

Development, Optimisation and Characterization of *Bacopa monnieri* Extract-Loaded Nanoemulsion (BMNE) System for Neuroprotective Applications and Enhanced Therapeutic Efficacy

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder (NDD) characterized by amyloid- β (A β) accumulation, synaptic dysfunction, and cortical neuronal loss. Current pharmacological interventions provide limited symptomatic benefit and are hindered by poor brain bioavailability and restricted blood-brain barrier (BBB) penetration. Nano-based delivery systems offer a promising strategy to enhance targeted central nervous system (CNS) delivery of phytoconstituents with neuroprotective potential. In the present study, a *Bacopa monnieri* extract-loaded nanoemulsion (BMNE) was developed and evaluated through physicochemical analyses. Excipients were screened for optimal solubilization, and the optimized BMNE exhibited thermodynamic stability with an average droplet size of ~212 nm and zeta potential of -28 mV. Transmission electron microscopy (TEM) confirmed spherical nanodroplets within the 100-250 nm range, and FT-IR analysis verified chemical bonds and functional groups present on the surface of the NE. These findings suggest that the developed and optimised BMNE demonstrates favourable physicochemical properties and promising multitarget interactions relevant for neuroprotection and enhancing its therapeutic efficacy.

1. Introduction

Alzheimer's disease (AD) is the most common form of dementia and represents a significant global health challenge, particularly in aging populations. It is characterized by the accumulation of amyloid- β (A β) peptides and neurofibrillary tangles (NFTs), cortical neuronal loss, synaptic dysfunction, and progressive cognitive decline. Despite extensive research into its molecular pathophysiology, most currently available treatments are symptomatic and do not modify disease progression [1]. As AD is a complex disorder, involving mitochondrial dysfunction, oxidative stress, neuroinflammation, excitotoxicity, poor proteostasis, and abnormal protein aggregation therefore, the treatment approaches that can target many pathogenic pathways at once are required. Excessive formation of reactive oxygen species (ROS) causes oxidative stress, which in turn leads to DNA damage followed by lipid peroxidation, and neuronal death [2]. At the same time, mitochondrial dysfunction speeds up synaptic loss by interfering with cellular energy metabolism and intensifying oxidative damage. While glutamate-mediated excitotoxicity encourages calcium dysregulation and neuronal degeneration, persistent stimulation of microglia and astrocytes maintains a pro-inflammatory environment that worsens neuronal damage. Furthermore, poor removal of misfolded proteins, such as hyperphosphorylated tau and A β , contributes to network disintegration and synaptic dysfunction. Because of the complex interactions between these processes, single-target therapies have had little long-term efficacy in modifying the course of the disease

[3].

Due to their inherent poly pharmacological qualities, bioactive chemicals originating from plants have attracted a lot of attention in this regard. By altering several signalling cascades, including as antioxidant defence systems, inflammatory mediators, neurotransmitter pathways, and apoptotic regulators, phytochemicals frequently produce combinatorial effects. Their structural diversity not only allows treating certain symptoms but also allows them to interact with a variety of molecular targets, potentially restoring cellular balance. Additionally, many natural agents have lower systemic toxicity and relatively good safety profiles, which makes them appealing options for the long-term treatment of chronic NDDs like AD [4].

Brahmi, also known as *Bacopa monnieri* (L.), is a classic Ayurvedic medicinal plant that is acknowledged as a “*Medhya Rasayana*” for improving cognitive function. It has a wide range of bioactive components, including flavonoids, alkaloids, sterols, amino acids, and triterpenoid saponins called bacosides. *Bacopa monnieri* may have neuroprotective effects through antioxidant activity, acetylcholinesterase inhibition, dopaminergic and cholinergic signalling modulation, A β aggregation attenuation, and synaptic plasticity improvement, according to the reported experimental studies. These multitarget mechanisms make it a promising candidate for AD management. Nevertheless, its clinical applicability remains constrained by poor aqueous solubility, limited BBB permeability, and rapid metabolic degradation, resulting in reduced bioavailability [5].

Nanotechnology-based drug delivery systems have emerged as effective strategies to overcome pharmacokinetic limitations and enhance therapeutic efficacy and brain targeting. Among these, nanoemulsions offer advantages such as improved absorption, controlled release, increased stability, and enhanced solubilization of lipophilic phytoconstituents. Considering these factors, the present study focuses on the development and characterization of a *Bacopa monnieri* extract-loaded nanoemulsion (BMNEs) aimed at improving its bioavailability and neuroprotective efficacy in AD [6].

2. Method

2.1. Preparation of *Bacopa monnieri* Extract

The *Bacopa monnieri* crude extract was sourced from Vital Herbs Company, Delhi. Extraction was carried out using aqueous and hydroalcoholic solvents in a Soxhlet apparatus. The solvent was evaporated under reduced pressure using a rotary evaporator, and the remaining extract was dried at 37°C. Until further examination, the dried extract was kept at 4°C in sealed glass containers that were shielded from the light [7].

2.2. Chromatographic Analysis of *Bacopa monnieri*

Liquid Chromatography-Mass Spectrometry (LC-MS) was used for phytochemical profiling of the extract of *Bacopa monnieri*, making it possible to detect polar and thermally labile bioactive components with sensitivity and selectivity. L-ascorbic acid was identified and measured as a representative marker compound in the extract. Analysis was conducted using an LC-MS system with an electrospray ionization (ESI) interface. Acetonitrile, formic acid, and HPLC-grade methanol were employed as solvents. L-ascorbic acid was used as the reference standard. Methanol and 0.3% phosphoric acid made up the mobile phase, which was filtered via a 0.45 μ m membrane and degassed before use. Under isocratic circumstances, separation was carried out. Mass spectra

were acquired using mass-to-charge (m/z) ratios, and ESI was used for detection in positive ion mode. To create a calibration curve, a stock solution of L-ascorbic acid (100 ng/mL) in 80% methanol was made. After being dissolved in 80% methanol and passed through a 0.45 μm syringe filter, the *Bacopa monnieri* extract (1 mg/mL) was added to the LC-MS apparatus. The sample's peak area was compared to the standard calibration curve in order to do quantification [8].

2.3. Preparation of *Bacopa monnieri* Extract-Loaded Nanoemulsion (BMNEs)

The solubility of *Bacopa monnieri* extract in different oils, surfactants, and co-surfactants was assessed in order to determine appropriate formulation components and 1ml of each excipient was mixed with an excess of extract. The mixture was agitated for a whole day at room temperature. Following equilibrium, the phenolic and flavonoid estimation was done to quantify the dissolved concentration of excipients. Further, to develop the nanoemulsion (NE), excipients with the highest solubility were chosen and water titration method was used to develop the NE. A transparent and stable system was then achieved by adding the aqueous phase dropwise while continuously mixing. Stable compositions were selected for additional analysis after formulations were visually evaluated for transparency, phase separation, creaming, or precipitation [9].

2.4. Characterization of optimized *Bacopa monnieri* Nanoemulsion (BMNE)

2.4.1. Thermodynamic Stability

Thermodynamic stress testing was used to assess the physical stability of optimized nanoemulsion compositions. To identify phase separation, creaming, or precipitation, formulations were subjected to high-speed centrifugation, freeze-thaw cycles (-20°C to 25°C), and heating-cooling cycles (4°C to 45°C). Formulations with no discernible instability were the only ones chosen for additional examination. To verify the robustness of the system, dispersibility in excess water was also investigated [10].

2.4.2. Particle Size (PSA) and Polydispersity Index (PDI)

Dynamic light scattering was used to measure the droplet size and PDI. Before being measured, samples were suitably diluted and sonicated. To evaluate homogeneity and average size of the formulated NE, the mean particle size and PDI values were recorded [11].

2.4.3. Zeta Potential Analysis

A Zetasizer Nano ZS was used to detect the surface charge of the nanoemulsion droplets. To assess colloidal stability, zeta potential values were measured; greater the absolute values signify better aggregation resistance [12].

2.4.4. Transmission Electron Microscopy (TEM)

Transmission electron microscopy was employed to analyze the droplet structure and morphology. To confirm size and spherical shape, diluted samples were placed on carbon-coated copper grids, negatively stained, and examined at appropriate magnifications [13].

2.4.5. Physicochemical and Rheological Evaluation

Examination of rheological behaviour, density, conductivity, pH, and viscosity of the optimized formulation was done. pH along with conductivity was measured at 37°C and Brookfield viscometer was used to test viscosity. To ascertain viscoelastic qualities, flow behaviour was assessed at different shear rates using a rheometer [14].

2.4.6. Fourier Transform Infrared (FT-IR) Analysis

FT-IR spectroscopy was used to analyse topologically available molecular arrangement on the BMNE surface. To assess the structural interactions between the extract and formulation components, IR radiation was transmitted across the optimised formulation stock and the spectra were acquired in the 4000-400 cm⁻¹ region, and distinctive functional group peaks were examined [15].

3. Results and Discussion

3.1. LC-MS Analysis

The significance of L-ascorbic acid as a detectable phytoconstituent in the *Bacopa monnieri* extract was confirmed by further analysis using LC-MS. Calibration curves created from standard solutions were used for quantification. Thus, making the extract's phytochemical a potential antioxidant.

3.2. Preparation and Optimization of *Bacopa monnieri* Nanoemulsion System

3.2.1. Screening of Excipients and Solubility Studies

Formulation components were selected based on their hydrophile-lipophile balance (HLB) values, appropriate for oil-in-water nanoemulsions. To identify suitable excipients, the solubility of *Bacopa monnieri* extract was evaluated across various oils, surfactants, and co-surfactants. Among the screened excipients, almond oil (17.81 ± 0.98 mg/mL), triacetin (28.08 ± 0.87 mg/mL), and ethanol (19.21 ± 1.44 mg/mL) demonstrated the highest solubility for the extract. Based on these findings, almond oil was selected as the oil phase, triacetin as the surfactant, and ethanol as the co-surfactant, as compiled in **Table 1**. Distilled water was used as the aqueous phase for nanoemulsion preparation.

Table 1. Solubility analysis of various oils, surfactant and co surfactant with *Bacopa monnieri* extract

Excipient (Oil)	<i>Bacopa monnieri</i> Solubility (mg/ml)	Excipient (Surfactant)	<i>Bacopa monnieri</i> Solubility (mg/ml)	Excipient (Co-surfactant)	<i>Bacopa monnieri</i> Solubility (mg/ml)
Linseed Oil	13.51 ± 0.83	PEG	16.21 ± 1.23	Ethanol	19.21 ± 1.44

Castor Oil	12.02 ± 1.22	Triacetin	28.08 ± 0.87	Isopropanol	16.99 ± 0.87
Tea Tree Oil	14.67 ± 1.57	Cween 20	14.53 ± 0.98		
Almond Oil	17.81 ± 0.98	Cween 80	16.01 ± 1.21		
Jjoba Oil	10.02 ± 1.11	Poly acrylic acid	12.01 ± 0.43		
Isopropyl Myristate (IPM)	10.86 ± 0.66	TPP	12.02 ± 1.56		
		PVP	12.67 ± 0.97		
		Pluronic acid	8.09 ± 1.23		

3.2.2. Optimized of *Bacopa monnieri* Nanoemulsion

The pseudo ternary phase diagram plot was created to reveal the existence range of nano-emulsions by showing the relationship between the phase behavior of a mixture and its composition. To obtain o/w nano-emulsions, pseudo ternary phase diagrams were created individually for each Smix ratio. The isotropic zone shifted towards Smix when the surfactant ratio was raised. The Smix form of these two components must have higher solubility and stability. The clear and homogeneous solutions are indicated by the area of solubilization in phase diagrams

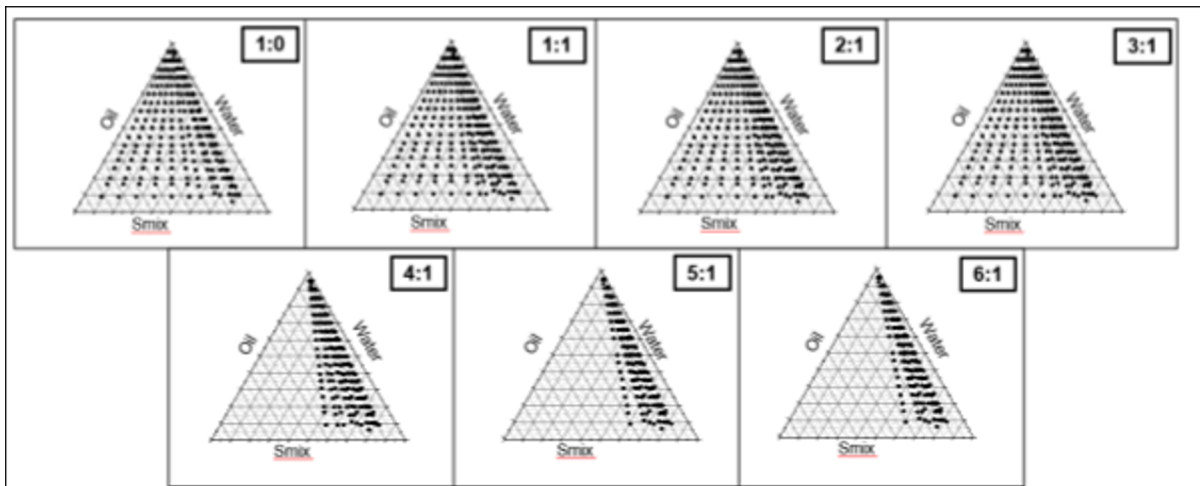


Fig:1 Showing pseudo ternary phase diagram indicating o/w nano-emulsions using Almond oil (Oil), Triacetin (Surfactant) and ethanol (Co surfactant) with all Smix ratio of *Bacopa monnieri*

3.2.3. Thermodynamic Stability

The optimized *Bacopa monnieri* nanoemulsion formulations were tested for thermodynamic stability under a variety of stress conditions, such as centrifugation ($1250 \times g$), freeze-thaw cycles, temperature changes (4°C , 37°C , and 45°C), and evaluation of water dispersibility. Several formulations successfully withstood all stress conditions without evidence of phase separation, creaming, or precipitation. Based on stability performance, selected Smix ratios (1:0, 1:1, 2:1, and 3:1) were considered stable and shortlisted for further physicochemical evaluation.

3.3.4. Particle Size and Polydispersity Index

Formulations that demonstrated thermodynamic stability were further analyzed for droplet size and polydispersity index (PDI) using a particle size analyzer. The optimized nanoemulsions exhibited droplet sizes within the nanometric range. PDI values were below 0.5, indicating uniform droplet distribution and good dispersion stability. These results confirmed the suitability of the selected formulation for further characterization and biological evaluation. Experiments with the other combination of excipients revealed a smaller size range when compared to the size of nano-emulsions reported in the literature. In the current work, however, the spherical radius (nm) is significantly larger, which could be related to the fact that both extracts contain a complex combination rather than a pure chemical. The PDI value of the optimized extract nano-emulsions is 1 and 0.540, indicating a moderately homogeneous distribution of nano-emulsions.

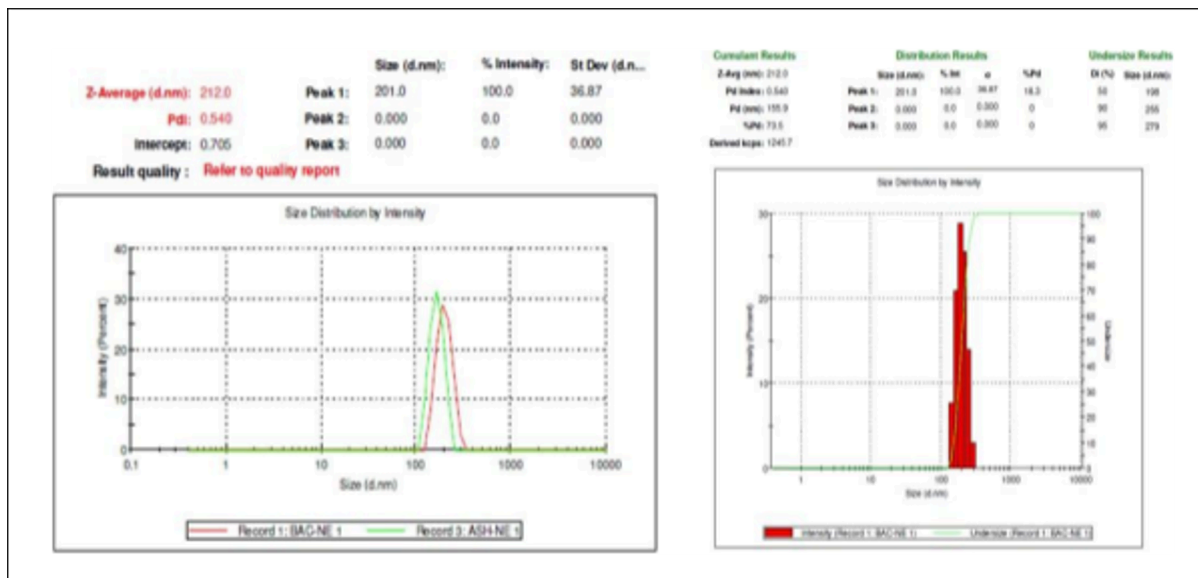


Fig:2 Summarization of Particle size and Polydispersity Index of the optimized nano-emulsions of *Bacopa monnieri*

3.3.5. Zeta Potential Analysis

The surface charge of the optimized *Bacopa monnieri* nanoemulsion was evaluated to assess colloidal stability. The measured zeta potential was **-28.4 mV**, indicating sufficient electrostatic repulsion between droplets to prevent aggregation. The negative surface charge suggests stabilization of the system through interfacial arrangement of the selected surfactant system. This range of zeta potential values promotes dispersion stability and lowers the risk of flocculation, indicating that the improved formulation is appropriate for more research.

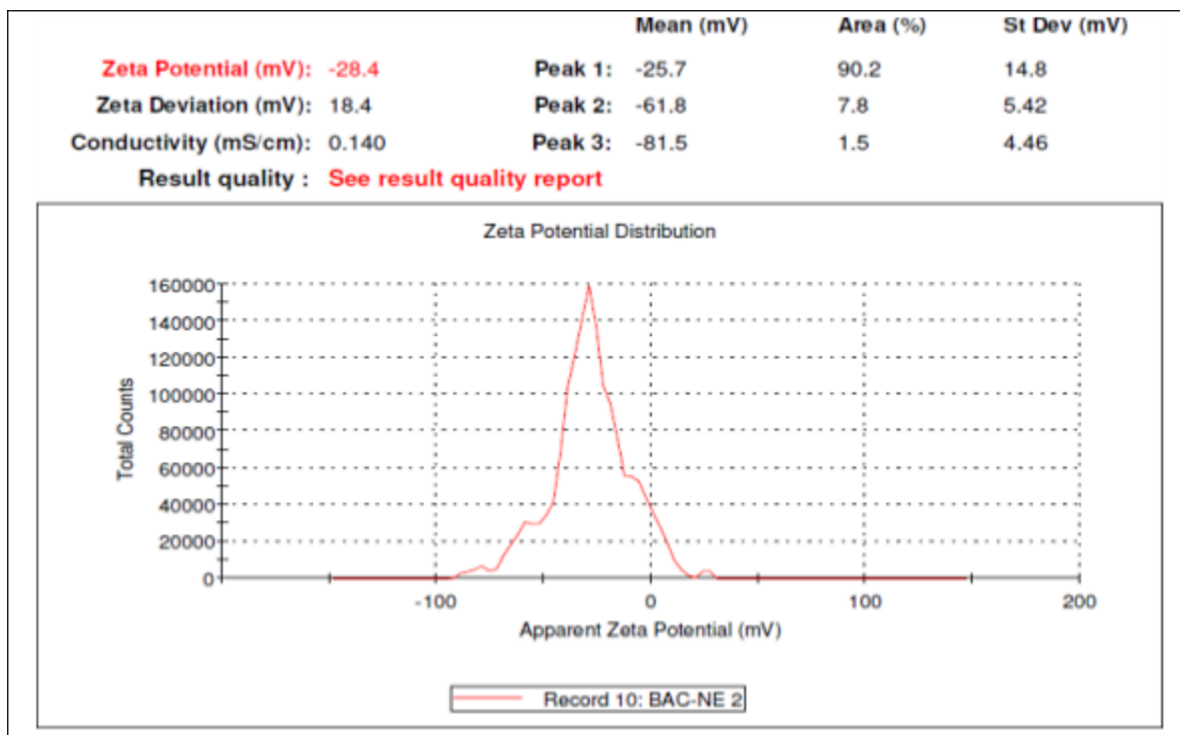
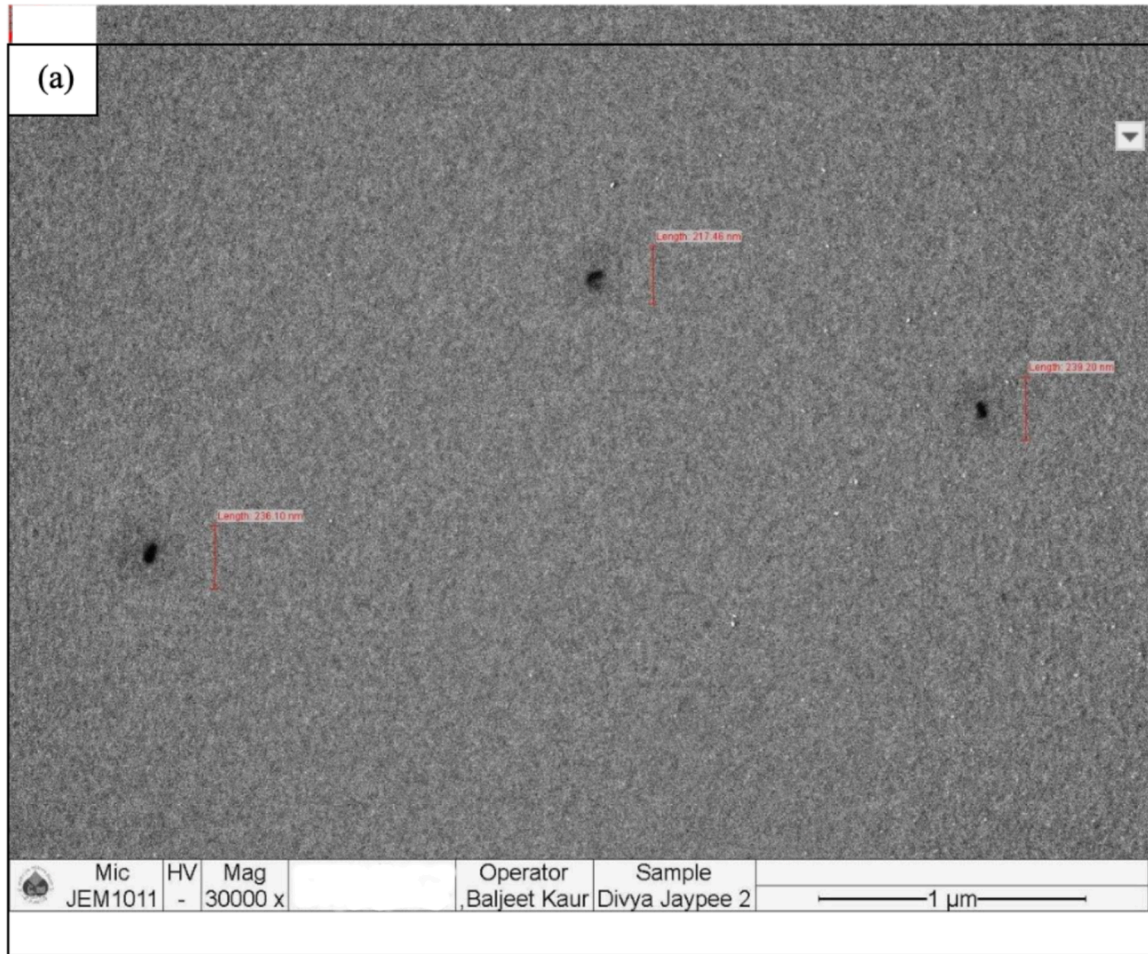


Fig: 3 Zeta Potential of the optimized nano-emulsions of *Bacopa monnieri*

3.3.6. Transmission Electron Microscopy (TEM) Analysis

Transmission electron microscopy analysis was used to investigate the improved *Bacopa monnieri* nanoemulsion's shape and droplet size. The micrographs developed showed spherically distributed, well-dispersed droplets in the 150-300 nm nanometric range. Homogeneity and structural integrity of the formulation were confirmed by the observed particle size, which matched the values derived from dynamic light scattering analysis.



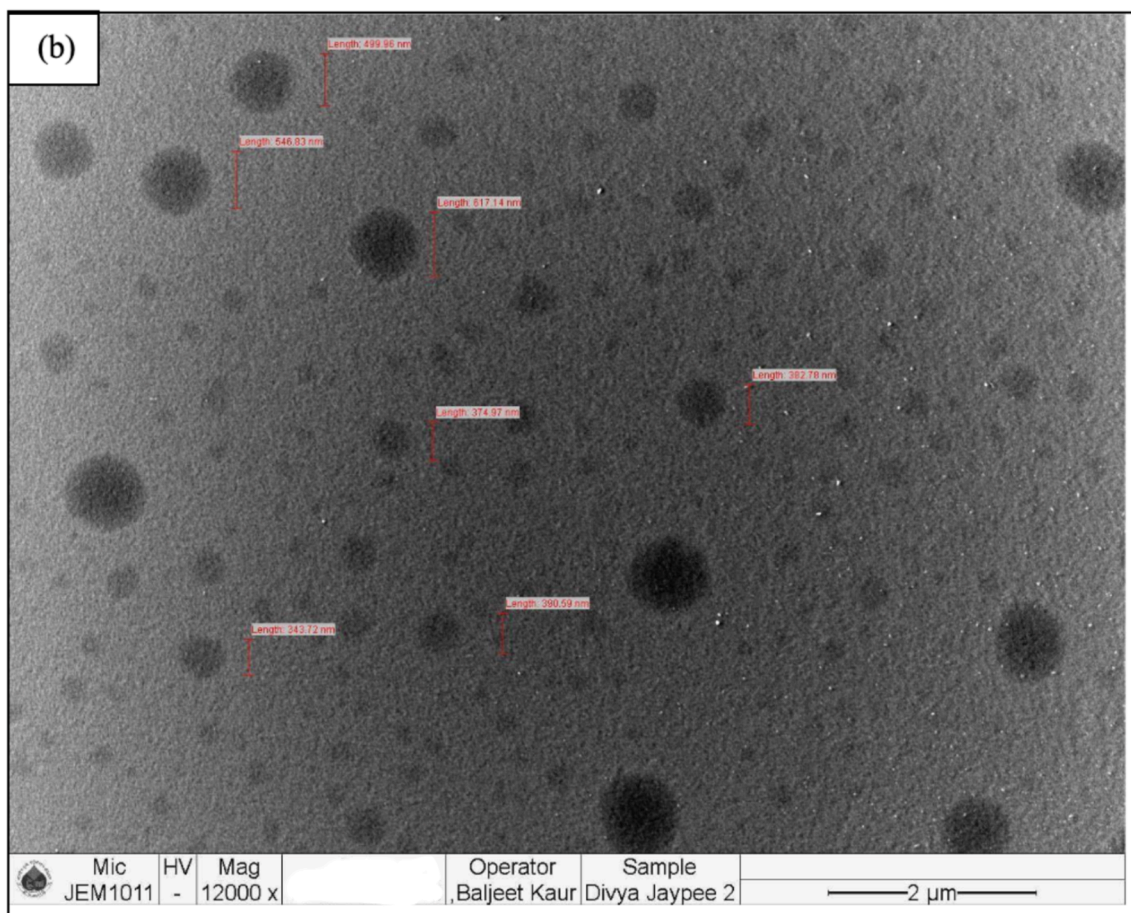


Figure 4 : (a) and (b) TEM image of the optimized *Bacopa monnieri* extract loaded nano-emulsions at 30,000x and 12,000x magnification (scale bar showing 500 and 1000 nm range)

3.3.7. MEASUREMENT OF RHEOLOGICAL AND OTHER PARAMETERS

The optimized extract-loaded oil-in-water (O/W) nanoemulsion was rheologically and physicochemically evaluated to assess its formulation stability and suitability for biomedical applications. The viscosity-shear rate profile exhibited shear-thinning behavior under applied stress, indicating that viscosity decreased with increasing shear rate. This behavior suggests structural rearrangement of the dispersed droplets within the nanoemulsion. According to the power law model, pseudo-plastic (non-Newtonian) flow behavior was confirmed by the matching shear stress versus shear rate curve, which showed a non-linear connection. These shear-dependent characteristics are beneficial because they preserve stability at rest and make administration easier under mechanical stress. Furthermore, nanoemulsions containing extracts of *Bacopa monnieri* had measured pH values of 3.74. These findings indicate the need for possible buffering changes to improve compatibility for neurological applications while retaining formulation stability, even though they are slightly acidic in comparison to the physiological brain pH (~7.2) as stated in Table:2.

Table:2 Summarization of rheological parameters of the optimized nano-emulsions.

Sample	pH	Conductivity (mS/cm)	Viscosity (cP)
BAC Bare nano-emulsion	3.88 ± 0.27	66.1 ± 0.6	32.3 ± 0.2
BAC-ALGA	3.74 ± 0.5	86.8 ± 0.5	41.1 ± 0.6

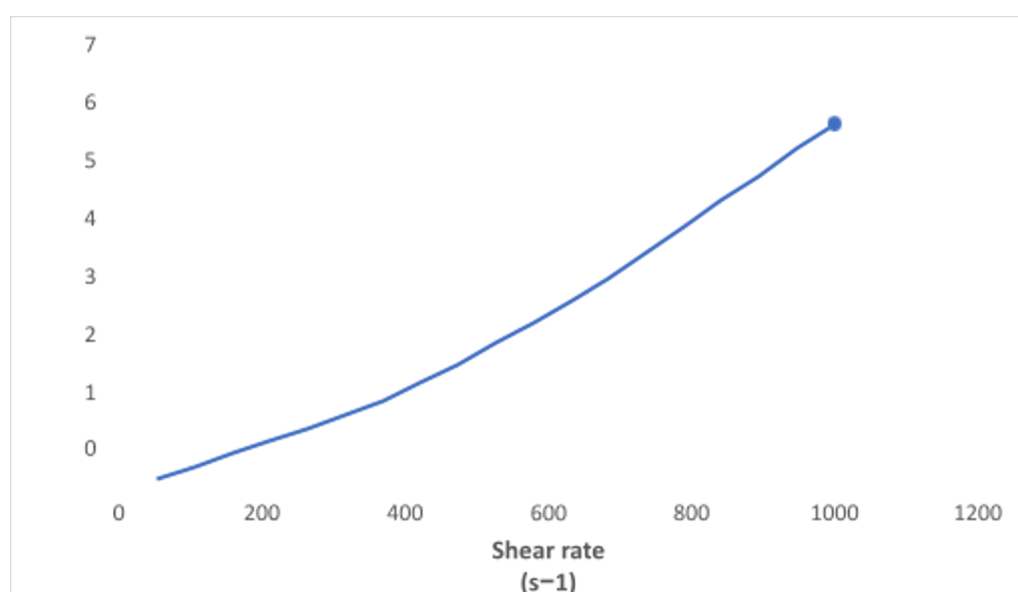


Fig:5 Graph of steady state test: shear stress (Pa) vs shear rate (s⁻¹) of the optimized ASH-ALGA & BAC-ALGA nano emulsion *Bacopa monnieri*

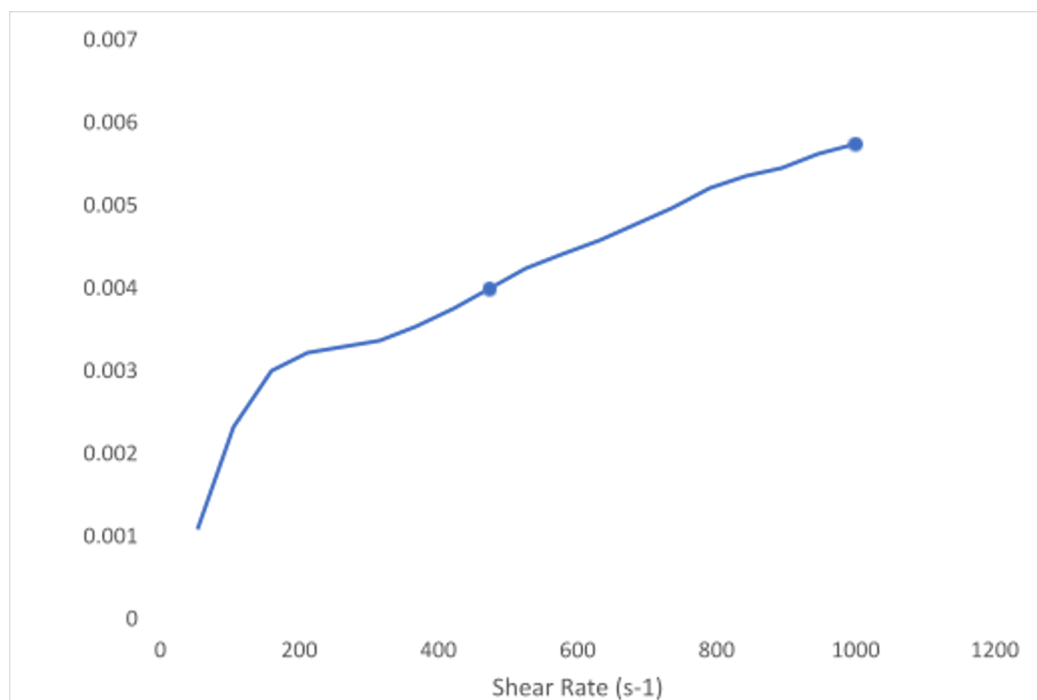


Fig:6 Graph of viscosity (Pa. s) vs shear rate (s⁻¹) of the optimized ASH-ALGA & BAC-ALGA nanoemulsion *Bacopa monnieri*

3.3.8. FT-IR Analysis

Fourier Transform Infrared (FT-IR) spectroscopy was carried out to evaluate the incorporation of *Bacopa monnieri* extract into the optimized nanoemulsion. Spectral analysis was performed over the range of 4000–400 cm⁻¹. Characteristic peaks corresponding to flavonoid functional groups (approximately 1028–1649 cm⁻¹) were observed in the pure extract. However, these characteristic bands were significantly reduced or masked in the extract-loaded nanoemulsion spectrum, suggesting successful encapsulation of the extract within the formulation matrix. The absence of prominent extract peaks on the surface indicates effective internalization of phytoconstituents within the nanoemulsion system.

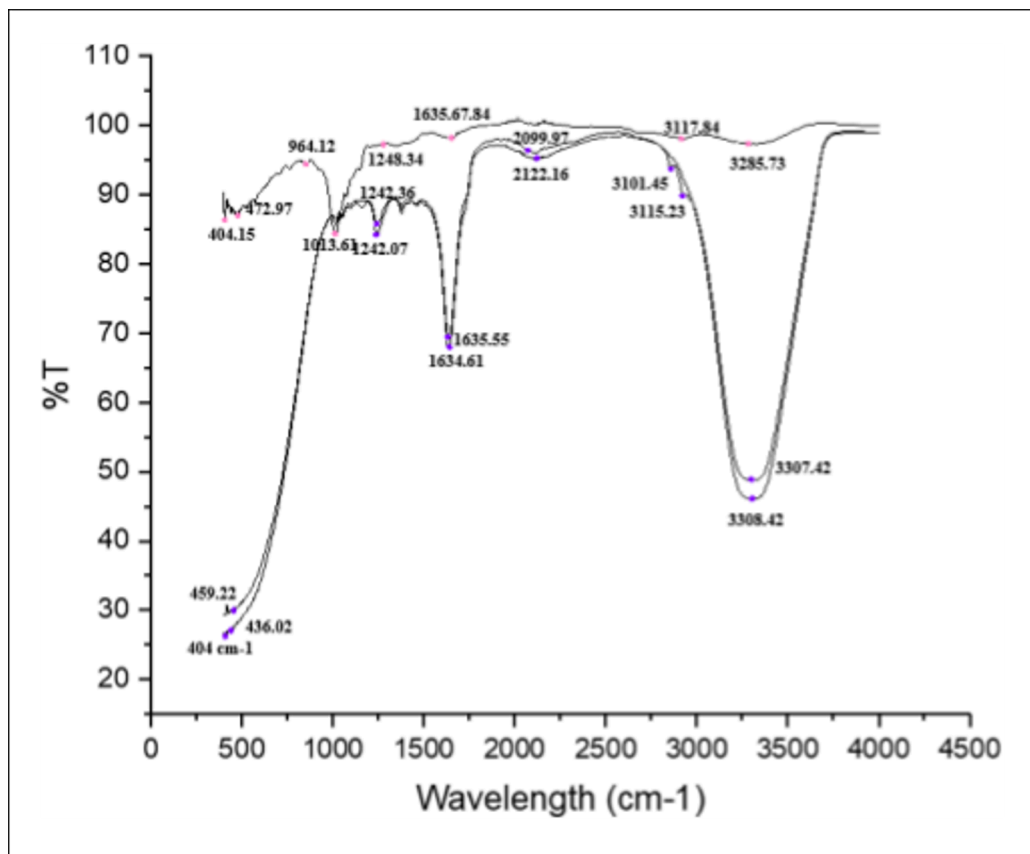


Fig:7 FT – IR analysis of the plain *Bacopa monnieri* extract, optimized *Bacopa monnieri* loaded nano-emulsions and bare nano-emulsions.

4. Conclusion

Systematic evaluation of phytochemical and pharmacological relevance of *Bacopa monnieri* followed by the development of a nanoemulsion-based formulation to enhance its delivery profile. Bioactivity prediction and target-based assessment of *Bacopa* active constituents suggested that multiple molecular pathways implicated in neurodegeneration, supporting its traditional use in cognitive disorders. To improve solubility, stability, and potential bioavailability, an optimized oil-in-water nanoemulsion system was formulated and characterized. The physicochemical and rheological properties indicated a stable, shear-responsive formulation suitable for further biological evaluation and advances in targeting neurodegenerative disorders.

5. References

- [1] M. Agarwal, M. R. Alam, M. K. Haider, Md. Z. Malik, and D.-K. Kim, “Alzheimer’s Disease: An Overview of Major Hypotheses and Therapeutic Options in Nanotechnology,” *Nanomaterials*, vol. 11, no. 1, p. 59, Dec. 2020, doi: 10.3390/nano11010059.
- [2] A. Kumar, J. Sidhu, F. Lui, and J. W. Tsao, “Alzheimer Disease,” in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2025. Accessed: Feb. 16, 2026. [Online]. Available: <http://www.ncbi.nlm.nih.gov/books/NBK499922/>
- [3] X. Du, X. Wang, and M. Geng, “Alzheimer’s disease hypothesis and related therapies,” *Transl. Neurodegener.*, vol. 7, no. 1, p. 2, Jan. 2018, doi: 10.1186/s40035-018-0107-y.

- [4] “Naturally occurring phytochemicals for the prevention of Alzheimer’s disease - Kim - 2010 - Journal of Neurochemistry - Wiley Online Library.” Accessed: Mar. 11, 2026. [Online]. Available: <https://onlinelibrary.wiley.com/doi/full/10.1111/j.1471-4159.2009.06562.x>
- [5] A. S. Abdul Manap *et al.*, “Bacopa monnieri, a Neuroprotective Lead in Alzheimer Disease: A Review on Its Properties, Mechanisms of Action, and Preclinical and Clinical Studies,” *Drug Target Insights*, vol. 13, p. 1177392819866412, Jul. 2019, doi: 10.1177/1177392819866412.
- [6] M. Wiciński, A. Fajkiel-Madajczyk, J. Wójcicki, M. Ozorowski, and M. Szambelan, “The Role of Bacopa monnieri in Alzheimer’s Disease: Mechanisms and Potential Clinical Use—A Review,” *Nutrients*, vol. 17, no. 22, p. 3538, Nov. 2025, doi: 10.3390/nu17223538.
- [7] “Using Soxhlet Ethanol Extraction to Produce and Test Plant Material (Essential Oils) for Their Antimicrobial Properties | Journal of Microbiology & Biology Education.” Accessed: Feb. 16, 2026. [Online]. Available: <https://journals.asm.org/doi/10.1128/jmbe.v15i1.656>
- [8] “GC/MS and LC/MS Phytochemical Analysis of Vigna unguiculata L. Walp Pod - Mahavirsing Dinore - 2023 - Chemistry & Biodiversity - Wiley Online Library.” Accessed: Mar. 11, 2026. [Online]. Available: <https://onlinelibrary.wiley.com/doi/10.1002/cbdv.202200048>
- [9] N. Sharma, S. Mishra, S. Sharma, R. Deshpande, and R. Sharma, “Preparation and Optimization of Nanoemulsions for targeting Drug Delivery,” *Int. J. Drug Dev. Res.*, vol. 5, pp. 37–48, Oct. 2013.
- [10] C. L. Ngan, M. Basri, M. Tripathy, R. Abedi Karjiban, and E. Abdul-Malek, “Physicochemical Characterization and Thermodynamic Studies of Nanoemulsion-Based Transdermal Delivery System for Fullerene,” *Sci. World J.*, vol. 2014, no. 1, p. 219035, 2014, doi: 10.1155/2014/219035.
- [11] J. Stetefeld, S. A. McKenna, and T. R. Patel, “Dynamic light scattering: a practical guide and applications in biomedical sciences,” *Biophys. Rev.*, vol. 8, no. 4, pp. 409–427, Dec. 2016, doi: 10.1007/s12551-016-0218-6.
- [12] V. Uskoković, R. Odsinada, S. Djordjevic, and S. Habelitz, “Dynamic Light Scattering and Zeta Potential of Colloidal Mixtures of Amelogenin and Hydroxyapatite in Calcium and Phosphate Rich Ionic Milieus,” *Arch. Oral Biol.*, vol. 56, no. 6, pp. 521–532, Jun. 2011, doi: 10.1016/j.archoralbio.2010.11.011.
- [13] M. R. McLaughlin, S. A. Weaver, F. Syed, and C. Evans-Molina, “Advanced Imaging Techniques for the Characterization of Subcellular Organelle Structure in Pancreatic Islet β Cells,” *Compr. Physiol.*, vol. 14, no. 1, pp. 5243–5267, Dec. 2023, doi: 10.1002/cphy.c230002.
- [14] M. Gupta, S. THAKUR, A. SHARMA, and S. Gupta, “Qualitative and Quantitative Analysis of Phytochemicals and Pharmacological Value of Some Dye Yielding Medicinal Plants,” *Orient. J. Chem.*, vol. 29, pp. 475–481, May 2013, doi: 10.13005/ojc/290211.
- [15] S. Kumar, M. Prabhakaran, and P. Giridhar, “Fourier transform infrared spectroscopy (FTIR) analysis, chlorophyll content and antioxidant properties of native and defatted foliage of green leafy vegetables,” *J. Food Sci. Technol.*, vol. 52, Aug. 2015, doi: 10.1007/s13197-015-1959-0.