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Original Article

Formulation, Optimization and Evaluation of Naringenin Loaded Cubosomes for Effective Management of Alzheimer's Disease

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ABSTRACT

Objectives: The goal of this work was to formulate a stable naringenin-loaded cubosomal *in situ* gel to improve naringenin absorption into the brain by direct nose-to-brain transfer. **Materials and Methods:** This investigation aimed to prepare, optimize and evaluate a cubosomal *in situ* gel containing naringenin, aimed at facilitating the delivery of the drug to the brain via nasal administration. Cubosomes were fabricated using a top-down methodology and optimization was conducted using 3^2 factorial designs. Cubosomes were analyzed for particle size, encapsulation efficiency, zeta potential and cumulative percent drug release. Poloxamer-407 was used as a thermoreversible and mucoadhesive polymer for preparing naringenin-loaded cubosomal *in situ* gel. **Results:** The optimized cubosome formulation (F3) containing 3% Glyceryl Monooleate (GMO) and 1.5% poloxamer, exhibited a particle size of 123.2 nm, an encapsulation efficiency of 71.12% and a zeta potential of -38.61. The Transmission Electron Microscopy (TEM) image represented the smooth cubic structure of naringenin-loaded cubosomes. The thermoreversible *in situ* gel demonstrated appropriate gelation temperatures, viscosities, pH levels and *in vitro* drug diffusion characteristics. The *in vitro* drug diffusion study revealed a higher percentage cumulative drug release, reaching 82.21%. **Conclusion:** The results obtained demonstrated enhanced capability of the developed naringenin-loaded cubosomes to facilitate drug permeation through the nasal cavity.

Keywords: Alzheimer's disease, Brain targeting, Cubosomes, Naringenin, Nasal delivery.

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INTRODUCTION

The nose filter and humidifies breathed air, protecting the airway from hazardous particles. It also functions as the sensory organ responsible for smell. Nasal delivery has several advantages to oral administration, including faster start of action, less drug degradation and higher absorption rates. Compared to intravenous administration, nasal administration allows for excellent patient compliance, self-administration and direct distribution to the brain via olfactory nerve pathways, bypassing the blood-brain barrier.[1]

Alzheimer's Disease (AD), given the name German psychiatrist Alois Alzheimer, is a very common form of dementia and a gradually progressive neurodegenerative illness. It is distinguished by an elevated level of Amyloid-beta (Aβ) peptides, leading to the formation of plaques with neuritis and neurofibrillary tangles. These degenerative alterations largely impact the middle part of the temporal lobe and neocortical regions, which are critical for memory and cognitive function. Globally, approximately 50 million people are affected by AD, a number expected to double every five years, reaching 152 million by 2050. Although there is currently no cure, available treatments focus on alleviating symptoms and slowing cognitive decline. The disease is marked by significant brain atrophy caused by the degeneration of neurons, synaptic loss and neuropil deterioration. Additionally, factors such as neuroinflammation, oxidative stress, cholinergic neuron injury and excitotoxicity contribute to neurodegeneration. Cholinergic nerve dysfunction, a higher oxidative stress load with a compromised protective antioxidant system and an enhanced inflammatory response are the primary pathogenic causes underlying Alzheimer's disease. Despite the lack of a cure, ongoing research aims to address these multiple mechanisms to develop therapies that could slow or stop the progression of the illness.[2,3]

In addition, negative lesions (owing to losses) are characterized by extensive atrophy caused by neuronal, neuropil and synaptic loss.[4] Other variables that might induce neurodegeneration

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include neurological inflammation, stress caused by oxidation and damage to cholinergic neurons.[5]

Naringenin is a flavanone found in abundance in citrus fruits. It has a wide range of pharmacological actions, including anti-inflammatory, antioxidant, immunological modulatory and neuroprotective activities, as well as peroxisome proliferator-activated receptor stimulation and NF-κB inhibition.[6,7]

Naringenin has antioxidant properties, enhances brain insulin signaling and cognitive functioning and alleviates AD-type neurodegeneration caused by intracerebroventricular streptozotocin. Furthermore, naringenin has anti-inflammatory properties, acts as a neuroprotective agent in a 6-Hydroxydopamine (6-OHDA) induced Parkinson's disease model and protects against 6-OHDA neurotoxicity.[8,9]

Naringenin is thought to improve Alzheimer's disease pathology and memory problems by targeting CRMP2 and lowering its phosphorylation in produced primary neurons.[10] Similarly, naringenin decreased tau protein phosphorylation and Aß plaque formation, promoting axonal development while improving overall cognition and learning.[11]

Cubosomes are nanostructured particles in the bicontinuous hexagonal liquid crystalline phase, measuring sub-micron in size. Cubosomes are self-assembling liquid crystalline particles with a solid-like rheology that move quickly and effectively. Cubosomes and the parent cubic phase have the same microstructure; cubosome dispersions have significantly lower viscosity than the cubic phase in bulk. Cubosomes take shape at specified temperatures. They occur in three distinct phases: for primitive, gyroid and diamond structures, P-surface, G-surface and D-surface are used.[12,13]

Cubosomes are reversed bicontinuous cubic phases with unique physicochemical properties, making them attractive for delivering hydrophobic, hydrophilic and amphiphilic drugs due to their enhanced bioavailability and loading potential. Monoolein–water cubosomes, particularly in binary systems, can self-assemble into thermodynamically stable cubic crystalline structures. Cubosomes are characterized by their unique structure, versatile composition, efficient preparation methods, significant advantages and extensive potential in drug delivery applications.[14,15]

The '*in situ* gel' system is one of the most successful novel drug delivery methods, allowing for controlled and sustained pharmaceutical release while also improving patient compliance and comfort.[16] It maintains a constant plasma drug pattern in the body by delayed the release of a drug, permitting it to be bound and absorbed in gel form and it has been shown to extend the drug's life in the mucosa.[17,18]

Ahmad N *et al.* developed a Naringenin-loaded nano-emulsion *in situ* gel (NAR-NE-gel + 0.50% CS) with Poloxamer-407 (20% w/v) and Chitosan (0.50% w/v). The investigation revealed substantial muco-adhesion (6245.38 dynes/cm²). The gel was formed at a temperature of 28.3°C. The micelle size was observed to be 98.31 nm and AUC_{0-24} values of 995.60 and 5600.99 ng min/mL for plasma and brain, respectively. There was no injury identified in the brain or nasal tissues.[19]

Salimi A, *et al.* formulated a Naringenin Liposomal Formulation (NLF) by thin lipid film process and analyzed using SEM and Dynamic Light Scattering (DLS), generating particles ranging in size from 148 to 215 nm with encapsulation efficiencies of 43% and 66%. After causing retinopathy with α-AAA, NLF was administered for three weeks. The 800 µg/mL dose significantly decreased neovascularization and retinal damage. NLF had significant anti-neovascularization effects, suggesting that it might be a viable treatment for diabetic retinopathy.[20]

Mohammed EE *et al.* developed Naringin-Dextrin Nanoparticles (NDNPs) as a treatment for Hepatocellular Carcinoma (HCC) in male Wistar rats. HCC was caused by Diethyl Nitrosamine (DEN) and 2-acetylaminofluorene (2AAF). After 24 weeks of therapy with either vehicle 10 mg/kg naringin, or 10 mg/kg NDNP, both naringin and NDNP improved liver function and reduced tumor markers, as shown by histological investigations. They decreased DEN-induced oxidative stress and inflammation, altered apoptosis-related protein expressions and affected IQGAP and Ras signalling. Notably, NDNP outperformed free naringin, suggesting higher bioavailability at tumor sites and stronger anticancer effects.[21]

Thus, cubosomes outperform liposomes (like NLF), Nanoemulsions (like NAR-NE-gel) and Nanoparticles (NDNPs) in terms of surface area, stability and controlled drug release. Cubosomes are ideal for intranasal delivery because they enhance drug penetration, encapsulation efficiency and degradation resistance compared to traditional formulations. Cubosomes are perfect for increasing naringenin's bioavailability and therapeutic advantages, especially for brain targeting, because they can encapsulate both hydrophilic and hydrophobic medications.

MATERIALS AND METHODS

Naringenin was purchased from Fine chem Ltd., Mumbai. Glyceryl Mono Oleate (GMO) was purchased from Mohini Pharmaceuticals, Mumbai. Poloxamer-407 was purchased from Sigma Aldrich Pvt. Ltd., Mumbai. All the other ingredients like Methanol, Di-sodium Hydrogen Orthophosphate, Sodium Di-hydrogen Orthophosphate; Sodium chloride was acquired from Sd Fine-chem Limited in Mumbai. All chemicals and solvents utilized in this study were of analytical grade.

Methods

Preparation of Cubosomes

Cubosomes were formulated using the top-down method.[22] Accurate quantities of Glyceryl Monooleate (GMO), Poloxamer 407 and the drug (naringenin) were weighed on a calibrated balance. Poloxamer 407, the stabilizer, was heated on a hot pan at a constant temperature of 60°C. GMO and naringenin were mixed to form a uniform blend at 500-550 rpm using a magnetic stirrer and then combined with the pre-melted Poloxamer 407. The mixture was stirred until homogeneous. Pre-heated water, maintained at 60°C, was gradually added to the mixture using a syringe to prevent phase separation or disruption of the formulation (Table 1).[23,24] The resulting formulation was then homogenized at 15,000 rpm for 10 min to achieve uniform nanoparticles. Finally, the mixture was sonicated using an ultrasonic probe sonicator at not more than 35% amplitude for 10 min, alternating 5-min ON and OFF cycles.[25]

Characterization

Determination of Particle Size, Poly Dispersity Index and Zeta Potential

The analysis required diluting 1 mL of the sample to 10 mL using Milli-Q water (10-fold dilution). The diluted sample was evaluated using laser diffraction or DLS method to assess particle size, zeta potential and Polydispersity Index (PDI) with the Malvern Zeta Sizer instrument.[26,27]

Determination of entrapment efficiency

Entrapment efficiency of naringenin-loaded cubosomes was determined by taking 1.5 mL of sample and then centrifuging at 15,000 rpm at 4°C for 30 min.[25] The sediment and supernatant were separated, before spectrophotometric analysis 0.1 mL of supernatant was collected and diluted (100 folds) up to 10 mL using methanol and checked for absorption at 288 nm.[28] The entrapment efficiency was calculated, as

$$\%\ Entrapment\ efficiency = \frac{Amount\ of\ drug\ entrapped}{Total\ drug\ content} \times 100$$

Transmission Electron Microscopy (TEM)

The structure of NAR-cubosomes was examined using a field emission gun transmission electron microscope (JEOL® JEM-2100F, Japan) at a voltage of 120/200 kV. A drop of NAR-cubosome dispersion was applied on a grid of copper coated with carbon and dyed with 1% phosphotungstic acid for 2-3 min. Following air drying, the sample was inspected under a microscope to assess its morphology.[29]

Optimization studies by Design of Experiments (DoE)

The GMO and Poloxamer concentrations were considered as independent variables, while particle size and entrapment efficiency were selected as dependent variables. These factors were optimized using DESIGN EXPERT® software [version 13] via a two-factor, three-level (3^2) factorial design. The drug concentration remained constant at 1% across all formulations.[30,31] The main objective of the design of the experiment is to minimize the particle size and maximize the entrapment efficiency.

Formulation of *in situ* Gel from optimized Cubosomal dispersion

To formulate the naringenin-loaded cubosomal *in situ* gel, 18% of Poloxamer 407 was added gradually in small portions with constant stirring for 30 min in a 10 mL of cubosomal dispersion and mixed on a magnetic stirrer at 600-700 rpm while keeping the temperature below 10°C.[32]

Viscosity

The viscosity of *in situ* gel was measured using a Spindle No. 01 CAP501, Brookfield CAP 2000+ digital viscometer at 10°C and 37°C (body temperature) at optimum speed (20 rpm). Various viscosity-affecting parameters, such as temperatures and RPM, were maintained throughout the operation. The viscosity measurements were taken in triplicate.

Determination of Gelation Temperature and Gelation Time

The gelation or transition temperature is defined as the temperature at which stirring terminates because of sol-to-gel formation. To determine this, 10 mL of the solution was stirring at 100 rpm using a magnetic stirrer while under pre-heating conditions. To achieve uniform and reproducible measurements, the operation was carried out three times.

In vitro Drug Diffusion Study of Naringenin Loaded Cubosomal Gel

In vitro permeation of naringenin-loaded cubosomal *in situ* gel was tested by carrying out diffusion studies using Franz Diffusion cell with cellophane membrane. A cubosomal gel containing 1 g was placed in the donor compartment of the Franz diffusion cell, above the cellophane membrane. The receptor chamber was filled with phosphate buffer (pH=6.4). The receptor chamber utilized a magnetic stirrer with a bead to maintain a speed of 500 rpm. The temperature of 37°C was maintained during the procedure. 1 mL of the sample was taken from the port used for sampling at 0 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr and 24 hr. The withdrawn amount of sample (1 mL) was replaced with buffer 6.4 pH. The samples are examined in the UV spectrophotometer at 288 nm after diluting the sample to 10 mL with 6.4 pH phosphate buffer.

RESULTS

Determination of Particle Size, Poly Dispersity Index and Zeta Potential

As depicted in Table 2 the particle size of cubosomes ranged from 123.20-256.09 nm. The PDI of optimized formulation (F3) was found to be 0.3165. The particle size distribution has a narrow peak at 100 nm, indicating homogenous particles. The zeta potential has a sharp peak, which indicates high stability. Overall, the system looks monodispersed, with probable aggregation tendencies.

Percentage Entrapment Efficiency

The Efficiency of Encapsulation (% EE) percentage plays a crucial role in assessing the drug transport capability of cubosomal nano-formulation. The %EE was found to fall within the range from 65.55% to 82.10%. The polynomial equation demonstrates how various independent variables affect the entrapment efficiency of the cubosomes.

Transmission Electron Microscopy (TEM)

Transmission Electron Microscopy (TEM) is an important technique for evaluating surface morphology. The two-dimensional images produced by TEM illustrate that the small particles are cubic with smooth surfaces. Morphological analysis shows that the particles exhibit well-defined borders and are distinctly separated, suggesting that the NAR-loaded cubosomes maintain their stability.

Design of Experiments (DoE)

The dispersion of the cubosomes formulation was carried out using the top-down method, which was then optimized through a two-factor, three-level factorial design (3^2). This design involved 09 experimental runs aimed at examining the impact of independent variables such as GMO and poloxamer 407 on dependent variables including particle size and entrapment efficiency.

As shown in Figure 1(a). The equation 1 indicates that GMO has a significant impact on increasing Cubosome particle size, with a considerable initial increase that plateaus and reduces at higher concentrations, as indicated by the positive linear (+234.18) and negative quadratic (-73.55) variables. Poloxamer 407 similarly increases particle size, although very slightly (+64.67) and has a modest quadratic impact (+1.13). The interaction between GMO and Poloxamer 407 is small yet favourable (+0.3025), indicating a modest increase in size. Overall, GMO has the most impact, with Poloxamer 407 playing a smaller but steady contribution.

$$PS=+234.18+64.67\ A-22.05\ B+0.3025AB-73.55A^2+1.13B^2 \quad \text{(Equation 1)}$$

As shown in Figure 1(b). The equation 2 illustrates that GMO increases the zeta potential, making it less negative, as confirmed by the positive linear (+3.27) and quadratic (+8.32) variables. Poloxamer 407 marginally lowers the zeta potential, making it more negative, as seen by the modest negative linear (-0.2133) and quadratic (-0.1150) variables. The combination of GMO and Poloxamer 407 (+0.6575) produces a little beneficial impact, slightly offsetting the negative. Overall, GMO is the most effective in increasing zeta potential, with Poloxamer 407 having just a modest negative impact.

$$ZP=-43.24+3.27A-0.2133\ B+0.6575\ AB+8.32\ A^2-0.1150\ B^2 \quad \text{(Equation 2)}$$

As shown in Figure 1(c). The equation 3 below illustrates how GMO (A) and Poloxamer 407 (B) impact cubosome Encapsulation Efficiency (%EE). GMO has a considerable positive influence on %EE, as seen by the large positive linear coefficient (+5.78), implying that increasing GMO content results in a significant improvement in encapsulation efficiency. Poloxamer 407 likewise has a beneficial effect on %EE, but to a lesser amount, as seen by the lower coefficient (+0.9417). This shows that, while both components increase encapsulation efficiency, GMO has a far more significant influence than Poloxamer 407.

$$\%EE=+75.25+5.78\ A+0.9417B \quad \text{(Equation 3)}$$

Evaluation of Cubosomal *in situ* Gel

In situ gel was evaluated for viscosity, gelation temperature, gelation time and *in vitro* diffusion studies.

Viscosity

To remain at an application site, the gel should have a low viscosity and then to a high viscosity following instillation. Table 3 shows the viscosity values of the *in situ* gel formulation at 10°C and 37°C, which were 1427.66±1.699 cP and 74.00±0.816 cP, respectively.

Table 1: 3^2 full factorial design space: parameters and their corresponding levels.

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Naringenin	30 mg	30 mg	30 mg	30 mg	30 mg	30 mg	30 mg	30 mg	30 mg
Glyceryl Monooleate	3%	3%	3%	5.5%	5.5%	5.5%	8%	8%	8%
Poloxamer 407	0.5%	1%	1.5%	0.5%	1%	1.5%	0.5%	1%	1.5%
Water	30 mL	30 mL	30 mL	30 mL	30 mL	30 mL	30 mL	30 mL	30 mL

Table 2: Experimental Design, formulation, composition and characterization of Cubosomes.

Formulation	GMO%	Poloxamer%	Particle Size (nm)	Zeta (mV)	EE%	Cumulative percentage drug release at 24 Hr
F1	3	0.5	139.90	-37.67	68.20	67.43
F2	3	1.0	131.50	-38.10	65.65	70.73
F3	3	1.5	123.20	-38.61	71.12	82.21
F4	5.5	0.5	245.71	-42.18	72.50	70.35
F5	5.5	1.0	234.50	-43.57	78.15	66.73
F6	5.5	1.5	224.60	-4/4.21	76.30	64.78
F7	8	0.5	256.09	-32.95	79.70	60.17
F8	8	1.0	221.00	-30.99	80.70	53.37
F9	8	1.5	201.10	-31.26	82.10	53.47

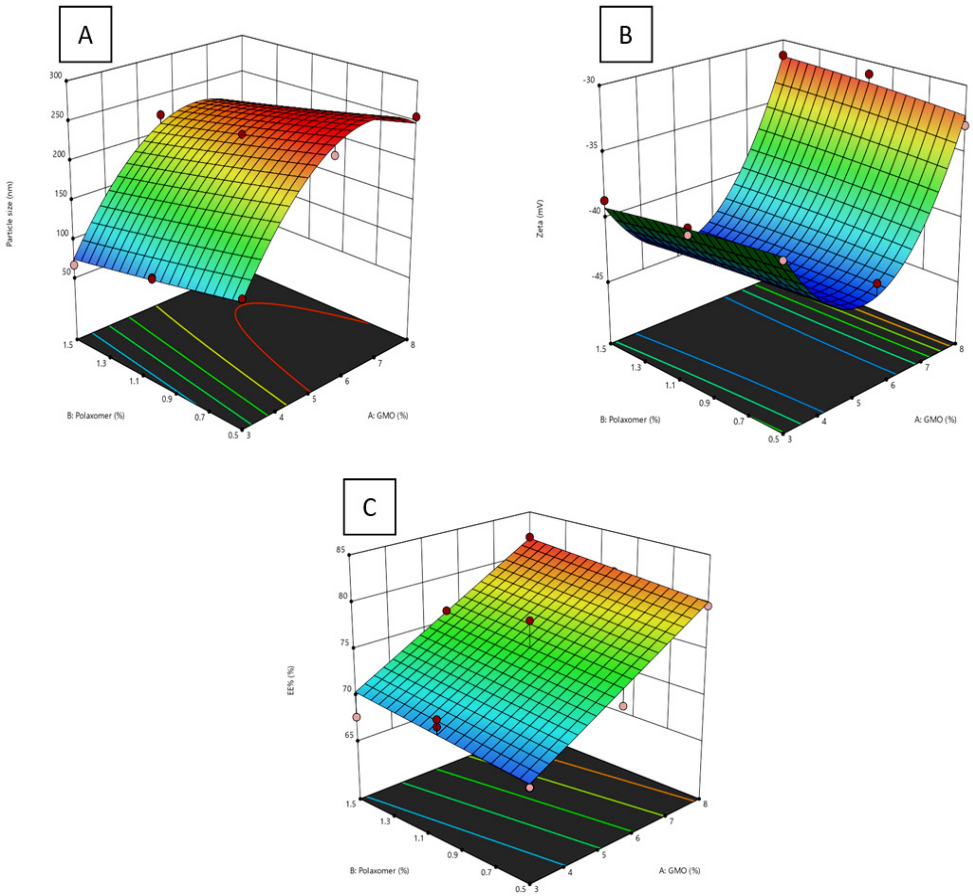

Figure 1: (a): 3D response surface chart exhibiting the influence of GMO and Poloxamer 407 on particle size of Cubosomes. (b): 3D response surface chart exhibiting the influence of GMO and Poloxamer 407 on zeta potential of Cubosomes. (c): 3D response surface chart exhibiting the influence of GMO and Poloxamer 407 on entrapment efficiency of Cubosomes.

Determination of Gelation Temperature and Gelation Time

The gelation temperature was determined using the visual inspection method. Thermoreversible gel should be cemented at temperatures ranging from 25°C to 34°C. This research revealed a gelation temperature of 35.00±0.816°C. If thermoreversible gel does not turn into gel within the required temperature range, it stays liquid at body temperature and is readily washed out of the nasal cavity. The number of micelles produced increases in proportion to the temperature. These micelles become tightly packed, making the fluid immobile and producing a gel (Table 3).

In vitro Drug Diffusion Study of Naringenin Loaded Cubosomal Gel

An *in vitro* drug diffusion study of naringenin-loaded cubosomal gel was conducted utilizing a Franz diffusion cell. The diffusion

Table 3: Viscosity, Gelation Temperature and time of Optimized Naringenin-Loaded Cubosomal *In Situ* Gel.

Viscosity			
Formulation Viscosity (Temperature and RPM)	**Mean Viscosity (Cp)**	**Mean Gelation Temperature (°C)**	**Mean Gelation Time (Seconds)**
F3 (37°C and 20 RPM)	1427.66±1.699	33.333±0.471	35.00±0.816
F3 (10°C and 20 RPM)	74.00±0.816		

Gelation temperature and time.

study was carried out using a 6.4 pH phosphate buffer to mimic the nasal cavity pH. The samples were collected at 0 min, 30 min, 1 hr, 2 hr, 3 hr, 4 hr, 5 hr, 6 hr, 8 hr and 24 hr.

The *in vitro* release profiles of naringenin from the optimized batch F3 at pH 6.4, which were created by plotting the cumulative percentage of drug release over time and the data represented illustrates that the optimized naringenin F3 batch exhibits an initial burst release of about 21.12% within the 1st hr, followed by a continuous and extended release of 82.21% of drug release noted up to 24 hr. This indicates a sustained and prolonged drug release profile.

DISCUSSION

The objective of this project was to develop an *in situ* gel containing naringenin-loaded cubosomes for delivering the drug intranasally for brain targeting. The Fourier Transform Infrared Spectroscopy (FTIR) analysis was used to determine the drug's compatibility with the excipients exhibited no interactions between the drug and the polymers employed in the solution. Naringenin was shown to be the higher soluble in methanol. The cubosomal formulation (F3), which was optimized with 3% GMO and 1.5% poloxamer, exhibited a particle size of 123.2 nm, an encapsulation efficiency of 71.12% and a Zeta potential of -38.61mV.

Based on the results obtained, an *in situ* gel containing naringenin was formulated by incorporating the optimized formulation F3 into a gel base. This *in situ* gel utilized poloxamer-407 as mucoadhesive polymers and for thermoreversible properties. The thermoreversible *in situ* gel exhibited suitable gelation temperatures, gelation time, viscosities, pH levels and *in vitro* drug diffusion characteristics. The *in vitro* drug diffusion study indicated that a higher percentage of cumulative drug release was observed, reaching 82.21%.

CONCLUSION

The novel naringenin-loaded cubosomal *in situ* gel shows potential as a better therapeutic strategy for Alzheimer's disease. This formulation uses the nasal route to access the brain directly, bypassing the blood-brain barrier and increasing therapeutic effectiveness. The addition of cubosomes improves stability, drug encapsulation and controlled release, while the thermoreversible *in situ* gel allows easy application and long-term drug administration. This method emphasizes the efficacy of novel nanostructured formulations in managing alzheimer's and improving patient outcomes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

AD: Alzheimer's Disease; **GMO:** Glyceryl Monooleate; **FTIR:** Fourier Transform Infrared Spectroscopy; **TEM:** Transmission Electron Microscopy; **DLS:** Dynamic Light Scattering; **%EE:** Percentage Entrapment Efficiency; **UHPLC:** Ultra High Performance Liquid Chromatography; **ESI:** Electrospray Ionization; **Q-TOF:** Quadrupole Time of Flight: **MS:** Mass Spectrometry; **NF-κB:** Nuclear Factor kappa light chain enhancer of activated B cells; **CRMP2:** Collapsin Response Mediator Protein 2; **PDI:** Poly Dispersity Index.

SUMMARY

- The study focused on developing an *in situ* gel for intranasal administration of naringenin-loaded cubosomes to enhance brain targeting.
- FTIR analysis confirmed the compatibility of naringenin with the selected excipients.
- The optimized cubosomal formulation was incorporated into a poloxamer-based *in situ* gel.
- The *in situ* gel exhibited mucoadhesive and thermoreversible properties.
- Key attributes such as gelation, viscosity and pH were appropriate for nasal application.
- The formulation demonstrated excellent *in vitro* drug release, indicating the potential for improved brain delivery of naringenin.
- The future scope of the presented work

- Animal Studies: Conduct *in vivo* studies to evaluate safety, efficacy and brain-targeting efficiency.
- Combination Therapies: Co-deliver naringenin with synergistic drugs.
- Pharmacokinetics: Study brain-targeting efficiency and dose optimization.

REFERENCES

1. Tai J, Han M, Lee D, Park IH, Lee SH, Kim TH. Different methods and formulations of drugs and vaccines for nasal administration. Pharmaceutics. 2022; 14(5): 1073.
2. Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease: causes and treatment. Molecules. 2020; 25(24): 5789.
3. Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen CE, *et al.* Alzheimer's disease. The Lancet. 2021; 397(10284): 1577-90.
4. Tahami Monfared AA, Byrnes MJ, White LA, Zhang Q. Alzheimer's disease: epidemiology and clinical progression. Neurology and therapy. 2022; 11(2): 553-69.
5. Singh SK, Srivastav S, Yadav AK, Srikrishna S, Perry G. Overview of Alzheimer's disease and some therapeutic approaches targeting Aβ by using several synthetic and herbal compounds. Oxidative medicine and cellular longevity; 2016(1): 7361613.
6. Zaki HF, Abd-El-Fattah MA, Attia AS. Naringenin protects against scopolamine-induced dementia in rats. Bulletin of Faculty of Pharmacy, Cairo University. 2014; 52(1): 15-25.
7. Ghofrani S, Joghataei MT, Mohseni S, Baluchnejadmojarad T, Bagheri M, Khamse S, *et al.* Naringenin improves learning and memory in an Alzheimer's disease rat model: Insights into the underlying mechanisms. European journal of pharmacology. 2015; 76(4): 195-201.
8. Lawal M, Olotu FA, Soliman ME. Across the blood-brain barrier: Neurotherapeutic screening and characterization of naringenin as a novel CRMP-2 inhibitor in the treatment of Alzheimer's disease using bioinformatics and computational tools. Computers in Biology and Medicine. 2018; 98: 168-77.
9. Zhu Y, Guo X, Li S, Wu Y, Zhu F, Qin C, *et al.* Naringenin ameliorates amyloid-β pathology and neuroinflammation in Alzheimer's disease. Communications Biology. 2024; 7(1): 912.
10. Li L, Lin Z, Yuan J, Li P, Wang Q, Cho N, *et al.* The neuroprotective mechanisms of naringenin: Inhibition of apoptosis through the PI3K/AKT pathway after hypoxic-ischemic brain damage. Journal of Ethnopharmacology. 2024; 318: 116941.
11. Surabhi S, Krishnananda K, Shabaraya AR. Cubosomes and its applications-A review. Eur. J. Biomed. Pharm. Sci. 2022; 9: 111-6.
12. Vibha B. *In situ* gel nasal drug delivery system-a review. International Journal of Pharma Sciences. 2014; 4(3): 577-80.
13. Palma AS, Casadei BR, Lotierzo MC, de Castro RD, Barbosa LR. A short review on the applicability and use of cubosomes as nanocarriers. Biophysical Reviews. 2023; 15(4): 553-67.
14. Karami Z, Hamidi M. Cubosomes: remarkable drug delivery potential. Drug discovery today. 2016; 21(5): 789-801.
15. Rizwan SB, Boyd BJ. Cubosomes: structure, preparation and use as an antigen delivery system. In Subunit Vaccine Delivery 2014 (pp. 125-40). New York, NY: Springer New York.
16. Kolawole OM, Cook MT. *In situ* gelling drug delivery systems for topical drug delivery. European Journal of Pharmaceutics and Biopharmaceutics. 2023; 184: 36-49.
17. Ahmad N, Ahmad R, Ahmad FJ, Ahmad W, Alam MA, Amir M, *et al.* Poloxamer-chitosan-based Naringenin nanoformulation used in brain targeting for the treatment of cerebral ischemia. Saudi Journal of Biological Sciences. 2020; 27(1): 500-17.
18. Feng Y, Zhang Z, Tang W, Dai Y. Gel/hydrogel-based *in situ* biomaterial platforms for cancer postoperative treatment and recovery. In Exploration 2023; (3): 20220173.
19. Ahmad N, Ahmad R, Ahmad FJ, Ahmad W, Alam MA, Amir M, *et al.* Poloxamer-chitosan-based Naringenin nanoformulation used in brain targeting for the treatment of cerebral ischemia. Saudi Journal of Biological Sciences. 20201; 27(1): 500-17.
20. Salimi A, Makhmalzadeh BS, Feghhi M, Rezai A, Bagheri F. Evaluation of the Effect of Naringenin Liposomal Formulation on Retinopathy in an Experimental Rabbit Model. KAFKAS ÜNİVERSİTESİ VETERİNER FAKÜLTESİ DERGİSİ. 2022; 28(4).
21. Mohamed EE, Ahmed OM, Abdel-Moneim A, Zoheir KM, Elesawy BH, Al Askary A, Hassaballa A, El-Shahawy AA. Protective Effects of Naringin-Dextrin Nanoformula against Chemically Induced Hepatocellular Carcinoma in Wistar Rats: Roles of Oxidative Stress, Inflammation, Cell Apoptosis and Proliferation. Pharmaceuticals. 2022; 15(12): 1558.
22. Sivadasan D, Sultan MH, Alqahtani SS, Javed S. Cubosomes in drug delivery-a comprehensive review on its structural components, preparation techniques and therapeutic applications. Biomedicines. 2023; 11(4): 1114.
23. Thakkar H, Modi B, Patel B. Intranasal spray of cubosomal tizanidine hydrochloride for brain targeting *in vitro* and *in vivo* characterization. Journal of Microencapsulation. 2023; 40(5): 366-83.
24. Varghese R, Salvi S, Sood P, Kulkarni B, Kumar D. Cubosomes in cancer drug delivery: A review. Colloid and Interface Science Communications. 2022; 46: 100561.
25. Esposito E, Eblovi N, Rasi S, Drechsler M, Di Gregorio GM, Menegatti E, Cortesi R. Lipid-based supramolecular systems for topical application: a preformulatory study. Aaps Pharmsci. 2003; 5: 62-76.
26. Zaki RM, Alkharashi LA, Sarhan OM, Almurshedi AS, Aldosari BN, Said M. Box Behnken optimization of cubosomes for enhancing the anticancer activity of metformin: Design, characterization and *in vitro* cell proliferation assay on MDA-MB-231 breast and LOVO colon cancer cell lines. International Journal of Pharmaceutics: X. 2023; 6: 100208.
27. El-Shenawy AA, Elsayed MM, Atwa GM, Abourehab MA, Mohamed MS, Ghoneim MM, Mahmoud RA, Sabry SA, Anwar W, El-Sherbiny M, Hassan YA. Anti-tumor activity of orally administered gefitinib-loaded nanosized cubosomes against colon cancer. Pharmaceutics. 2023; 15(2): 680.
28. Zaki RM, Alkharashi LA, Sarhan OM, Almurshedi AS, Aldosari BN, Said M. Box Behnken optimization of cubosomes for enhancing the anticancer activity of metformin: Design, characterization and *in vitro* cell proliferation assay on MDA-MB-231 breast and LOVO colon cancer cell lines. International Journal of Pharmaceutics: X. 2023; 6: 100208.
29. Qin Y, Fang D, Wu Y, Wu Y, Yao W. Controllable Preparation of Gold Nanocrystals with Different Porous Structures for SERS Sensing. Molecules. 2023; 28(5): 2316.
30. Gorbounov M, Taylor J, Petrovic B, Soltani SM. To DoE or not to DoE? A technical review on and roadmap for optimisation of carbonaceous adsorbents and adsorption processes. South African Journal of Chemical Engineering. 2022; 41(1): 111-28.
31. Sabir F, Qindeel M, Rehman AU, Ahmad NM, Khan GM, Csoka I, Ahmed N. An efficient approach for development and optimisation of curcumin-loaded solid lipid nanoparticles' patch for transdermal delivery. Journal of Microencapsulation. 2021; 38(4): 233-48.
32. Dewan M, Adhikari A, Jana R, Chattopadhyay D. Development, evaluation and recent progress of ocular *in situ* gelling drug delivery vehicle based on poloxamer 407. Journal of Drug Delivery Science and Technology. 2023: 104885.

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