil (ASO) was stored at room temperature for further analysis. ASO was standardized for physicochemical parameters — specific gravity, saponification value, acid value, iodine value and peroxide value — using standard pharmacopoeial procedures, and characterized by differential scanning calorimetry (DSC). Excipients (candelilla wax, Carbopol 974P) were standardized against manufacturer certificates of analysis for appearance, colour, odour, solubility and, where applicable, melting point, saponification value, acid value and ester value.

In vitro anti-proliferative (SRB) assay

HaCaT cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum, 10 μ g/mL streptomycin and 10 U/mL penicillin

at 37 °C in 5% CO₂, and used at 60–90% confluence. ASO and CP were suspended in 2% acacia solution, filter-sterilized (0.2 µm) and tested at four concentrations (10, 20, 40 and 80 µg/mL) in 96-well plates (1 × 10⁵ cells/200 µL/well, six replicates per concentration) for 48 h. Cell proliferation was measured by the sulforhodamine-B (SRB) assay: cells were fixed with cold 30% (w/v) trichloroacetic acid, stained with 0.4% (w/v) SRB in 1% acetic acid, washed, and the bound dye eluted with 10 mM Tris base; absorbance was read at 540 nm (690 nm reference). Percentage cell growth was expressed relative to control wells, and LC50 (50% lethal concentration), TGI (total growth inhibition) and GI50 (50% growth-inhibitory concentration) were derived graphically.

HPLC method development and validation

A reverse-phase HPLC method was developed on an Agilent 1200 series liquid chromatograph (quaternary pump, UV-visible detector, EZChrom Elite software) using a Qualisil C18 column (250 × 4.6 mm i.d., 5 µm) with a mobile phase of acetonitrile:methanol:tetrahydrofuran:water containing 0.5% glacial acetic acid (36:32:31:1 v/v) at a flow rate of 0.5 mL/min, column temperature 40 °C and detection at 236 nm; linoleic acid (LA) was used as the marker for ASO and quantified by the area-under-curve (AUC) method. The method was validated for linearity, intra- and inter-day precision, specificity, limit of detection (LOD) and limit of quantitation (LOQ), and separately validated for extraction and quantification of LA from the nanoformulations (using methanol:THF 1:1% v/v as extraction solvent) and from the in vitro release medium (methanol:water:THF, 50:25:25).

Formulation of ASO nanoemulsion (ANE)

ANE was formulated as an oil-in-water system comprising ASO (10% w/w) as the oil phase, Span 80 as the lipophilic surfactant, Tween 80 as the hydrophilic surfactant, and water as the continuous phase. The lipid phase (ASO + Span 80) was dispersed into the aqueous phase (water + Tween 80) under high-speed stirring for 1 h, and the resulting pre-emulsion was subjected to high-pressure homogenization (Panda Plus, GEA Niro Soavi) at pressures of 200–1000 bar (four cycles at each pressure). Surfactant concentration was optimized using a 3² factorial design (Tween 80 and Span 80 each at three levels) with sedimentation volume after 24 h and post-centrifugation stability as response variables; homogenization pressure and cycle number were then optimized against particle size and polydispersity index (PI) determined by photon correlation spectroscopy (Malvern particle size analyzer).

Formulation of ASO nanostructured lipid carrier (ANLC)

ANLC was prepared using candelilla wax as the solid lipid matrix. The solid lipid was melted 5 °C above its melting point; the lipophilic surfactant (Myrj S40) was mixed with liquid lipid (ASO) at the same temperature and combined with the molten solid lipid. This lipid phase was emulsified into the aqueous phase (Tween 80

+ water) using an Ultra-Turrax T25 high-speed disperser to obtain a pre-dispersion, which was then hot-homogenized (Panda Plus homogenizer) to yield the nanostructured lipid carrier. Solid lipid:liquid lipid ratio (screened from 70:30 to 20:80), surfactant combination/concentration (3² factorial design for Myrj S40 and Span 80) and homogenization parameters (pressure 200–1000 bar, four cycles) were optimized using sedimentation volume, particle size and PI as response variables.

Preparation and characterization of topical gels

The optimized ANE and ANLC dispersions were incorporated into 1% w/w Carbopol 974P gel under continuous stirring to obtain nanoemulgel (ANE gel) and nanolipogel (ANLC gel) respectively; the gels were neutralized with 0.1% triethanolamine. Formulations (dispersions and gels) were evaluated for physicochemical properties (colour, odour, appearance, pH), drug content (marker-based HPLC), particle size, polydispersity index and zeta potential (Malvern Zetasizer, photon correlation spectroscopy, Laser Doppler electrophoresis), surface morphology (Cryo Field-Emission-Gun Scanning Electron Microscopy, 50× magnification, 15.0 kV accelerating voltage), and thermal behaviour (DSC, Q10 calorimeter, 4–110 °C at 10 °C/min under nitrogen). Rheological evaluation comprised spreadability (parallel-plate slip-drag method, 100 g load) and viscosity (Brookfield R/S-CPS+ rheometer, spindle C25-2 DIN, shear rate 0–100 s⁻¹ ascending/descending).

In vitro drug release studies

In vitro release of LA from plain ASO, ANE gel and ANLC gel was studied using modified Franz diffusion cells (10 mm orifice, 10 mL receptor volume) fitted with cellulose nitrate membranes (0.1 µm pore size), maintained at 32 ± 1 °C (OECD Guideline 428) with continuous magnetic stirring of the receptor fluid. Samples were withdrawn at predetermined intervals over 24 h, analysed by the validated HPLC method, and cumulative percentage release of LA was plotted against time.

Stability studies

Stability studies were conducted in accordance with ICH Q1A(R2) guidance, storing the optimized ANE, ANLC and corresponding gels in USP Type I glass vials under refrigeration, 25 ± 2 °C/60 ± 5% RH, 30 ± 2 °C/65 ± 5% RH and 40 ± 2 °C/75 ± 5% RH for 12 months, with sampling at 0, 1, 2, 3, 6 and 12 months for appearance, centrifugation stability, pH, particle size, PI, spreadability and drug content.

Statistical analysis

Formulation optimization data (sedimentation volume, particle size, polydispersity index, zeta potential) were analysed by response-surface methodology using a 3² factorial design (Design-Expert software); response variables were fitted to a second-order polynomial model to generate 3-D response surface and contour plots. SRB assay data are expressed as mean % cell growth ± SEM (n = 4); HPLC precision data are

expressed as mean $\pm$ % relative standard deviation (% RSD, $n = 3$ or 6 as indicated).

RESULTS

Standardization of ASO and excipients

Percentage yield of ASO from dried, powdered *Annona squamosa* seeds was 25–30% w/w. The physicochemical characteristics of ASO are summarised in Table 1. The high saponification value and specific-gravity profile confirmed the oily, triglyceride-rich nature of the extract; the relatively low

Parameter	Result
Colour	Pale yellow
Specific gravity	0.897
Saponification value (mg KOH/g)	167.8

Table: 1 Physicochemical standardization of *Annona squamosa* seed oil (ASO)

Sample	Endothermic peak (°C)
ASO	50.0

In vitro anti-proliferative activity

ASO inhibited proliferation of HaCaT keratinocytes in a concentration-dependent manner over the range 10–80 $\mu\text{g/mL}$ (Figure 1). The calculated GI50 for ASO (32.4 $\mu\text{g/mL}$) was markedly lower than that of the

Agent	LC50 ($\mu\text{g/mL}$)	TGI ($\mu\text{g/mL}$)	GI50 ($\mu\text{g/mL}$)
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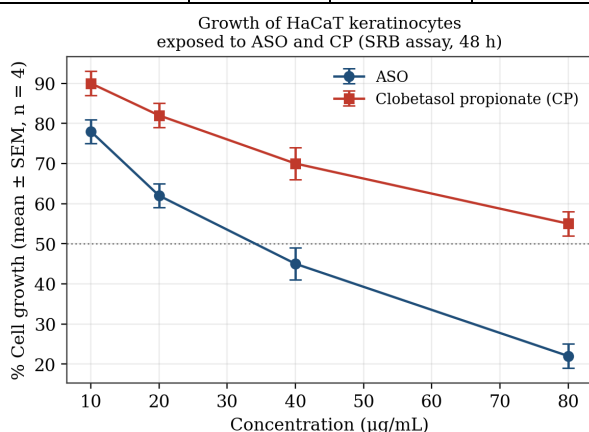


Figure: 2 Concentration-dependent growth of HaCaT keratinocytes mean $\pm$ SEM, $n = 4$.

HPLC method development and validation for linoleic acid (LA)

Table: 4 Mobile phases screened for HPLC analysis of linoleic acid

Mobile phase	Ratio
Acetonitrile: Water	90:10
Acetonitrile: Methanol: Water	80:10:10

acid value indicated a low free-fatty-acid content, while the iodine value confirmed a high degree of unsaturation, consistent with a linoleic-acid-rich fatty-acid profile. DSC analysis of ASO showed a single sharp endothermic peak at 50 °C, and candelilla wax showed a corresponding peak at 68.5 °C (Table 2). All excipients (candelilla wax, Carbopol 974P) complied with manufacturer certificate-of-analysis specifications for appearance, colour, odour and solubility.

oil)	
Acid value (mg KOH/g oil)	2.68
Iodine value (g ICl/100 g oil)	95.7
Peroxide value (meq/kg)	6.10

Candelilla wax	68.5
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reference corticosteroid clobetasol propionate (75.9 $\mu\text{g/mL}$), indicating greater potency in suppressing keratinocyte growth, while LC50 for both agents exceeded 80 $\mu\text{g/mL}$, indicating comparably low cytotoxicity at the concentrations tested (Table 3).

Clobetasol propionate	> 80	> 80	75.9
ASO	> 80	> 80	32.4

A mobile phase of acetonitrile: methanol: THF: water containing 0.5% glacial acetic acid (36:32:31:1 v/v) gave a well-resolved, symmetrical LA peak at a retention time of 6.35 min and was selected for further validation (Table 4). The calibration curve for LA was linear over 4–100 $\mu\text{g/mL}$ ($y = 50.248x$, $r^2 = 0.9998$; Figure 2). Intra- and inter-day precision showed % RSD < 1.1% at all tested concentrations (Tables 5 and 6), and LOD and LOQ were 0.164 and 0.496 $\mu\text{g/mL}$ respectively (Table 7). The same method, after re-validation for the nanoformulation matrix (linear range 10–100 $\mu\text{g/mL}$, $r^2 = 1.00$) and for the in vitro release medium ($r^2 = 0.9979$), gave % recovery of 98.8–99.1% (Table 8), confirming that the method was specific, linear, precise and accurate for quantification of LA in ASO, in the developed nanoformulations and in release-medium samples.

Acetonitrile: Methanol: THF: Water	50:30:10:10
Acetonitrile: Methanol: THF: Water + 0.5% glacial acetic acid	36:32:31:1 v/v

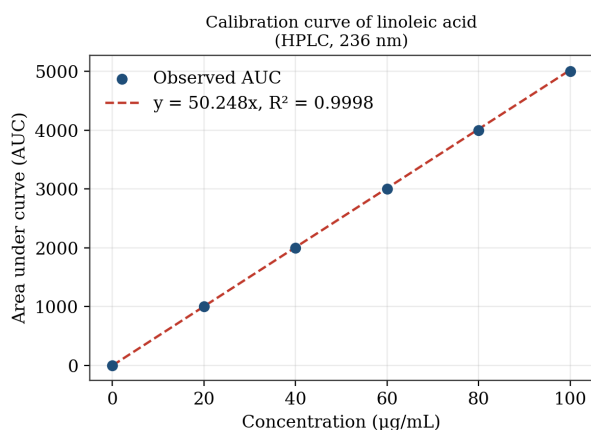


Figure: 2 Calibration curve of linoleic acid by HPLC (236 nm); linear over 4–100 µg/mL ($y = 50.248x$, $r^2 = 0.9998$).

Table: 5 Intra-day precision of the HPLC method for LA in ASO

Concentration (µg/mL)	Mean AUC	% RSD
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10	503.33	1.055
30	1513.67	0.189
50	2507.67	0.068

Table: 6 Inter-day precision of the HPLC method for LA in ASO

Day	Concentration (µg/mL)	Mean AUC	% RSD
1	10 / 30 / 50	505.67 / 1511.33 / 2504.33	0.336 / 0.205 / 0.167
2	10 / 30 / 50	502.33 /	0.497 /

		1510.67 / 2503.67	0.113 / 0.105
3	10 / 30 / 50	501.00 / 1512.00 / 2506.33	0.163 / 0.216 / 0.105

Table: 7 Detection and quantitation limits for linoleic acid

Parameter	Value (µg/mL)
Limit of detection (LOD)	0.164

Limit of quantitation (LOQ)	0.496
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Table: 8 Recovery of LA from ASO nanoformulations (accuracy)

Spike level	% Recovery	% RSD
80% (40 + 32 µg/mL)	98.84	0.165

100% (40 + 40 µg/mL)	99.13	0.103
120% (40 + 48 µg/mL)	98.89	0.120

Optimization and characterization of ASO nanoemulsion (ANE)

Of the nine batches generated by the 3^2 factorial design for surfactant concentration (Tween 80, Span 80), four batches (ANE-2, ANE-5, ANE-6, ANE-8) were physically stable after 24 h and on centrifugation (Table 9). These stable batches were homogenized at 600–

1000 bar; particle size decreased and PI improved with increasing pressure for all batches, and batch ANE-5 (medium Tween 80, medium Span 80) gave the smallest particle size (33.8 nm) with the lowest PI (0.101) at 1000 bar/4 cycles and was selected as the optimized batch (Table 10).

Bat ch	Twe en 80 (lev el)	Spa n 80 (lev el)	24-h observ ation	Sediment ation volume	Centrifug ation
AN	-1	-1	Creami	0.94	Creaming

E-1			ng		
AN E-2	-1	0	Stable	1.00	Stable
AN E-3	-1	+1	Creami ng	0.97	Creaming
AN E-4	0	-1	Stable	0.98	Creaming
AN	0	0	Stable	1.00	Stable

E-5					
AN E-6	0	+1	Stable	1.00	Stable
AN E-7	+1	-1	Oil separate	–	–

Table: 10 Effect of homogenization pressure on particle size and PI of stable ANE batches

Batch	600 bar PS (nm)/PI	800 bar PS (nm)/PI	1000 bar PS (nm)/PI
ANE-2	125.6 / 0.567	100.4 / 0.329	46.4 / 0.242
ANE-5	109.2 /	76.5 / 0.170	33.8 / 0.101

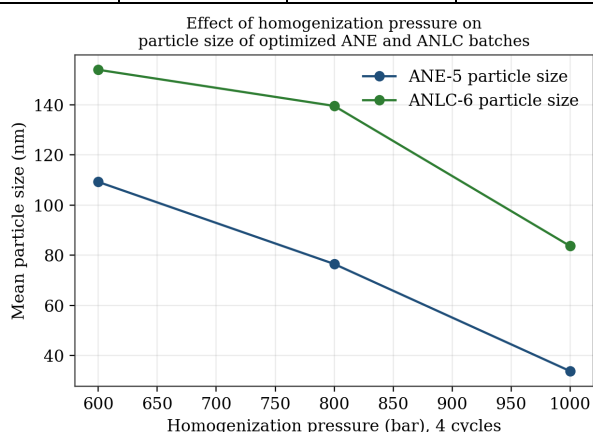


Figure: 3 Effect of homogenization pressure on mean particle size of the optimized ANE-5 and ANLC.

The optimized ANE was a white, transparent dispersion, stable on centrifugation (3000 rpm, 20 min), with pH 6.0–7.0, particle size in the range 30–100 nm and negative zeta potential (–25 to –33 mV across the Table: 11 Drug (LA) content of optimized ANE batches

Batch	Mean AUC	% Drug content	% RSD
ANE-2	736.33	98.59	0.169

Optimization and characterization of ASO nanostructured lipid carrier (ANLC)

Preliminary lipid-ratio screening identified 50:50 candelilla wax:ASO as the minimum solid-lipid proportion able to yield a coherent solid matrix on cooling; higher liquid-lipid proportions produced soft, non-solidifying mixtures. Of the nine batches from the 3² factorial design for surfactant concentration (Myrj Table: 12 Response of the 3² factorial design (surfactant concentration) for ANLC

Batch	Myrj S40 (level)	Span 80 (level)	24-h observation	Sedimentation volume
ANLC -1	-1	-1	Cream	0.85
ANLC -2	-1	0	Cream	0.90
ANLC	-1	+1	Thick	0.97

			d		
AN E-8	+1	0	Stable	1.00	Stable
AN E-9	+1	+1	Creaming	0.80	Creaming

	0.193		
ANE-6	136.5 / 0.346	106.8 / 0.275	78.8 / 0.231
ANE-8	172.9 / 0.306	114.7 / 0.255	98.2 / 0.158

design space), consistent with electrostatically stabilized droplets. SEM confirmed spherical droplets with a homogeneous surfactant coating. Drug content of the four optimized batches (as LA) ranged from 98.6–99.4% with % RSD < 0.36 (Table 11)

ANE-5	741.33	99.26	0.168
ANE-6	742.00	99.35	0.291
ANE-8	738.33	98.86	0.355

S40, Span 80), three batches (ANLC-5, ANLC-6, ANLC-8) were stable after 24 h and on centrifugation (Table 12) and were carried forward for homogenization optimization. Batch ANLC-6 (medium Myrj S40, high Span 80) gave the smallest particle size (83.7 nm, PI 0.151) at 1000 bar/4 cycles and was selected as the optimized ANLC batch, with a zeta potential of –22.1 mV (Table 13).

-3			cream	
ANLC -4	0	-1	Cream	0.84
ANLC -5	0	0	Stable	1.00
ANLC -6	0	+1	Stable	1.00
ANLC -7	+1	-1	Creaming	0.95
ANLC -8	+1	0	Stable	1.00

ANLC-9	+1	+1	Thick cream	0.94
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Table: 13 Effect of homogenization pressure on particle size and PI of stable ANLC batches

Batch	600 bar PS (nm)/PI	800 bar PS (nm)/PI	1000 bar PS (nm)/PI
ANLC-5	154.7 / 0.496	140.7 / 0.257	109.4 / 0.198

ANLC-8 165.8 / 0.462 146.8 / 0.226 123.1 / 0.212

The optimized ANLC was a homogeneous, translucent, pale-yellow dispersion with a typical odour, pH 6.0–7.0, stable on centrifugation. Drug (LA) content of the three optimized batches ranged from 98.3–99.1% with Table: 14 Drug (LA) content of optimized ANLC batches

Batch	Mean AUC	% Drug content	% RSD
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Rheological evaluation of topical gels

The spreadability of ANE gel (8.5–9.5 s/g) and ANLC gel (9.0–9.8 s/g) was comparable to, and only marginally different from, that of the plain Carbopol 974P gel base (6.5–7.5 s/g), indicating that incorporation of the nanocarriers did not compromise Table: 15 Spreadability of nanocarrier-enriched gels versus plain Carbopol 974P gel

Formulation	Spreadability (s/g)
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ANLC gel (nanolipogel) 9.00–9.80

In vitro drug release

Plain ASO released its LA content almost completely (~100%) within 30 min through the cellulose-nitrate membrane, whereas incorporation into the nanocarrier gels markedly prolonged release: ANE gel showed an initial burst (~20% in 30 min) followed by sustained release reaching ~98% by 8 h, while ANLC gel showed a smaller initial burst (~12–20%) and a more gradual profile, reaching ~50% release at 5 h and ~90–96% by 8–10 h (Figure 4). The initial burst is attributable to drug adsorbed at or near the particle surface, while the extended release phase reflects diffusion of LA from within the surfactant/lipid matrix, modulated by surfactant type/concentration, gelling polymer and homogenization conditions.

ANLC-6	153.9 / 0.561	139.5 / 0.299	83.66 / 0.151
ANLC-8	165.8 / 0.462	146.8 / 0.226	123.1 / 0.212

% RSD < 0.45 (Table 14). SEM revealed spherical, smooth-surfaced nanoparticles, and DSC showed a reduction in melting endotherm and enthalpy for candelilla wax on incorporation into ANLC, consistent with a less-ordered crystal lattice and good entrapment of ASO within the wax matrix.

ANLC-5	740.33	99.12	0.278
ANLC-6	734.33	98.32	0.449
ANLC-8	736.33	98.59	0.231

ease of application (Table 15). Both nanoemulgel and nanolipogel exhibited pseudoplastic (shear-thinning) flow with negligible thixotropy on the Brookfield rheometer, consistent with good spreadability and retention on the skin.

Plain gel base (Carbopol 974P)	6.50–7.50
ANE gel (nanoemulgel)	8.50–9.50
ANLC gel (nanolipogel)	9.00–9.80

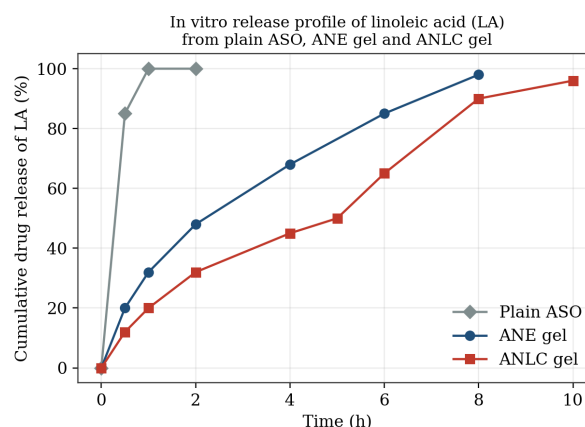


Figure: 4 In vitro cumulative release profile of linoleic acid from plain ASO, ANE gel and ANLC gel

Stability studies

ANE was physically stable at refrigeration, 25 ± 2 °C/60 ± 5% RH and 30 ± 2 °C/65 ± 5% RH over 12 months, with particle size increasing only marginally (34.6→39.6–41.2 nm) and PI remaining < 0.31; however, at the accelerated condition of 40 ± 2 °C/75 ± 5% RH, particle size increased more appreciably (34.6→45.9 nm) and PI rose to 0.324, indicating comparatively lower stability under this stress condition (Table 16, Figure 5). ANE gel similarly showed acceptable stability at refrigeration and 25 ± 2 °C/60 ± 5% RH but a reduction in consistency and spreadability

by 6–12 months at 30 ± 2 °C/ $65 \pm 5\%$ RH and 40 ± 2 °C/ $75 \pm 5\%$ RH. In contrast, ANLC and ANLC gel remained stable across all four storage conditions over the full 12-month period, with particle size changing by less than 6 nm at any condition and no phase separation

Table: 16 Twelve-month stability of ANE at three storage conditions (particle size and pH)

Time (months)	25 ± 2 °C/60% RH — PS (nm)/pH	30 ± 2 °C/65% RH — PS (nm)/pH	40 ± 2 °C/75% RH — PS (nm)/pH
0	34.55 / 6.80	34.55 / 6.80	34.55 / 6.80

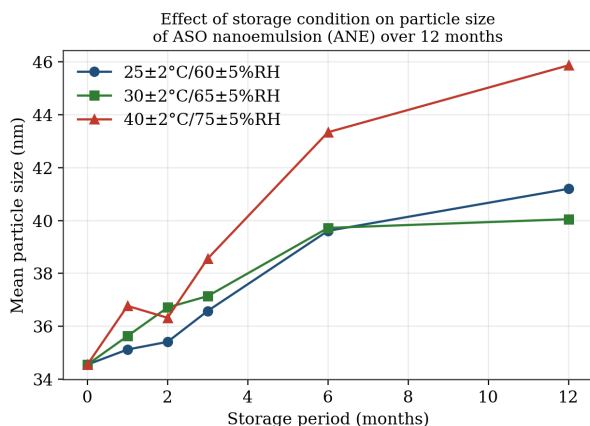


Figure: 5 Effect of storage condition on mean particle size of ASO nanoemulsion (ANE) over 12 months

Discussion

The physicochemical profile obtained for ASO in this study — a low acid value, high saponification value and high iodine value — is consistent with a triglyceride oil rich in unsaturated fatty acids, in agreement with earlier fatty-acid-composition studies of *Annona squamosa* seed kernels reporting linoleic acid as the predominant fatty acid [29, 30]. This unsaturated-fatty-acid-rich composition provides a plausible mechanistic basis for the anti-proliferative activity observed in the present HaCaT assay, since polyunsaturated fatty acids such as linoleic acid are reported to down-regulate NF-κB signalling and scavenge reactive oxygen species that would otherwise drive keratinocyte hyperproliferation [31,32].

The anti-proliferative data reported here (GI₅₀ 32.4 µg/mL for ASO versus 75.9 µg/mL for clobetasol propionate) are consistent with, and extend, the only directly comparable prior report identified in the literature [26], who described anti-psoriatic activity of *Annona squamosa* seed oil in vitro and in vivo. The present study adds a validated analytical basis (marker-based HPLC quantification of linoleic acid) and a full nanocarrier development programme that was not addressed in that earlier work. The observation that ASO was more potent than CP in inhibiting keratinocyte growth while showing comparable cytotoxicity thresholds (LC₅₀ > 80 µg/mL for both agents) suggests a wide margin between anti-proliferative and cytotoxic concentrations for ASO, a

on centrifugation, reflecting the greater physical robustness conferred by the solid-lipid matrix. Drug content of all formulations remained within the acceptable $\pm 5\%$ range throughout the study period.

1	35.12 / 6.91	35.63 / 6.74	36.77 / 6.85
2	35.41 / 6.97	36.71 / 6.97	36.32 / 6.94
3	36.58 / 6.95	37.14 / 7.10	38.56 / 7.32
6	39.61 / 7.02	39.72 / 7.12	43.34 / 6.90
12	41.20 / 6.92	40.05 / 7.27	45.87 / 7.10

favourable profile relative to corticosteroids, whose long-term topical use is constrained principally by atrophic and rebound effects rather than acute cytotoxicity [13–15].

The particle sizes achieved for ANE (33.8 nm) and ANLC (83.7 nm) fall within, or below, the sub-100 nm to 200 nm range generally considered optimal for enhanced follicular and intercellular penetration of nanocarriers into the stratum corneum, and are comparable to nanoemulsion-based antipsoriatic systems reported [16], who similarly used factorial-design optimization of Tween/Span systems to achieve stable antipsoriatic nanoemulsions. The negative zeta potentials obtained here (-22 to -33 mV) are within the range generally regarded as sufficient for electrostatic stabilization of nanoemulsions and NLC dispersions, consistent with the stability principles [18–20] for lipid-based colloidal carriers.

The sustained-release behaviour of both nanocarrier gels relative to plain ASO parallels findings for other NLC- and NE-based topical systems, where incorporation of an active into a nanoparticulate matrix consistently prolongs cutaneous release compared with the free drug, [21, 22] for a range of anti-inflammatory and antifungal actives. The comparatively slower and more sustained release from ANLC gel relative to ANE gel is consistent with the additional diffusional barrier imposed by the solid-lipid matrix of NLC systems, a feature previously exploited to prolong release of celecoxib, flurbiprofen and other actives from NLC-based topical gels [21–23].

The superior long-term stability of ANLC relative to ANE across all four storage conditions mirrors the well-established advantage of solid-lipid-matrix carriers over purely liquid-oil nanoemulsions: the crystalline/semi-crystalline wax matrix restricts droplet mobility and Ostwald ripening, the dominant destabilization mechanism in nanoemulsions, thereby conferring greater resistance to particle-size growth on storage, particularly under accelerated (40 °C/ 75% RH) conditions [17,18]. This is consistent with the rationale underlying the development of NLC as a "second generation" of lipid nanoparticles specifically to overcome the storage-stability limitations of nanoemulsions and solid lipid nanoparticles [23,24].

Collectively, these findings position ASO-loaded nanocarriers, and particularly the candelilla-wax-based ANLC gel, as a promising herbal, corticosteroid-sparing platform for topical psoriasis management, combining (i) intrinsic anti-proliferative activity attributable to the unsaturated fatty-acid content of ASO, (ii) enhanced and sustained cutaneous availability afforded by nanoencapsulation, and (iii) long-term physical and chemical stability suitable for pharmaceutical development.

Conclusion

Annona squamosa seed oil was successfully extracted, standardized and incorporated into two complementary nanocarrier platforms — a nanoemulsion (ANE) and a nanostructured lipid carrier (ANLC) — using a factorial-design optimization approach, and further formulated into Carbopol-974P-based topical gels. Both nanocarrier gels exhibited sub-100 nm to sub-200 nm particle sizes, narrow polydispersity, negative zeta potential, pseudoplastic rheology and acceptable spreadability, and provided sustained release of linoleic acid relative to plain ASO. ASO demonstrated potent, corticosteroid-comparable-to-superior anti-proliferative activity against HaCaT keratinocytes with a favourable cytotoxicity margin. Twelve-month stability studies confirmed that the candelilla-wax-based ANLC and ANLC gel were more physically robust than the nanoemulsion-based counterparts across the range of storage conditions tested. Taken together, these results support ASO-loaded nanostructured lipid carrier gel as a promising, natural, corticosteroid-sparing alternative for the topical management of psoriasis, warranting further evaluation in ex vivo permeation and in vivo efficacy/safety models.

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