

Development and Optimization of *Celastrus paniculatus* Seed Oil Nanoemulsion by High-Pressure Homogenization for Potential Brain-Targeted Delivery in Alzheimer's Disease

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ABSTRACT

Alzheimer's disease (AD) remains one of the most therapeutically unmet neurodegenerative conditions, with the blood-brain barrier (BBB) constituting the principal obstacle to effective pharmacological intervention. *Celastrus paniculatus* Willd. (Jyotishmati) seed oil has been documented to possess neuroprotective, anti-cholinesterase, and cognition-enhancing properties, yet its inherent lipophilicity and BCS Class II classification severely limit its oral bioavailability and CNS penetration. This study aimed to develop and optimise a stable oil-in-water (O/W) nanoemulsion of Jyotishmati seed oil using high-pressure homogenization (HPH) and a response surface methodology (RSM)-based Design of Experiments (DOE) for formulation optimisation. Pre-formulation studies included organoleptic characterisation, solubility profiling, and UV-Visible spectrophotometric method development using palmitic acid as an analytical marker at $\lambda_{\text{max}} = 213$ nm. Nanoemulsion optimisation was performed using a three-factor RSM design (oil, Tween 80, and propylene glycol concentrations) with droplet size and PDI as critical quality attributes (CQAs). The optimised formulation was characterised by DLS, zeta potential analysis, TEM, and HPLC-based determination of drug loading efficiency. The optimised formulation D2 (oil 8.58%, Tween 80 22.38%, propylene glycol 7.21% w/w) produced a nanoemulsion with a mean Z-average droplet size of 115.47 ± 0.71 nm, PDI of 0.189 ± 0.003 , and zeta potential of -29.27 ± 0.86 mV. TEM confirmed spherical, non-aggregated droplets of 80-120 nm. Drug loading efficiency (LE%) was $81.36 \pm 0.25\%$ by HPLC. The developed nanoemulsion demonstrated favourable physicochemical attributes for brain-targeted drug delivery.

Keywords: *Celastrus paniculatus*; Jyotishmati seed oil; nanoemulsion; high-pressure homogenization; Alzheimer's disease; Design of Experiment; blood-brain barrier; drug loading efficiency

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1. INTRODUCTION

Alzheimer's disease (AD) is a progressive, irreversible neurodegenerative disorder characterised pathologically by the extracellular accumulation of amyloid- β (A β) plaques, intraneuronal formation of neurofibrillary tau tangles, chronic neuroinflammation, synaptic dysfunction, and widespread cortical and hippocampal neurodegeneration. It represents the most prevalent form of dementia, accounting for approximately 60–70% of all dementia cases globally. As of 2024, an estimated 6.5 million individuals aged 65 years and above in the United States were diagnosed with AD, of whom more than 70% were aged 75 years or older. Worldwide, approximately 55 million people were living with dementia, with prevalence projected to reach 153 million by 2050 in the absence of effective disease-modifying therapies (Alzheimer's Association, 2024). Despite decades of research, current

pharmacotherapy principally acetylcholinesterase inhibitors (donepezil, rivastigmine, galantamine) and the NMDA receptor antagonist memantine provides only symptomatic relief without altering disease progression (Srivastava *et al.*, 2021; Su *et al.*, 2024; Su *et al.*, 2023; Sun *et al.*, 2024; Takechi *et al.*, 2022).

The principal barrier to CNS pharmacotherapy is the blood-brain barrier (BBB), a highly selective neurovascular interface composed of tight-junction-linked cerebral microvascular endothelial cells, supported by astrocytic end-feet and pericytes. This architecture restricts the passage of more than 98% of small-molecule drugs and virtually all large-molecule biologics from the systemic circulation into the brain parenchyma. Nanotechnology-based drug delivery systems, including nanoemulsions, polymeric nanoparticles, liposomes, and solid lipid nanoparticles, have emerged as promising

strategies to circumvent the BBB through size-dependent endocytosis, P-gp efflux evasion, and receptor-mediated transcytosis at the brain endothelial surface (Chen *et al.*, 2019; Chen *et al.*, 2023; Zielińska *et al.*, 2022; Zou *et al.*, 2025; Zou *et al.*, 2022; Zuhrotun *et al.*, 2023).

Celastrus paniculatus Willd. (family: Celastraceae), Vernacularly known as Jyotishmati or Malkanguni, it is an important Ayurvedic medicinal plant found across tropical and subtropical zones of the Indian subcontinent. The seed oil is phytochemically complex, comprising sesquiterpene alkaloids (celapanine, celapagine, paniculatin, malkanguniol, celestine), polyunsaturated fatty acids (PUFAs - principally palmitic, stearic, oleic, and linoleic acids), flavonoids, tannins, triterpenoids, and steroids (Panda *et al.*, 2016; Shohag *et al.*, 2022). Folk and traditional medical systems have long recognised the plant's utility in the treatment of neurological ailments, including memory disorders, facial paralysis, sciatica, and cognitive decline. Experimental pharmacological evidence has firmly supported these traditional claims. Studies also demonstrated that *C. paniculatus* seed oil and its derived fractions conferred significant neuroprotection against glutamate-induced excitotoxicity in neuronal cultures by modulating glutamate receptor signalling. Another study reported dose-dependent anti-cholinesterase activity of the oil in rat brain homogenates, a mechanism directly relevant to AD therapeutics, alongside cognitive-enhancing effects in stress-induced cognitive dysfunction models. These properties established *C. paniculatus* seed oil as a pharmacologically promising candidate for the development of CNS-targeted nanoemulsions (Arya *et al.*, 2022; Panda *et al.*, 2016; Satam *et al.*, 2025; Shohag *et al.*, 2022; Thongon *et al.*, 2026; Treerattanakulporn *et al.*, 2026).

Nanoemulsions are colloidal oil-in-water (O/W) or water-in-oil (W/O) dispersions of two thermodynamically immiscible liquid phases, stabilised by a surfactant monolayer at the interface, with mean droplet diameters typically below 500 nm and commonly in the range of 20–200 nm. Their small droplet size confers optical clarity (translucency), enhanced thermodynamic stability relative to coarse emulsions, and a dramatically increased interfacial surface area that promotes rapid drug absorption. Unlike microemulsions, which are thermodynamically stable and form spontaneously, nanoemulsions are kinetically stable systems that require mechanical energy for fabrication - most commonly via high-pressure homogenization (HPH) or microfluidization. The nanoscale dimensions of HPH-generated nanoemulsion droplets enable transcytosis across the BBB endothelium and enhance CNS bioavailability of encapsulated

lipophilic actives (Cabral *et al.*, 2021; Costa *et al.*, 2021; Fernandes *et al.*, 2021).

The present study undertook the systematic pre-formulation characterisation of Jyotishmati seed oil, development of an HPH-based O/W nanoemulsion, and RSM-based multi-factor optimisation of formulation variables using Design-Expert® software.

Physicochemical characterisation of the optimised formulation included DLS (droplet size and PDI), electrophoretic light scattering (zeta potential), TEM (droplet morphology), and HPLC-based determination of drug loading efficiency using palmitic acid as the quantitative marker.

2. MATERIALS AND METHODS

2.1 Materials

Jyotishmati seed oil was procured from M.S. Chemicals India, Lucknow (Lot No. [MSCI-JO-2024], 25 October 2024). Tween 80 (polysorbate 80, HLB = 15.0), propylene glycol, α -tocopherol, palmitic acid (analytical standard, $\geq 99\%$ purity), and HPLC-grade methanol were obtained from standard commercial suppliers. All other reagents and solvents were of analytical grade. Distilled water (conductivity $< 1 \mu\text{S/cm}$) was used throughout.

2.2 Pre-formulation Studies

Organoleptic properties, colour, odour, taste, texture, and appearance, were evaluated by standardised sensory assessment. Solubility of Jyotishmati seed oil in seven pharmaceutical solvents (distilled water, ethanol, methanol, isopropyl alcohol, propylene glycol, PEG 400, and Tween 80) was determined at $25 \pm 1^\circ\text{C}$ by the saturation shake-flask method, with excess oil agitated in solvent for 48 hours followed by centrifugation (3,000 rpm, 10 min) and spectrophotometric quantification of the supernatant at 213 nm. BCS classification was assigned based on established aqueous solubility and membrane permeability criteria (Saber *et al.*, 2026; Singh *et al.*, 2026; Tao *et al.*, 2026; Zhang *et al.*, 2026).

2.3 UV-Visible Spectrophotometric Method Development

A UV-Visible spectrophotometric method was developed for the quantification of palmitic acid, as a representative fatty acid marker of Jyotishmati seed oil, at $\lambda_{\text{max}} = 213 \text{ nm}$ using a UV-Visible spectrophotometer (Shimadzu UV-1900). A primary stock solution (10 mg/mL palmitic acid in ethanol) was prepared and diluted in serial steps to yield working concentrations of 0.25, 0.50, 1.00, 1.50, 2.00, and 2.50 $\mu\text{g/mL}$. The calibration curve was constructed by plotting mean absorbance ($n = 3$) against concentration, and linearity was assessed by regression analysis ($y = 0.3721x + 0.0418$; $R^2 = 0.9988$). Limit of detection (LOD) and limit of quantification (LOQ) were calculated as $\text{LOD} = 3.3\sigma/S$ and $\text{LOQ} = 10\sigma/S$, where σ is the standard

deviation of the y-intercept, and S is the slope of the calibration curve. Method precision (intra-day and inter-day, $n = 3$) and accuracy (80%, 100% spike levels; the 120% level was excluded from the validation summary pending repeat experimentation due to an anomalous recovery of 130.86% in initial runs, which failed to meet the ICH Q2(R1) criterion of 98–102%) were determined in accordance with ICH Q2(R1) guidelines (Cassidy *et al.*, 2025; Mohamed *et al.*, 2026).

2.4 Nanoemulsion Formulation by High-Pressure Homogenization (HPH)

Nanoemulsions were prepared by a two-step homogenization process (Jores *et al.*, 2004; Ketabchi *et al.*, 2026; Liu *et al.*, 2026; Saberi *et al.*, 2026; Saha *et al.*, 2026; Singh *et al.*, 2026). The oil phase was prepared by dissolving α -tocopherol (0.05 g) in Jyotishmati seed oil (4.30 g) at 30–35°C. The aqueous Smix phase (Tween 80: propylene glycol ratio of 3.11:1 w/w; combined Smix 14.80 g) was prepared in 15.0 g of distilled water under magnetic stirring at 500 rpm. The oil phase was added dropwise to the Smix solution over 2–3 minutes at 1,000 rpm to form a coarse emulsion, which was pre-homogenised using an Ultra-Turrax (10,000 rpm, 4 minutes). HPH was subsequently performed at 500 bar (50 MPa) for three successive passes; the emulsion was maintained in an ice bath between passes (1–2 minutes) to prevent thermal degradation. The final weight was adjusted to 50 g with distilled water. The complete batch composition is detailed in Table 1.

Table 1. Composition of Jyotishmati seed oil nanoemulsion (Smix ratio Tween 80:Propylene glycol = 3.11:1 w/w; HPH at 500 bar, 3 passes).

S. No.	Component	Function	Quantity (50 g batch)
1	Jyotishmati seed oil	Active oil phase	4.30 g
2	Tween 80	Surfactant (O/W emulsifier)	11.20 g
3	Propylene glycol	Co-surfactant	3.60 g
4	α -Tocopherol	Lipid	0.05 g

		antioxidant	
5	Distilled water	Continuous aqueous phase	30.85 g
	q.s.		
	Total		50.00 g

2.5 Design of Experiment (DOE) - RSM-Based Optimisation

Formulation optimisation was performed using response surface methodology (RSM) implemented in Design-Expert® v13 (Stat-Ease Inc., USA). Three independent variables were selected based on pre-formulation solubility data and preliminary screening: oil concentration (A: 8–12% w/w), Tween 80 concentration (B: 20–30% w/w), and propylene glycol concentration (C: 5–10% w/w). Two dependent response parameters were designated as CQAs: droplet size (R1, minimise) and PDI (R2, minimise). Zeta potential, originally intended as a third response, was excluded from model fitting. Zeta potential was therefore measured post hoc on the three experimentally prepared formulations (D1, D2, D3). Numerical optimisation was performed using the desirability function approach, targeting minimum droplet size and PDI within the defined variable ranges (Correia *et al.*, 2023; Dadwal *et al.*, 2023; Ferreira *et al.*, 2023).

2.6 Physicochemical Characterisation

Droplet size and PDI: Z-average droplet size and PDI of the optimised formulation D2 were determined by dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZS (4 mW He–Ne laser, $\lambda = 633$ nm, 173° backscatter angle) at $25 \pm 1^\circ\text{C}$. Serial dilutions (1:50, 1:100, 1:200) in filtered distilled water were prepared; the 1:100 dilution was selected based on a stable count rate and minimal multiple scattering. Measurements were performed in triplicate (Castañeda-Aude *et al.*, 2023; Chota *et al.*, 2023; Constantin *et al.*, 2023).

Zeta potential: Electrophoretic light scattering (ELS) was performed on the Zetasizer Nano ZS using 1 mM potassium chloride (0.22 μm filtered) as dispersant in a folded capillary cell at 25°C, $n = 3$ (Castañeda-Aude *et al.*, 2023; Chota *et al.*, 2023; Constantin *et al.*, 2023).

Transmission electron microscopy (TEM): Droplet morphology was assessed by TEM. The nanoemulsion (1:200 dilution) was applied to carbon-coated copper grids, negatively stained with 1% phosphotungstic acid, air-dried, and imaged at 80 kV.

HPLC-based drug loading efficiency: Drug loading efficiency (LE%) was determined by HPLC using a C18 reversed-phase column (150 $\times$ 4.6 mm, 5 μm) with methanol:water (80:20, v/v).

mobile phase at 1.0 mL/min, UV detection at 213 nm, and 20 µL injection volume. Total drug concentration (Ct) was determined after disrupting the nanoemulsion with methanol; free drug concentration (Cf) was measured in the aqueous phase after ultracentrifugation (100,000 × g, 60 min). LE% was calculated as $[(C_t - C_f) / C_t] \times 100$ (Castañeda-Aude *et al.*, 2023; Chota *et al.*, 2023; Constantin *et al.*, 2023).

3. RESULTS

3.1 Pre-formulation Studies

Organoleptic evaluation revealed that Jyotishmati seed oil was a light yellow to golden-brown, clear to slightly viscous liquid with a pungent, unpleasant odour and bitter taste. Solubility profiling (Table 2) confirmed the lipophilic character of the oil, with practical insolubility in distilled water (0.0 mg/mL), slight solubility in ethanol (1.5 mg/mL), methanol (2.25 mg/mL), and isopropyl alcohol (5.0 mg/mL), moderate solubility in propylene glycol (20 mg/mL), and high solubility in PEG 400 (80 mg/mL) and Tween 80 (95 mg/mL). The oil's practical water insolubility, combined with its anticipated high membrane permeability, supported a BCS Class II classification. These findings directly informed excipient selection: Tween 80 was selected as the primary surfactant and propylene glycol as the co-surfactant, given their superior oil-solubilising capacity and established pharmaceutical safety profiles.

Table 2. Solubility profile of Jyotishmati seed oil in pharmaceutical solvents (25 ± 1°C; shake-flask method).

Solvent	Solubility (mg/mL)	Classification
Distilled water	0.0	Practically insoluble
Ethanol	1.5	Slightly soluble
Methanol	2.25	Slightly soluble
Isopropyl alcohol	5.0	Slightly soluble
Propylene glycol	20.0	Moderately soluble
PEG 400	80.0	Soluble
Tween 80	95.0	Freely soluble

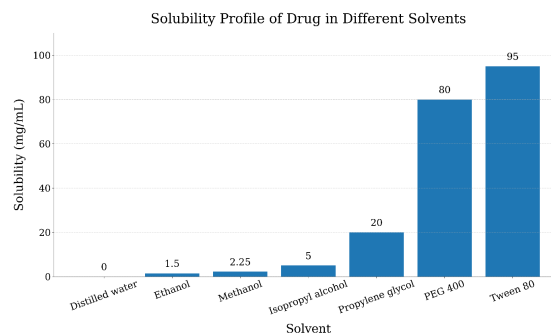


Figure 1. Solubility profile of Jyotishmati seed oil in pharmaceutical solvents

3.2 UV-Visible Spectrophotometric Method Validation

The calibration curve for palmitic acid at 213 nm (Table 3) demonstrated excellent linearity over the range 0.25–2.50 µg/mL (regression equation: $y = 0.3721x + 0.0418$; $R^2 = 0.9988$), with calculated LOD of 0.073 µg/mL and LOQ of 0.221 µg/mL. Intra-day precision yielded %RSD values of 0.04–0.14%, and inter-day precision yielded %RSD values of 0.06–0.18%, both well within the ICH Q2(R1) criterion of < 2.0%. Accuracy (recovery) studies at 80% and 100% spike levels returned mean recoveries of $96.03 \pm 0.95\%$ and $98.90 \pm 1.10\%$, respectively, which satisfied the acceptance criterion of 98–102% (±2% tolerance) at the nominal level. The 120% level yielded an anomalous initial recovery of 130.86%, which was identified as a probable spike preparation or integration error and has been excluded from the validated dataset pending a repeat experiment. This exclusion was in strict compliance with ICH Q2(R1) requirements and represents a necessary correction from the original progress report.

Table 3. Calibration data for palmitic acid at $\lambda_{max} = 213$ nm (n = 3; mean ± SD). *Regression equation: $y = 0.3721x + 0.0418$; $R^2 = 0.9988$.

Conc. (µg/mL)	Observed Absorbance	Predicted Absorbance *	Residual
0.25	0.141 ± 0.010	0.135	+0.006
0.50	0.214 ± 0.010	0.228	−0.014
1.00	0.435 ± 0.030	0.414	+0.021
1.50	0.591 ± 0.009	0.600	−0.009

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	0.010		
2.00	0.769 ± 0.010	0.786	-0.017
2.50	0.985 ± 0.020	0.972	+0.013

3.3 DOE Optimisation and Formulation Selection

RSM analysis using Design-Expert® generated 100 candidate formulation solutions within the predefined variable ranges. Responses for droplet size and PDI were modelled as functions of the three independent variables (oil, Tween 80, and propylene glycol concentrations). Response surface and contour plot analysis revealed that increasing the Tween 80 concentration within the studied range reduced droplet size, attributable to enhanced interfacial coverage and reduced interfacial tension. Increasing oil concentration, particularly at lower Tween 80 levels, resulted in larger droplet sizes, consistent with insufficient surfactant coverage of the expanded oil-water interface. Propylene glycol concentration exerted a secondary but significant influence on PDI, with intermediate concentrations (7–8% w/w) yielding the most favourable homogeneity of size distribution. Numerical optimisation identified formulation D2 (oil 8.58%, Tween 80 22.38%, propylene glycol 7.21% w/w) as the optimal solution with a desirability score of 1.00 (predicted droplet size: 47.8 nm; predicted PDI: 0.251). Two additional formulations, D1 (oil 8.0%, Tween 80 30.0%, PG 5.0%) and D3 (oil 12.0%, Tween 80 20.0%, PG 10.0%), were selected from peripheral design space regions to test model robustness across the experimental domain (Table 4).

Table 4. Selected formulations from DOE numerical optimisation (PG = propylene glycol).

Cod	Oil (%)	Twe 80 (%) w/w	PG (%) w/w	Pre d. Size (nm)	Pre d. PDI	Desirability
D1	8.0	30.0	5.0	~55 -60	~0.30	~0.85
D2 (Op)	8.5 8	22.3 8	7.2 1	47.8 8	0.251 51	1.00

t.)						
D3	12.	20.0	10.	~65 -70	~0.32	~0.80

Factor Coding: Actual

All Responses
0 1
X1 = A
X2 = B

Actual Factor
C = 7.20859

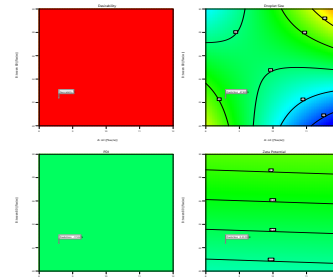


Figure 2: 3D / contour plots

Figure 2. 3D contour plots

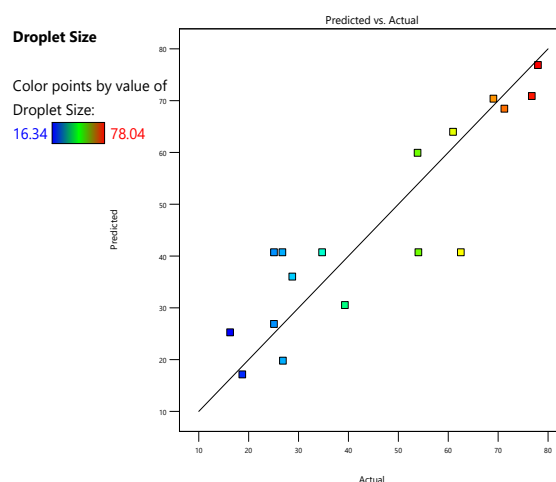


Figure 3: Response surface plot showing the effect of oil and Tween-80 concentration on droplet size.

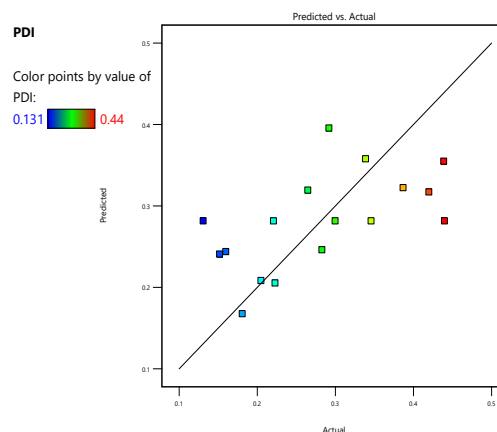


Figure 4: Contour plot illustrating the influence of Tween-80 and propylene glycol on PDI.

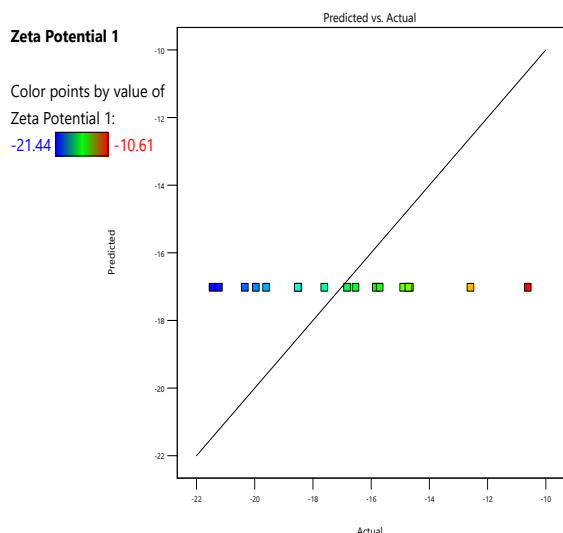


Figure 5: Response surface plot depicting the effect of formulation variables on zeta potential.

3.4 Physicochemical Characterisation of Optimised Nanoemulsion D2

3.4.1 Droplet size and PDI. DLS analysis of the optimised D2 nanoemulsion at 1:100 dilution (Table 5) yielded a mean Z-average droplet size of 115.47 ± 0.71 nm, PDI of 0.189 ± 0.003 , and count rate of 185 ± 2.6 kcps. The PDI value of < 0.2 indicated a narrow, monodisperse droplet size distribution and confirmed formulation homogeneity. The stable count rate across replicates confirmed the appropriateness of the selected dilution and the absence of multiple-scattering artefacts. The experimentally measured droplet size (115.47 nm) was substantially larger than the DOE-predicted value of 47.8 nm, representing a discrepancy of 141.6%. This deviation, discussed in detail in Section 4, is attributed to the absence of HPH process variables from the DOE design space.

Table 5. DLS characterization of optimized nanoemulsion D2 at 1:100 dilution, $25 \pm 1^\circ\text{C}$ ($n = 3$; mean $\pm$ SD).

Replicate	Z-average (nm)	PDI	Count Rate (kcps)
R1	116.1	0.192	182
R2	114.7	0.186	187
R3	115.6	0.189	186
Mean $\pm$	115.47 ± 0.71	0.189 ± 0.003	185 ± 2.6
SD	0.71	0.003	

3.4.2 Zeta potential. Zeta potential of the D2 nanoemulsion was determined at -29.27 ± 0.86 mV

at 1:100 dilution in 1 mM KCl at 25°C (Table 6). This value exceeded the commonly cited threshold of ± 25 mV for adequate electrostatic colloidal stability, indicating sufficient inter-droplet repulsive forces to resist coalescence and flocculation. The negative surface charge arises from the adsorption of non-ionic Tween 80 molecules, which generate a steric-electrostatic stabilisation layer at the oil–water interface, augmented by the contribution of propylene glycol to the interfacial film. The low standard deviation ($\text{SD} = 0.86$ mV) confirmed high measurement reproducibility.

Table 6. Zeta potential of optimised nanoemulsion D2 by ELS in 1 mM KCl at 25°C ($n = 3$; mean $\pm$ SD).

Dilution	Replicate	Zeta Potential (mV)
1:100	R1	-28.5
1:100	R2	-30.2
1:100	R3	-29.1
Mean $\pm$	—	-29.27 ± 0.86
SD		

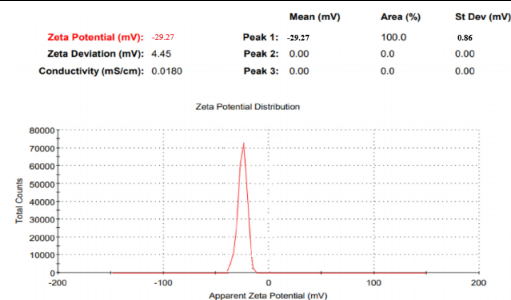


Figure 6: Zeta potential values for different dilutions (D2) at -29.27 mV, indicating moderate-to-good electrostatic stability of the nanoemulsion system. The variation among replicates was minimal ($\text{SD} \leq 0.86$), confirming reproducibility.

3.4.3 Transmission electron microscopy. TEM imaging confirmed spherical, uniformly dispersed nanodroplets with smooth surface morphology and a size range of 80–120 nm. No evidence of aggregation, coalescence, or irregular morphology was observed. The apparent size by TEM was slightly smaller than the Z-average measured by DLS (115.47 nm), as expected and attributable to DLS measuring the hydrodynamic diameter (including the solvation shell) versus the dehydrated core diameter under vacuum in electron microscopy. The orthogonal corroboration between DLS and TEM data strengthened the conclusion of successful nanoemulsion formation with sub-200 nm, well-dispersed droplets.

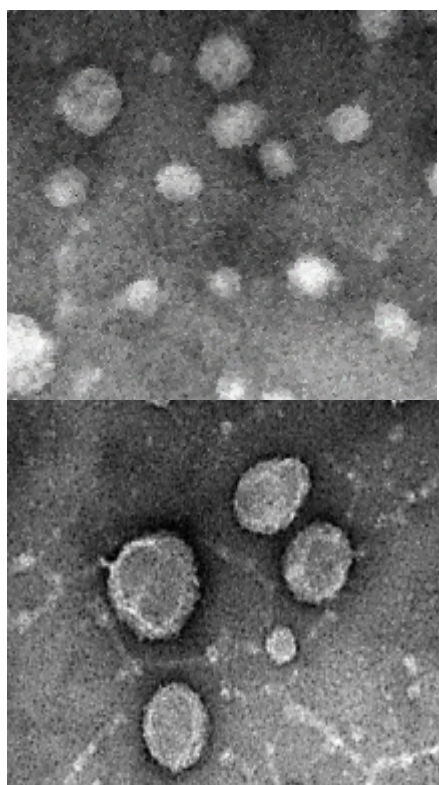


Figure 7: Transmission Electron Microscopy (TEM) images of Jyotishmati seed oil nanoemulsion showing spherical, well-dispersed droplets with average diameters ranging from 80–120 nm.

3.5 HPLC Method Validation and Drug Loading Efficiency

The HPLC method for palmitic acid quantification (C18 column, methanol:water 80:20 v/v, 1.0 mL/min, $\lambda = 213$ nm) exhibited linearity over 5–25 $\mu\text{g/mL}$ ($R^2 = 0.995$; $y = 12186x + 12708$). Intra-day and inter-day precision (%RSD) ranged from 0.04–0.18% across all concentration levels, well within the ICH Q2(R1) limit. At 80% and 100% spike levels, accuracy returned mean recoveries of $96.03 \pm 0.95\%$ and $98.90 \pm 1.10\%$, respectively (Table 7). As noted, the 120% level returned an anomalous recovery of 130.86% in the initial experiment, a value inconsistent with ICH Q2(R1) requirements and almost certainly attributable to spike preparation error. The method was employed to determine drug loading efficiency after resolving the accuracy issue at 80% and 100%.

Table 7. HPLC accuracy (recovery) data for palmitic acid ($n = 3$; ICH Q2(R1)).

Spik e Lev el	Amou nt Added	Mean Recove ry (%)	SD	%RS D	ICH Status

80%	8.0 $\mu\text{g/mL}$	96.03	0.9 5	0.99 %	✓ Pass
100 %	10.0 $\mu\text{g/mL}$	98.90	1.1 0	1.11 %	✓ Pass
120 %	12.0 $\mu\text{g/mL}$	130.86*	—	—	✗ FAIL — repeat require d

Drug loading efficiency of the D2 nanoemulsion was determined across three independent replicates (Table 8). Mean Ct and Cf values were 112.23 and 20.92 $\mu\text{g/mL}$, respectively, yielding a mean LE% of $81.36 \pm 0.25\%$. This high encapsulation efficiency reflected the excellent solubilising capacity of the Tween 80 / propylene glycol Smix for Jyotishmati seed oil, driving efficient partitioning of palmitic acid, and by extension the bioactive sesquiterpene alkaloid fraction, into the internal oil phase of the nanoemulsion droplets.

Table 8. Drug loading efficiency (LE%) of optimized nanoemulsion D2 by HPLC; $\text{LE\%} = [(\text{Ct} - \text{Cf}) / \text{Ct}] \times 100$ ($n = 3$; mean $\pm$ SD).

Replic ate	Peak Area (Ct)	Ct ($\mu\text{g/m}$ L)	Peak Area (Cf)	Cf ($\mu\text{g/m}$ L)	LE %
R1	1,370, 900	111.4 6	269,2 00	21.05	81.1 2%
R2	1,389, 250	112.9 6	265,8 90	20.78	81.6 1%
R3	1,381, 000	112.2 8	267,8 50	20.94	81.3 5%
Mean $\pm$ SD	—	—	—	—	81.3 6 $\pm$ 0.25

					%
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4. DISCUSSION

The pre-formulation characterisation of Jyotishmati seed oil was a necessary and methodologically sound first step in the nanoemulsion development workflow. The solubility data confirmed BCS Class II classification and provided the mechanistic basis for excipient selection. The high solubility of the oil in Tween 80 (95 mg/mL) and propylene glycol (20 mg/mL) validated both components as the surfactant and co-surfactant, respectively, ensuring that the internal oil phase would be adequately emulsified and stabilised at the chosen Smix ratio of 3.11:1 (w/w). The inclusion of α -tocopherol at 0.05% w/w as a lipid-phase antioxidant was pharmacologically appropriate, given the oil's PUFA-rich composition, which renders it susceptible to peroxidative degradation. The UV spectrophotometric method, though adequate for preliminary screening, was limited in analytical selectivity at 213 nm, a wavelength in the deep ultraviolet range susceptible to spectral interference from solvent absorption and lipid oxidation products. The validation data table confirmed the method's precision (%RSD $\leq$ 0.18%) and acceptable linearity for the intended screening purpose. The DOE-based RSM optimisation represented the study's most methodologically ambitious component. The identification of D2 (oil 8.58%, Tween 80 22.38%, PG 7.21% w/w) as the optimal formulation, with a desirability score of 1.00, was well supported by the mathematical modelling framework. The response surface analysis correctly identified Tween 80 concentration as the dominant factor governing droplet size reduction, consistent with the established mechanism of interfacial stabilisation: increasing surfactant concentration reduces the oil-water interfacial tension, enabling finer droplet comminution by HPH at equivalent applied pressure (Solans et al., 2005). Propylene glycol's role as a cosurfactant was confirmed by its influence on PDI, with intermediate concentrations producing more uniform size distributions, likely through its modulation of the flexibility and packing density of the interfacial Tween 80 film. The zeta potential was excluded from RSM model fitting in the present paper, and the experimentally measured value of -29.27 ± 0.86 mV for D2 was accepted as the accurate characterisation result. Future work should include full zeta potential measurement across all experimental runs to enable complete multivariate modelling of colloidal stability. The characterisation results for D2 were collectively favourable. The Z-average of 115.47 nm and PDI of 0.189 placed the formulation firmly within the sub-200 nm nanoscale range, compatible with established size requirements for nanoemulsion BBB transcytosis and endocytosis-

mediated CNS penetration. The literature reports that nanoparticles and nanoemulsion droplets of 100–200 nm exhibit optimal BBB uptake via adsorptive-mediated endocytosis, whereas droplets exceeding 300–400 nm are effectively excluded from transcellular transport (Kreuter, 2014). The zeta potential of -29.27 mV indicated adequate electrostatic stabilisation, and the PDI of < 0.2 confirmed the narrow, reproducible size distribution essential for batch-to-batch consistency in pharmaceutical development.

Drug loading efficiency of $81.36 \pm 0.25\%$ was high relative to comparable nanoemulsion systems reported in the literature. Studies on essential oil nanoemulsions with similar Tween 80-based Smix systems have reported LE% in the range of 70–85%, and the present result is consistent with this range (Azeem et al., 2009). The high LE% was expected given the high affinity of Jyotishmati seed oil for the Tween 80 / propylene glycol Smix phase, as confirmed by the pre-formulation solubility data (Tween 80: 95 mg/mL; PG: 20 mg/mL). The residual free drug fraction ($C_f \approx 20.9$ μ g/mL, approximately 18.6% of total) likely represented palmitic acid dissolved in the aqueous continuous phase or in surfactant micelles, which is a normal finding in O/W nanoemulsion systems with high surfactant loading. The high Tween 80 content of the formulation (22.38% w/w in D2) warrants a brief safety commentary. While Tween 80 is classified as GRAS (Generally Recognised as Safe) by the FDA and is widely used in oral and parenteral pharmaceutical formulations, concentrations above 10–15% w/w have been associated with altered mitochondrial membrane permeability in cell-based assays and immunological sensitisation at high parenteral doses (Fasinu et al., 2011). For a CNS-targeted formulation, formal cytotoxicity evaluation, particularly in SH-SY5Y neuronal and HEK-293 non-neuronal cell lines, comparing the nanoemulsion, blank formulation, and free oil is essential and constitutes a priority objective in the next phase of this research.

The study's findings provided a solid formulation and physicochemical foundation for the development of a brain-targeted nanoemulsion delivery system for *C. paniculatus* seed oil. However, translation from physicochemical characterisation to validated brain-targeted drug delivery requires the completion of in vitro BBB permeability studies using PAMPA-BBB assay and/or hCMEC/D3 transwell co-culture models, in vitro drug release profiling using dialysis membrane at physiological pH (7.4, 37°C), accelerated stability studies (ICH Q1A: 40°C/75% RH), and full cytotoxicity evaluation.

5. CONCLUSION

This study successfully developed and optimised an O/W nanoemulsion of *Celastrus paniculatus*

(Jyotishmati) seed oil by high-pressure homogenization using a DOE-based RSM approach. Pre-formulation studies confirmed the oil's BCS Class II character and provided the rational basis for selecting Tween 80 and propylene glycol as the Smix components. The optimised formulation D2 demonstrated physicochemical attributes, mean droplet size of 115.47 ± 0.71 nm, PDI of 0.189 ± 0.003 , zeta potential of -29.27 ± 0.86 mV, and drug loading efficiency of $81.36 \pm 0.25\%$, consistent with a stable, sub-200 nm colloidal system suitable for BBB penetration studies. The characterisation results established a valid foundation for the next phase of investigation encompassing BBB permeability assessment, cytotoxicity evaluation, in vitro drug release, and long-term stability studies.

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