Available online on 15.12.2024 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Research Article

Formulation, Optimization and Characterization: Thermosensitive Intranasal Nanostructured Lipid Carrier (NLC) *In-situ Gel* of Novel Agomelatine to Overcome the Limitations of Oral Delivery

Nabeela Zainab*, Syed Abdul Azeez Basha, Abdul Mannan

Department of Pharmaceutics, Deccan School of Pharmacy (Osmania University), Aghapura, Hyderabad, Telangana – 500 001, India

Article Info:

Article History:

Received 08 Sep 2024
Reviewed 22 Oct 2024
Accepted 19 Nov 2024
Published 15 Dec 2024

Cite this article as:

Zainab N, Basha SAA, Mannan A, Formulation, Optimization and Characterization: Thermosensitive Intranasal Nanostructured Lipid Carrier (NLC) *In-situ Gel* of Novel Agomelatine to Overcome the Limitations of Oral Delivery, Journal of Drug Delivery and Therapeutics. 2024; 14(12):123-142 *DOI: http://dx.doi.org/10.22270/jddt.v14i12.6889*

*Address for Correspondence:

Nabeela Zainab, Department of Pharmaceutics, Deccan School of Pharmacy (Osmania University), Aghapura, Hyderabad, Telangana – 500 001, India. Email; nabeelazainab15@gmail.com

Abstract

Agomelatine (AG), the first-in-class melatonergic antidepressant used in Major Depressive Disorder (MDD), belongs to BCS Class-II with low oral bioavailability (<5%) due to extensive hepatic metabolism. This research work developed a thermosensitive in-situ gel using nanostructured lipid carriers (NLC) for intranasal delivery as alternate route, aiming to bypass hepatic metabolism, enable controlled release, and enhance cerebral distribution. FTIR ensured compatibility with lipids, surfactants and polymers. AG-NLCs were synthesized utilizing hot high-speed homogenization with 5 mg agomelatine dose and optimized with 3^2 factorial design with total lipids (Precirol® ATO 5:Oleic acid – 70:30) and surfactant concentration (% of Poloxamer 188) as independent two-factor variables. The optimized AG-NLC (AF8) showed particle size of 159.3 nm, zeta potential of -37.0 mV, and 58.14% entrapment efficiency. AF8 was further fabricated into in-situ gels using thermosensitive polymer Poloxamer 407 and sodium alginate as mucoadhesive polymer, with NLCG-4 (with 8% Poloxamer 407) as the optimized in-situ gel formulation. NLCG-4 had 95.16±0.90% drug content, excellent viscosity (1690.65 ±0.40 cP at 37°C), and gelled at 33.24±0.11°C in 10.3±0.38 seconds, ideal for nasal mucosa. NLCG-4 exhibited a complete, prolonged release of 100.01±0.2% over 6 h, and SEM images confirmed spherical particles without aggregation. The above findings suggest that thermosensitive NLC in-situ gel could be a potential novel approach for enhanced direct nose-to-brain delivery of agomelatine, bypassing first-pass metabolism to treat depression. Further *in vivo* investigations are ongoing to establish and justify clinical applicability of the novel system.

Keywords: thermosensitive, in-situ gel, NLC, agomelatine, major depressive disorder, intranasal delivery, Design-Expert, poloxamer 407.

INTRODUCTION

Depression, a prevalent and debilitating mental disorder, seriously impairs cognitive performance, interferes with physical health, affects day-to-day functioning, causes social and financial challenges, and frequently results in high rates of suicide[1]. The effectiveness of current pharmacotherapy treatments, such as tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), is outweighed by serious side effects, including discontinuation symptoms, sexual dysfunction, and disturbed sleep patterns because their mechanisms of action are not fully understood and their interactions with non-targeted receptors[2].

Agomelatine (AG), a melatonin analogue, is an atypical antidepressant that combines melatonin receptor agonism and 5-HT_{2c} antagonism, offering a safer alternative to SSRIs and TCAs with fewer side effects and better tolerance[3]. It is marketed as a 25 mg oral tablet. However, AG has low water solubility and poor oral bioavailability (<5%), a short half-life of 1–1.5 hours, high protein binding, and extensive hepatic metabolism, which complicates longer dosing schedules and patient compliance[4]. High doses and liver metabolism can also pose a risk of liver damage[5]. As an alternative to the oral route, nasal route has been researched to target the brain tissues directly with lower concentrations of the drug.

Nose-to-brain is a non-invasive technique for targeting the brain directly, with high-level vasculature offering rapid absorption and bypassing the blood-brain barrier (BBB) especially for drugs under 200 nm in size[6]. It increases bioavailability by preventing plasma protein binding, making more of the drug available for therapeutic effects. However, conventional nasal delivery faces challenges such as difficulty targeting the upper nasal region, rapid mucociliary clearance of drugs, and low membrane permeability of nasal mucosa.

A promising solution is the use of nanosize-based in-situ gelling systems, which form a gel network in the nasal cavity, increasing residence time and allowing for more efficient drug delivery with accurate dose[7]. Thermosensitive in-situ gels, made from polymers like Poloxamer 407 (Pluronic® F127), which undergo reversible gelation in response to temperature changes[8] such as increment to temperature in physiological nasal range, improve the system's effectiveness. However, solution of poloxamer 407 that imparts gelling property has short residence time[9] due to it quickly dissolving in aqueous phase, limiting its effectiveness. Residence time can be extended by adding mucoadhesive polymers like sodium alginate, which is known for its strong adhesive properties and compatibility with Poloxamer 407[10], to enhance gel strength and pharmacological effectiveness without causing irritation or any significant side effects.

To further enhance intranasal administration and drug delivery, an attractive colloidal system of nanostructured lipid carriers (NLCs) with a size of less than 200 nm has been selected for efficient AG delivery. The next generation of solid lipid nanoparticles (SLNs) are NLCs, which are made up of a binary mixture of liquid and solid lipids stabilized by a surfactant system. Compared to conventional colloidal delivery systems (such as nanoemulsions), they have several benefits, including cell-specific controlled drug release, high stability, high drug entrapment, and decreased drug leakage[11]. Additionally, these nanoparticles enhance nasal penetration and inhibit drug deterioration.

The research strategy here seeks to get beyond the drawbacks of traditional nasal delivery by fusing nanotechnology with in-situ gelling technology to deliver neurotherapeutics like agomelatine to the brain effectively. In current investigation, effort was made to develop NLCs of agomelatine (AG-NLCs) using a modified high-shear homogenization method followed by converting them into thermoreversible in-situ gel by cold method with poloxamer 407 and sodium alginate as polymers to overcome associated oral delivery problems.

MATERIALS AND METHODS

Materials

Agomelatine was received as a gift sample from Precise ChemiPharma, Navi Mumbai, India. Compritol® 888 ATO, Precirol® ATO 5, Gelucire® 43/01 were kindly gifted by Gattefossé, Bangalore, India. Oleic acid, Capryol® 90, Labrafac™ PG, Lauroglycol™ FCC were received from SRL Chemicals, Mumbai, India. Poloxamer 188 and poloxamer 407 were generously supplied by BASF Pharma, Bangalore, India. Sodium alginate was purchased from S.D. Fine Chem Ltd., Mumbai, India. Ethanol and methanol were purchased from Ashland (Pvt. Ltd), Maharashtra., India. Dialysis Membrane-110 was obtained from HiMedia Laboratories, Mumbai, India. All other reagents were of analytical grade obtained from laboratory.

Selection of Excipients

Selection of the Solid Lipid and Liquid Lipid

Screening of solid lipids was done by measuring agomelatine's saturation solubility in various solid lipids – Compritol® 888 ATO, Precirol® ATO 5, Gelucire® 43/01. One gram of each solid lipid was put into separate test tubes, and heated in a water bath with a controlled temperature to 4-5°C above the respective solid lipid's melting point (MP) [12].

For selected liquid lipids – Capryol® 90, Oleic Acid, Lauroglycol™ FCC, Labrafac™ PG – 2 mL of each vehicle was added to 5 mL stopper vials.

50 mg of agomelatine was put in excess in each test tube and each vial with different solid lipids and liquid lipids respectively. Using a vortex shaker (CM101; Remi), the mixtures were agitated at 100 rpm, 25°C for 72 hours to reach a supersaturation state. After centrifuging the mixtures at 18,000 rpm (3300; Inkarp Instruments), the excess agomelatine was removed by passing the supernatant through a 0.20 µm syringe filter. Following the proper dilution with ethanol, the quantity of agomelatine in each filtrate was measured using a UV spectrophotometer (T60U; PG Instruments) at 229 nm (readings were taken thrice)[13].

Selection of Binary Mixture of Solid and Liquid Lipid based on Miscibility

To test the compatibility of chosen lipids in the ratio of solid lipid to liquid lipid ratio stated in literature[14], both lipids were mixed together in the given ratio 70:30 by heating to 15-20°C above melting point of selected solid lipid, agitated at 100 rpm for 1 h, brought to 25°C for 24 h. Obtained solidified binary mixture was assessed visually for phase separation or precipitation by smearing on filter paper to analyze for traces of any oil on the filter paper, absence of which indicates miscibility of the lipids[15].

Screening of Surfactant

100 mg $lipid_{mix}$, with a ratio of 70:30 solid:liquid lipid, was dissolved in 3 ml of methylene chloride and added to 10 ml of 5% aqueous surfactant solution. The solvent was evaporated and a colloidal system, consisting of NLC, was created by agitating the mixture constantly. UV spectrophotometer (T60U) was used to examine the solution in three batches for % transmittance at a wavelength of 229 nm after suitable dilution with deionized water[16].

Drug-Excipient Interaction study using FT-IR

By FTIR analysis on JASCO Inc. FT/IR-4X, FT-IR spectra were obtained for pure drug (Agomelatine) and mixtures of drug with excipients (Precirol® ATO 5, oleic Acid, poloxamer 188 and poloxamer 407). All solid samples were prepared using the potassium bromide (KBr) pellet technique[17] 1:10 of sample:KBr at 25°C, and ATR method[18] was used for liquid samples, spectra were recorded from 4000 to 400 cm^{-1}.

Design Of Experiment – 3^2 Full Factorial Design

A two-factor three-level factorial design[19] was employed using Stat-Ease Design-Expert v.12 (USA) to investigate the impact of independent variables on dependent variables. The quantity of total lipid (A) and surfactant

concentration (B) were chosen to serve as the independent variables (two-factors). Particle size (nm) at a range of (150 to 170), Zeta potential (mV) at range of (-3 to -40) & Entrapment efficiency (%) at a range of (55-65) were selected as dependent variables (three responses) (Table 1). We tested the impact of the two factors we chose A & B at three different levels (+1, 0, -1). Each factor's experimental range was established according to preliminary findings and the feasibility of manufacturing the NLC at extreme levels (Table 2). The experimental approach led to the development of nine different formulas, designated as AF1–AF9 and evaluated for chosen dependent responses PS, ZP, EE.

The applied factorial table shows the values of the independent factors and the dependent responses that will be assessed (Table 3).

Table 1: Experimental variables (factors and responses)

Independent Variables/Factors	Dependent Variables/Responses
A: Total Lipids (mg)	*Particle size (nm)*
B: Surfactant concentration (%)	*Zeta potential (mV)*
	Entrapment efficiency (%)

Table 2: Independent Factors at Low and High Level based on Design Expert

	Name	Units	Type	Changes	Std. Dev.	Low	High
	Total Lipids	mg	Factor	Easy	0	100	400
	Surfactant	%	Factor	Easy	0	1	2
	Particle Size	nm	Response				
	Zeta Potentia	mV	Response				
	Entrapment E	%	Response				

Table 3: Agomelatine NLC Trials based on Stat-Ease Design Expert

	Std	Run	Factor 1 A:Total Lipids mg	Factor 2 B:Surfactant %	Response 1 Particle Size nm	Response 2 Zeta Potential mV	Response 3 Entrapment Efficinecy %
	4	1	100	1.5			
	5	2	250	1.5			
	9	3	400	2			
	6	4	400	1.5			
	1	5	100	1			
	2	6	250	1			
	7	7	100	2			
	8	8	250	2			
	3	9	400	1			

Statistical Analysis:

Analysis of variance (ANOVA)[17] was conducted with a confidence interval of 95% to identify the statistical significance of each variable and differences across variables. For each response, a suitable mathematical model (regression equation) was generated to measure the impact of independent variables, by aid of statistical metrics such as the regression coefficient (R^2), p-value ($p<0.05$), and F-value. Contour plots and 3D response surface plots were created to establish the relationship between factors and dependent variables.

Preparation of AG-NLC

Using data from the nine separate runs by factorial design approach depicted in Table 3, Agomelatine-loaded NLCs were made using slightly modified hot high-speed homogenization (HSH) method[20] with solid lipid:liquid lipid ($lipid_{mix}$) in 70:30. Chosen solid lipid (here Precirol® ATO 5) was heated up on a water bath at 80°C, a temperature about 15-20°C above its MP, liquid lipid (i.e., Oleic acid) was added to melting solid lipid. Into the molten lipid phase, 5 mg of agomelatine in a little quantity of ethanol (1 mL) was placed. The lipid phase was transferred to the different concentrations of hot aqueous surfactant phase (double distilled water + Poloxamer 188) preheated at 80 °C, same as that of lipid

phase with constant stirring. This mixture was put through high-speed homogenizer (SS316; Bhuvan Engineering) at 18000 RPM for 30 minutes at 80°C, followed by probe sonication (VCX750; SONIC)[48] at 90 W and 40% amplitude for 5 minutes for increased size reduction. The NLCs were brought to ambient temperature, filtered (Whatman syringe filter; nylon; 0.45 µm) to remove unentrapped agomelatine, stored in sterile glass vials with screw caps at 6°C ± 2°C until they were further analyzed.

Table 4: Agomelatine NLC Formulations

S. No.	Ingredients	AF1	AF2	AF3	AF4	AF5	AF6	AF7	AF8	AF9
1	Agomelatine (mg)	5	5	5	5	5	5	5	5	5
2	Precirol® ATO 5 (mg)	70	175	280	280	70	175	70	175	280
3	Oleic Acid (mg)	30	75	120	120	30	75	30	75	120
4	Poloxamer 188 (%)	1.5	1.5	2	1.5	1	1	2	2	1
5	Ethanol (mL)	1	1	1	1	1	1	1	1	1
6	Total Lipids (mg)	100	250	400	400	100	250	100	250	400

Figure 1: Formulated Nanostructured Lipid Carriers for Agomelatine

Characterization of AG-NLCs

Particle size, Polydispersity Index (PDI) and Zeta Potential

The PS, polydispersity Index and ZP of prepared NLCs were analyzed by Nano Partica Nano particle analyser/Zetasizer – (SZ-100; Horiba Scientic) with 90° angle at 25°C. The samples have been adjusted to a 10% concentration using double distilled water to avoid multiple light scattering effects and contained in a disposable cuvette made of quartz, which included two electrodes[21].

Zetasizer uses dynamic light scattering (DLS) method to analyze PS and electrophoretic mobility for ZP determination.

Figure 2: Nano Partica Nano Particle Analyser

Entrapment Efficiency (%)

A slightly modified version of the previously published method was used to determine the %EE of agomelatine in the NLC[22]. After filtering out any traces of free agomelatine, 1 mL of the NLC solution was mixed with 2.5 mL of methanol to dissolve it entirely. Following a 15-minute centrifugation run at 4°C at 12,000 rpm (3300; Inkarp Instruments), the transparent supernatant liquid was collected, filtered, and subjected to UV analysis at 229 nm. The following equation was used to compute the %EE:

$$\%\ Entrapment\ Efficiency = \frac{\text{Actual amount of Agomelatine (mg) } in\ NLC}{\text{Total amount of Agomelatine (mg) } added\ in\ formulation} \ x\ 100$$

Formulation of Thermosensitive AG-NLC In-situ Gel

AG-NLC in-situ gels were prepared by cold method[23] (Table 5). Varied concentrations of poloxamer 407 solutions (2%, 4%, 6%, 8% and 10%) were prepared by dispersing the required amount of polymer in 25 ml cold deionized water (5 ˚C) with constant stirring on magnetic stirrer at 200 rpm for 1 hr, followed by refrigeration for 4 hours to get clear solutions by complete dissolution. 0.3% (0.075 g) sodium alginate (mucoadhesive polymer for controlled release of agomelatine from the gel) was mixed with each conc. of poloxamer 407 solution. 25 microliter (0.10% w/v) of optimized AG-NLC was added to each base solution at 5 °C with continuous stirring to form homogeneous phase. With incubation at 35 °C, gelation was allowed to happen. The gels were then refrigerated for further evaluation.

Table 5: AG-NLC In-situ Gel Formulation

S. No.	*In-situ gel formulations*	Poloxamer 407 (%)	Sod. Alginate (%)	AG-NLC (µL)	Ethanol (ml)	Deionized water (ml)
1	NLCG-1	2	0.3	25 µL	1	25
2	NLCG-2	4	0.3	25 µL	1	25
3	NLCG-3	6	0.3	25 µL	1	25
4	NLCG-4	8	0.3	25 µL	1	25
5	NLCG-5	10	0.3	25 µL	1	25

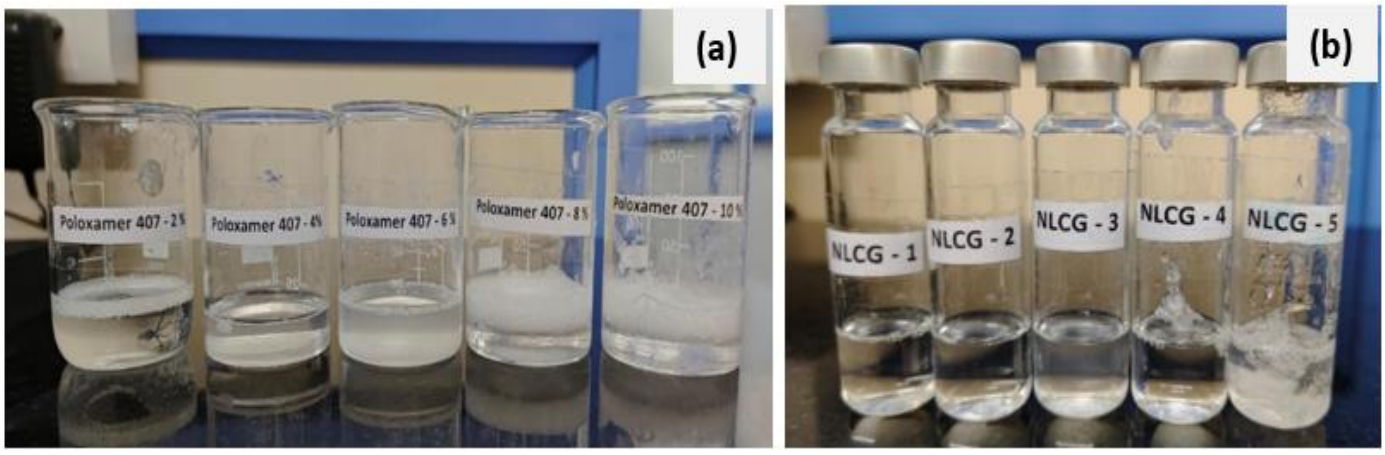


Figure 3: (a) Poloxamer solutions in Varied Concentrations (2-10%); (b)Thermosensitive AG-NLC In-Situ Gels (NLCG-1 to NLCG-5)

Evaluation of AG-NLC In-Situ Gel

Viscosity

The viscosity-related phase behavior changes were quantified using a Brookfield viscometer (Brookfield Engineering, MA, USA) equipped with a specific C50-1 spindle at 20 rpm. The temperature was recorded at 25 °C and 37 °C, with three measurements taken for each[24].

Determination of pH

pH of formulated AG-NLC in-situ gels was measured in triplicate with a calibrated digital pH meter (WPH-10; Wensar Instrument) at room temperature by immersing the glass electrode into it and recording the value[25].

Gelation temperature and gelation time.

5 mL of AG-NLC in-situ gel was placed in a clear vial containing a magnetic bead. Using a magnetic stirrer (MG-212; SISCO) and a heating pan, the vial was placed in a water bath set at 4°C. A 2°C/min increase in temperature at 100 rpm was achieved stirring in the range of transition temperature (25-37°C). The temperature when the AG-NLC in-situ gel in solution form converted to gel with non-flowing characteristic when turned to 90° angle was noted, when the bead stopped rotating, and the duration of the phase transition was documented as the gelation time[26]. The experiments were repeated three times.

Drug Content

A modified UV-Vis spectroscopic technique was used to ensure drug content uniformity. In a volumetric flask, 1 mL of the gel formulation—which is equal to 1 mg of Agomelatine—was combined with 100 mL of PBS (pH 6.8). The mixture was agitated for 2 hours and then filtered. One mL of the filtrate was diluted with ten mL of pH 6.8 phosphate buffer and then analyzed at 229 nm. Drug content was determined three times for each formulation[27].

In Vitro Drug Release Study

In-vitro diffusion experiments from in-situ gels were carried out using a Franz diffusion cell (JFDC-07; ORCHID Scientific) through dialysis membrane soaked in receptor dialysis medium of PBS 6.8 for 4 h before use. 1 mL of AG-NLC in-situ gel was added to the donor compartment, while 15 mL of PBS (pH 6.8) was added to the receiver compartment. The mixture was kept at 100 rpm with a magnetic stirrer at 37 ± 1 °C for 6 hours. The receptor compartment was sampled at regular intervals (every 1 mL) and restocked with new dialysis media at the same volume at time intervals 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 minutes[28]. The samples were taken three times and then passed through a 0.20 µm filter; after appropriate dilutions, they were analyzed spectrophotometrically (T60U) at 229 nm against PBS (pH 6.8) as blank[29].

Drug Release Kinetic Study

In vitro drug release data of optimized AG-NLC in-situ gel was fitted to four well-known release models like zero order, first order, Higuchi-equation, and Peppas-Korsemeyer[28] to know the mechanism of drug release of agomelatine from the formulation by regression analysis correlation coefficient (R^2) and release exponent (n) determination.

Scanning Electron Microscopy (SEM)

To envisage the surface morphology of the optimized AG-NLC in-situ gel formulation in terms of shape and texture of surface, scanning electron microscopic analysis (SEM) was done (S-3700N, Hitachi) at 15-18 Kv after gold sputtering at various magnifications[30]. It gives a qualitative description of the surface.

Stability Studies

Short-term stability investigations were conducted on the optimized AG-NLC in-situ gel as per ICH regulations. The samples were kept in glass vials with screw caps at temperatures 4°C±1°C & 25°C±1°C for a duration of 1 month in stability chamber (NLHC16SI; Newtronic). Results were evaluated for physical stability at day 30, for drug diffusion rate, gelation temperature, pH, and drug content using same methods stated earlier[27].

RESULTS AND DISCUSSION

Selection of excipients

Selection of Solid Lipid and Liquid Lipid

For high drug loading in NLC, drug should show high solubility in lipid matrix. Order of solubility of agomelatine in solid lipids was Precirol® ATO 5>Compritol® 888ATO>Gelucire® 43/01 (Figure 4a) and that in liquid lipids was oleic acid>Labrafac™ PG>Lauroglycol™ FCC>Capryol® 90 (Figure 4b). As maximum solubility was demonstrated in Precirol® ATO 5 (62 ± 0.147 mg/mL) and oleic acid (81.5 ± 0.91 mg/mL), they were chosen as solid lipid and liquid lipid respectively for AG-NLC preparation. AG's greater solubility in liquid lipid as opposed to solid lipid suggests it may be responsible for greater drug loading and retention[11].

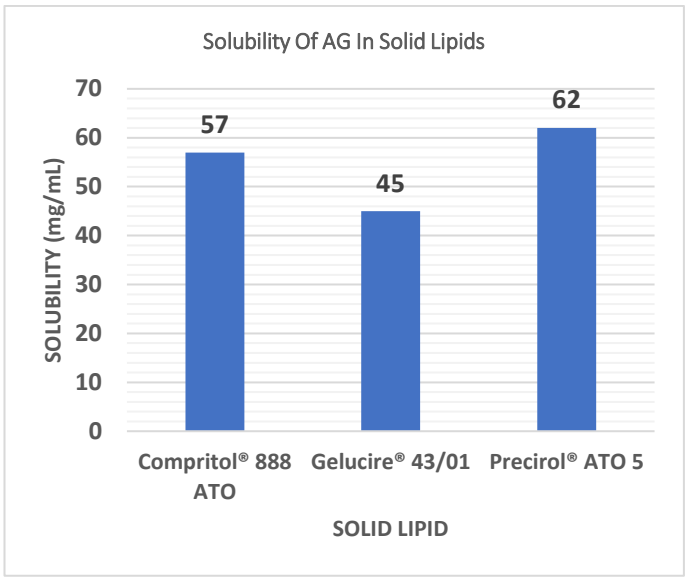
It has been demonstrated that Precirol® increases the EE of NLCs[31], could be because of its loose structure to trap lipophilic and hydrophilic molecules.

Miscibility assessment

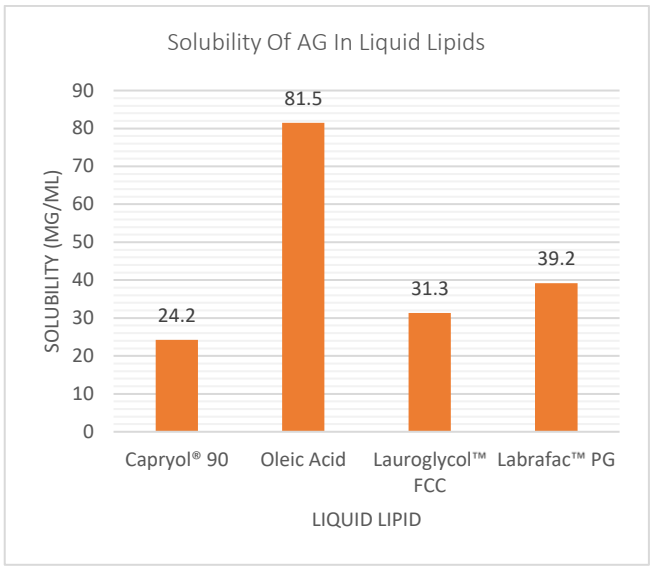
Lack of oil droplets from Precirol® ATO 5 and oleic acid mixture, and no sign of phase separation on filter paper confirmed the miscibility of the $Lipid_{mix}$ in 70:30 ratio. Based on published research, selected lipids demonstrated the necessary stability and were shown to be safe for the mucous layer and a variety of cell types[32].

Selection of Surfactant

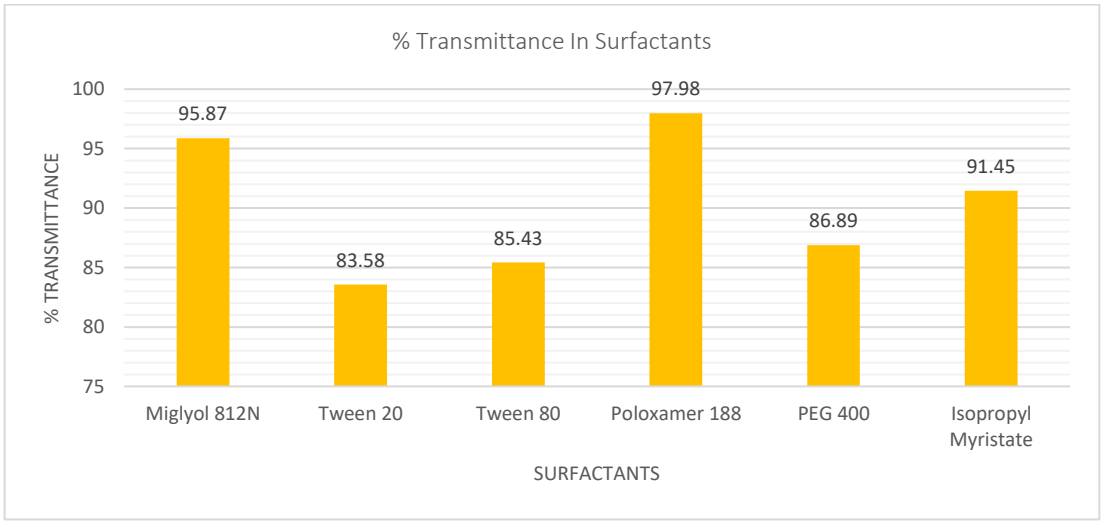
Figure 4c lists the surfactants that were considered for use in determining the Agomelatine transmittance %; poloxamer 188 (97.98 ± 0.45%) was chosen to generate NLC. More percentage of light transmittance indicates smaller vesicle size, hence greater emulsification. This occurs with surfactants having high HLB value, poloxamer 188 has HLB value of 29. Consistent with this, earlier studies demonstrated compatibility of poloxamer 188 with selected lipids[33]. As a surfactant, Poloxamer 188 has the capacity to emulsify selected $lipid_{mix}$, has a mild effect on the nasal mucosa without irritating it, and tends to lessen lipid polymorphic state transitions.



(a)



(b)



(c)

Figure 4: Solubility of AG in different selected (a) solid lipids, (b) liquid lipids, (c) surfactants. All measurements were repeated thrice.

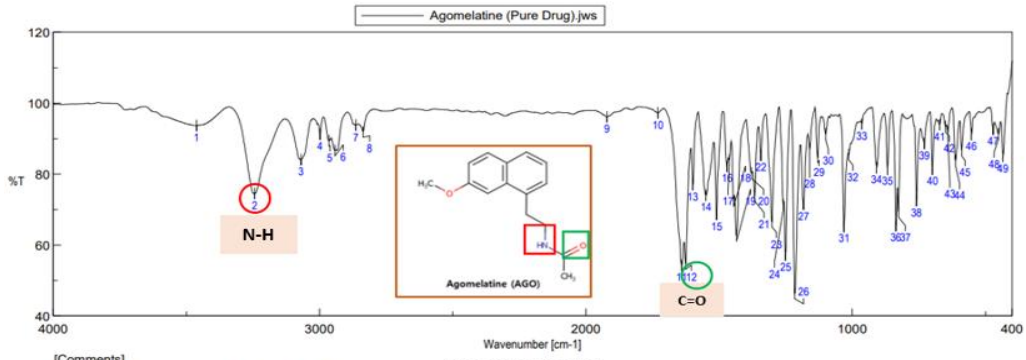
Fourier Transform Infrared Spectroscopy

The stretching of the N-H bond, of the C=O bond, and of the aliphatic C-H bond for the CH_3 group all caused broad peaks seen in the FTIR spectrum of pure agomelatine (Figure 5), at 3243.68 cm^{-1}, 1625.7 cm^{-1}, and 2941.88 cm^{-1} respectively; these are characteristic to agomelatine, they demonstrate similarity to and are in good occurrence with findings reported earlier[34] (Table 6).

FTIR spectra of NLC formulation mixture (Figure 7) and NLCG formulation mixture (Figure 8) reported same absorption peaks at 3005.52 cm^{-1} for amide (N-H) stretch and 1628.59 cm^{-1} for ester (C=O) stretch. This spectral data is also close to the major absorption peaks of respective groups reported in literature. The placements of the distinctive bands have not changed much which concludes that the drug was dispersed in the excipients molecularly thus retaining its unique characteristics while avoiding chemical reactions with the other excipients.

Table 6: FTIR characteristic peak comparison between pure agomelatine and AG with different excipients

Groups	Agomelatine in literature[34]	Agomelatine Pure Drug	Agomelatine with NLC Excipients	Agomelatine with NLCG Excipients
Amide (N-H) stretch	3,240 cm^{-1}	3243.68 cm^{-1}	3005.52 cm^{-1}	3005.52 cm^{-1}
Ester (C=O) stretch	1,620 cm^{-1}	1625.7 cm^{-1}	1628.59 cm^{-1}	1628.59 cm^{-1}



[Comments]
Sample name Agomelatine (Pure Drug)
Comment 031279
Username 1023
Workgroup QC
Division QC

[Measurement Information]
Model name FT/IR-4X1typeA
Serial No. A038962111 Instrument_ID : ABNL/QC/FTIR-01

Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity
1	3461.6	93.6413	2	3243.68	74.5291
3	3069.16	84.071	4	2997.8	91.1861
5	2965.02	89.3386	6	2941.88	86.6113
7	2863.77	93.7822	8	2835.81	91.885
9	1920.75	96.0691	10	1729.83	97.2132
11	1640.16	54.2148	12	1625.7	54.6945
13	1598.7	76.9164	14	1549.52	73.9814
15	1509.03	68.6482	16	1470.46	82.1252
17	1461.78	83.9598	18	1445.39	72.3486
19	1434.78	62.5672	20	1378.85	80.2549
21	1366.32	73.9102	22	1343.18	85.2734
23	1301.72	66.6081	24	1259.29	71.4167
25	1250.61	57.1114	26	1215.9	46.3992
27	1183.11	71.501	28	1159.97	87.0636
29	1130.08	84.7243	30	1100.19	91.3131
31	1030.77	65.2183	32	1013.41	85.4703
33	964.233	94.1003	34	908.308	81.7755
35	867.81	81.3076	36	835.99	65.4087
37	826.348	69.2611	38	758.852	72.5096
39	729.925	88.6016	40	699.069	81.3775
41	672.071	93.9054	42	649.893	93.3145
43	641.215	91.8845	44	612.288	83.7669
45	589.147	86.5407	46	552.506	91.1577
47	471.51	92.583	48	451.261	91.2954
49	433.905	84.9343			

Figure 5: FTIR Spectrum of Pure Agomelatine

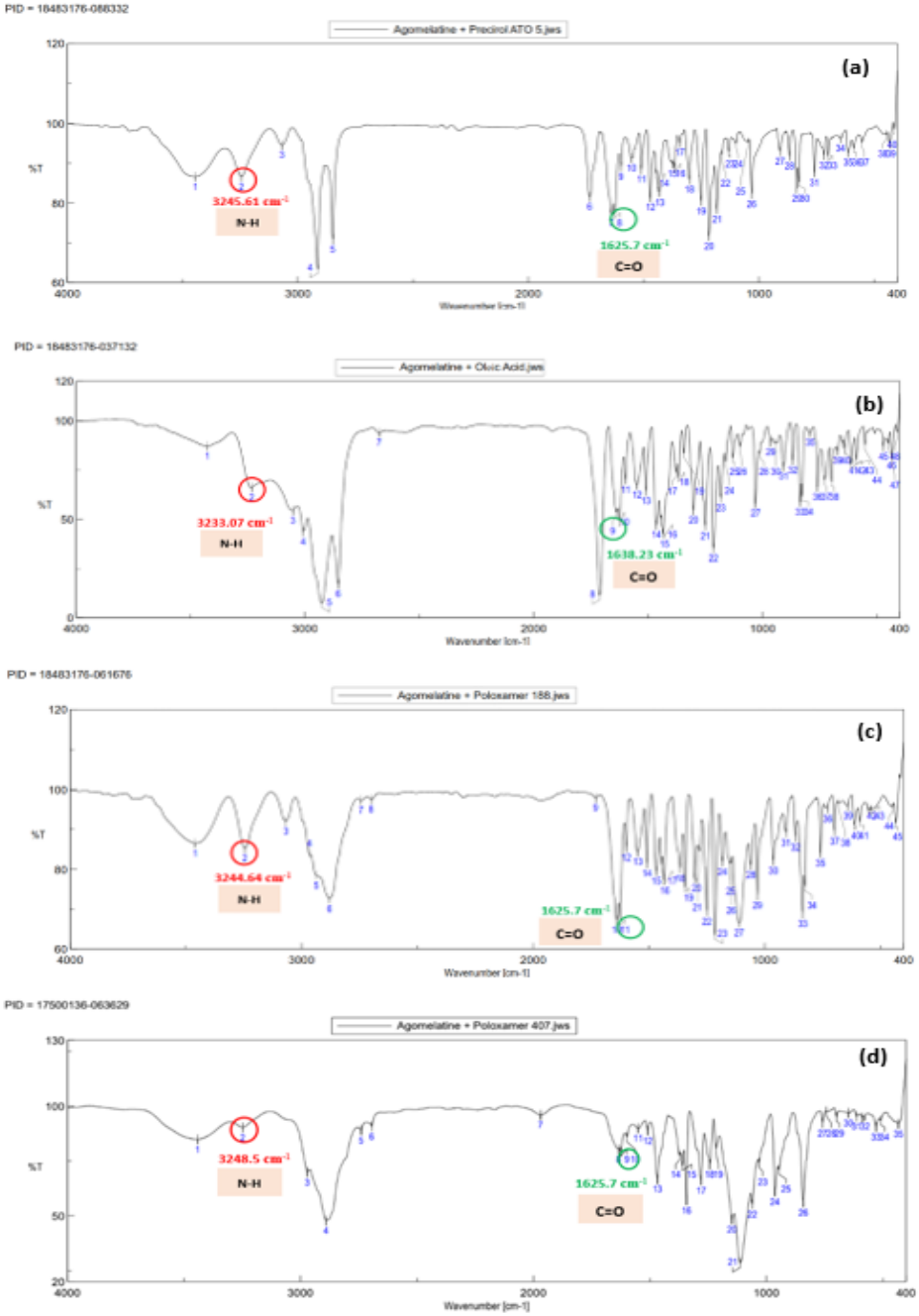
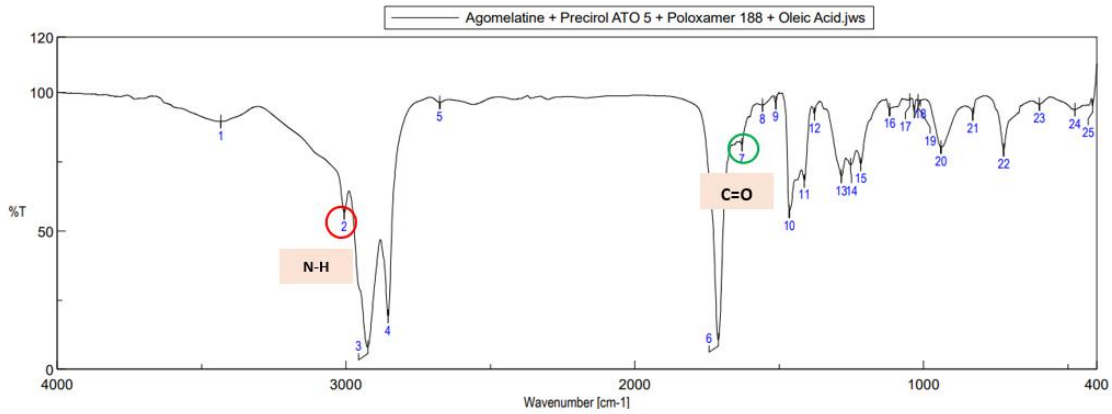


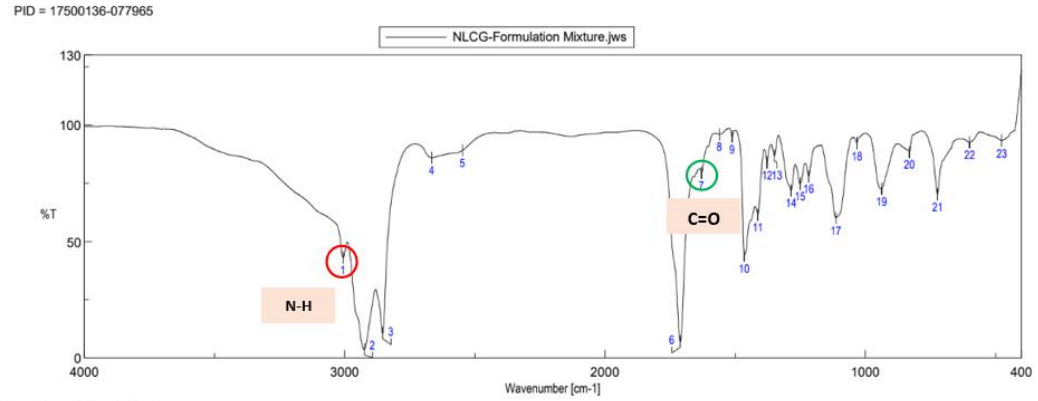
Figure 6: FTIR Spectra of Pure AG with (a)Precirol® ATO 5, (b)Oleic Acid, (c)Poloxamer 188, (d)Poloxamer 407



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity
1	3432.67	89.4988	2	3005.52	56.5204
3	2924.52	8.00143	4	2854.13	19.0643
5	2674.78	96.3765	6	1710.55	10.6124
7	1628.59	81.1205	8	1558.2	95.4702
9	1511.92	96.3143	10	1464.67	56.9465
11	1412.6	68.0263	12	1377.89	92.1895
13	1284.36	69.6848	14	1252.54	73.8409
15	1217.83	74.0059	16	1117.55	93.8389
17	1047.16	97.2527	18	1032.69	93.0439
19	1019.19	96.9651	20	940.128	80.2509
21	829.241	92.1785	22	722.211	79.2974
23	598.789	95.775	24	476.331	93.7891
25	414.62	95.1828			

Figure 7: FTIR Spectrum of NLC formulation mixture – Pure Agomelatine with Precirol® ATO 5, Oleic Acid and Poloxamer 188



Results of Peak Find

No.	Position	Intensity	No.	Position	Intensity
1	3005.52	42.967	2	2924.52	3.74706
3	2854.13	10.6831	4	2665.14	85.8869
5	2546.54	89.0784	6	1710.55	7.07763
7	1628.59	79.4586	8	1560.13	95.9581
9	1510.95	95.0029	10	1464.67	43.8479
11	1412.6	61.3004	12	1376.93	83.8967
13	1348.96	86.8323	14	1285.32	71.7826
15	1250.61	74.7965	16	1216.86	77.8302
17	1112.73	59.9972	18	1032.69	92.1981
19	937.235	72.5939	20	830.205	88.3008
21	723.175	70.2038	22	598.789	92.5124
23	476.331	93.3099			

Figure 8: FTIR Spectra of NLCG Formulation mixture – Pure Agomelatine with Precirol® ATO 5, Oleic Acid, Poloxamer 188, Poloxamer 407

Optimization & Statistical Analysis for Formulated AG-NLCs (AF1-AF9)

Using Design-Expert®, AG-NLCs were optimized. 9 Formulations (AF1-AF9) found in different levels of total lipids (A) and surfactant concentration (B) were prepared and evaluated for dependent responses – particle size (PS), zeta potential (ZP), entrapment efficiency (EE) depicted in Table 7 showing the design matrix of response results. This response data was fitted into different models. R^2 value of each model for each response – PS, ZP, EE – was obtained with 2FI model

being the best fitted model showing R^2 value of 0.9660, 0.9677, 0.9994 respectively (Table 8). Interactive optimization model, two-factor interaction (2FI) model was chosen also based on p-value which was lowest for this model compared to other models under observation. The effects of components A and B on PS, ZP, and EE are determined using ANOVA utilized by 2FI, which yields significant (*p<0.05*) or insignificant (*p>0.05*) values.

Table 7: Formulation Optimization of AG-NLC with their observed responses

		Factor 1	Factor 2	Response 1	Response 2	Response 3
Std	**Run**	**A: Total Lipids (mg)**	**B: Surfactant (%)**	**Particle Size (nm)**	**Zeta Potential (mV)**	**Entrapment Efficiency (%)**
4	**1**	100	1.5	130.2	-23.2	39.23
5	**2**	250	1.5	172.3	-29.3	53.08
9	**3**	400	2	235.3	-20.1	27.65
6	**4**	400	1.5	241.1	-18.1	23.28
1	**5**	100	1	152.1	-21.3	32.31
2	**6**	250	1	184	-32.2	48.41
7	**7**	100	2	115.9	-25.4	45.05
8	**8**	250	2	159.3	-37	58.14
3	**9**	400	1	265	-16.1	21.11

Table 8: 2FI model summary for PS, ZP, EE

Response	Mean	Std. Dev	R^2	Adjusted R^2	Predicted R^2
1: PS	183.91 nm	2.17	0.9660	0.9456	0.9267
2: ZP	-24.74	2.03	0.9677	0.9139	0.8573
3: EE	38.70%	0.5517	0.9994	0.9983	0.9922

Table 7 shows the summary of the ANOVA findings for response 1, 2, 3, i.e., PS, ZP, %EE using the 2FI model with a coefficient based on the coded variables provided by the Design-Expert®. As per ANOVA results from the software, 2FI model of response 1, 2, and 3 was best fitted as significant as F-value is 240.52, 17.97 and 938.92; and *p*-value was 0.0004, 0.0191, and < 0.0001 respectively. Observed *p<0.05* indicate accepted significance of coefficient terms. Model terms A, B, and A^2. are significant for PS; A and A^2 are significant model terms for ZP; A^2, AB, B, and A are significant model terms for %EE. These results indicated that independent selected variables – individually and in their interaction – are significantly influencing the three responses as shown in Table 9.

Table 9: F-ratio and associated p-values for the NLCs (AF1-AF9)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
For Response 1, PS						
Model	*21695.55*	*5*	*4339.11*	*240.52*	*0.0004*	*Significant*
A-Total Lipids	19631.04	1	19631.04	1088.16	< 0.0001	
B-Surfactant	1368.06	1	1368.06	75.83	0.0032	
AB	10.56	1	10.56	0.5855	0.4999	
A^2	652.81	1	652.81	36.19	0.0092	
B^2	33.08	1	33.08	1.83	0.2687	
Residual	54.12	3	18.04			
Cor Total	21749.67	8				
For Response 2, ZP						
Model	*369.33*	*5*	*73.87*	*17.97*	*0.0191*	*Significant*
A-Total Lipids	40.56	1	40.56	9.87	0.0516	
B-Surfactant	27.74	1	27.74	6.75	0.0805	
AB	0.0025	1	0.0025	0.0006	0.9819	
A^2	294.44	1	294.44	71.65	0.0035	
B^2	6.60	1	6.60	1.61	0.2945	
Residual	12.33	3	4.11			
Cor Total	381.66	8				
For Response 3, EE						
Model	*1428.79*	*5*	*285.76*	*938.92*	*< 0.0001*	*Significant*
A-Total Lipids	330.78	1	330.78	1086.86	< 0.0001	
B-Surfactant	140.26	1	140.26	460.86	0.0002	
AB	9.61	1	9.61	31.58	0.0111	
A^2	948.01	1	948.01	3114.89	< 0.0001	
B^2	0.1233	1	0.1233	0.4053	0.5696	
Residual	0.9130	3	0.3043			
Cor Total	1429.70	8				

Effect of Independent factors on Response 1: Particle Size

The polynomial equation with respect to coded factors was potentially utilized to identify the relative impact of given levels of each factor (A and B) on response 1 PS, by comparing the factor coefficients based on magnitude and mathematical sign (+/-). As a general rule, high levels are coded as +1 and low levels as -1.

The coded-factors equation for PS response given by Design-Expert® was represented as follows:

$$PS = +169.16 + 57.20\,A - 15.10\,B + 1.62\,AB + 18.07\,A^2 + 4.07\,B^2 \quad (Eq.1)$$

PS of all NLCs AF1-AF9 fell within 115.9-265.0 nm. The polynomial equation of PS suggests that factor B has a negative dominant effect and factors A, AB, A^2 & B^2 have positive dominant effects on PS. Figure 9a further shows that PS increases with increasing concentration of total lipids. This can be because more lipid creates increased core viscosity resulting in swollen core. As for the effect of factor B, increasing surfactant concentration decreases particle size due to reduced surface interfacial tension helping the NLC particles to breakdown into smaller sizes and remain disaggregated.

Effect of Independent Factors on Response 2: Zeta Potential

ZP response coded-factors equation given by Design-Expert® is represented as follows:

$$ZP = -31.62 + 2.60\,A - 2.15\,B + 0.0250\,AB + 12.13\,A^2 - 1.82\,B^2 \quad (Eq.2)$$

ZP of all NLCs AF1-AF9 was in the range of -16.1 to -37.0. Factor B & B^2 seem to show negative dominant effect on ZP and factors A, AB & A^2 show positive effects (Eq.2). ZP decreases with increasing concentration of surfactant B. Higher surfactant levels result in superposing of electric double layer and its compression due to its non-ionic nature which provides the material it is adsorbed on with a negative charge. The zeta potential of lipid nanoparticles has been shown to be enhanced by poloxymer 188 alone. Effect of A and B is also confirmed from ANOVA results and 3D response surface plot (Figure 9b).

Effect of Independent Factors on Response 3: Entrapment Efficiency of AG in AG-NLC

EE quadratic equation was given by Design-Expert®, represented as follows:

$$EE = +53.04 + 7.42\ A + 4.84\ B - 1.55\ AB - 21.77\ A^2 + 0.2483\ B^2\ B2 \quad (Eq.3)$$

EE of all NLCs AF1-AF9 was in the range of 21.11-58.14 %. Eq.3. suggests that A, B, AB, A^2 have antagonistic effect while B^2 show synergistic effect on EE. EE increases with increasing total lipids (A) as well as increasing surfactant concentration (B). Elevated surfactant levels increase solubility and stability of agomelatine in lipid matrix by decreasing partitioning between phases leading to micelle formation. Further, more amount of lipid (factor A) in the matrix results in a structure with less order and imperfections allowing more space for solubilization and entrapment of agomelatine[11], which can be attributed mainly to liquid lipid. The findings of the ANOVA and 3D response surface plot provide extra context for this (Figure 9c).

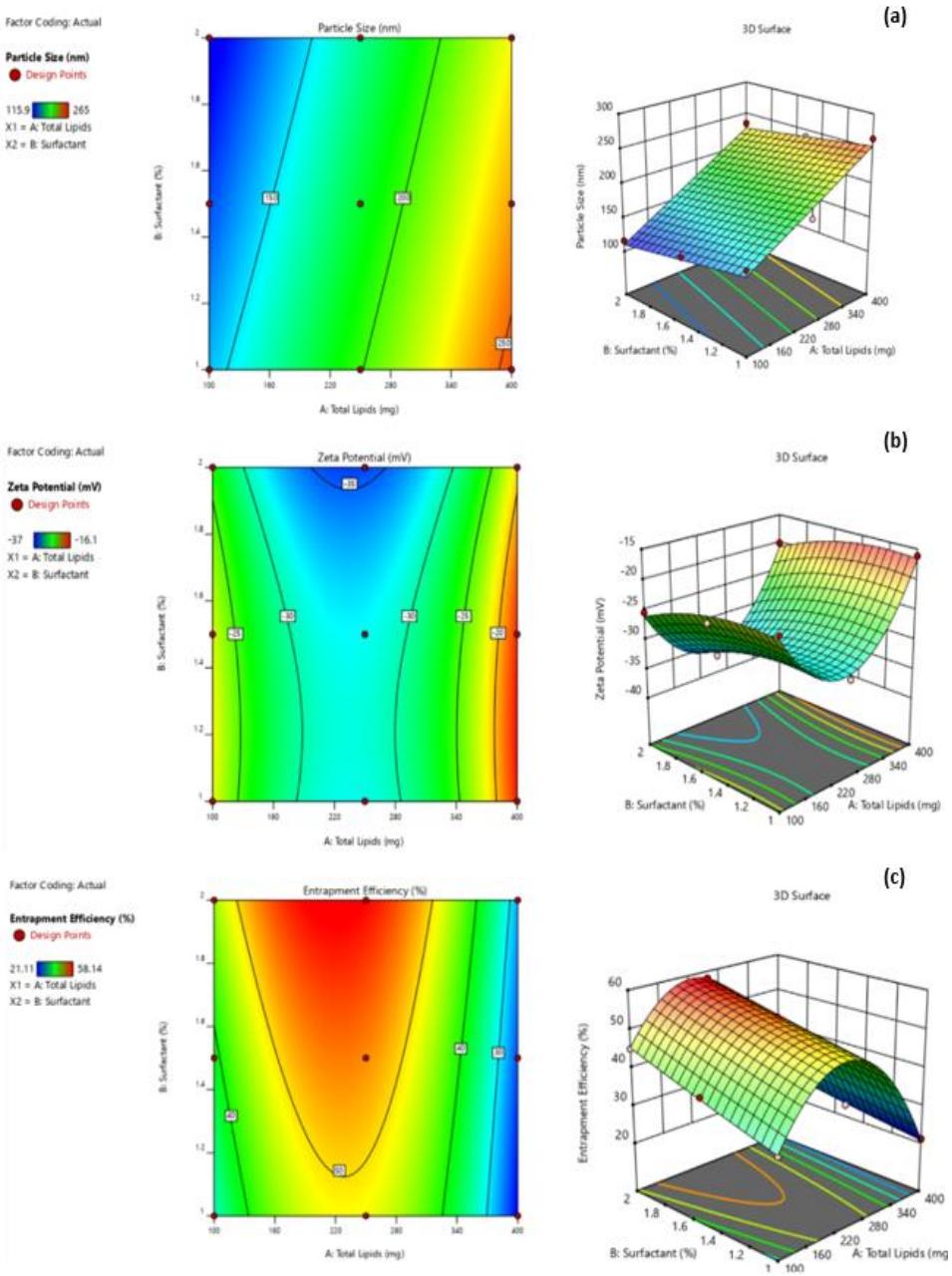


Figure 9: 3D Response Surface Plot for (a) Particle Size of NLCs, (b) Zeta Potential of NLCs, (c) Entrapment Efficiency of AG in NLCs

Selecting the optimized formulation

In order to optimize the formulation with the required responses, a multi-criteria decision optimization (MCDM) approach was used. For the optimization process, we applied the following constraints: PS: 150-170 nm; ZP: -40 to -30 mV; and EE: 55-65% to get optimal formula solutions with high desirability functions.

According to the response surface plots in Figure 10 and the data in Tables 10 & 11, the best formulation that fits the model is AF8 with its observed values very closely aligning with the predicted values of the software (Table 13-14).

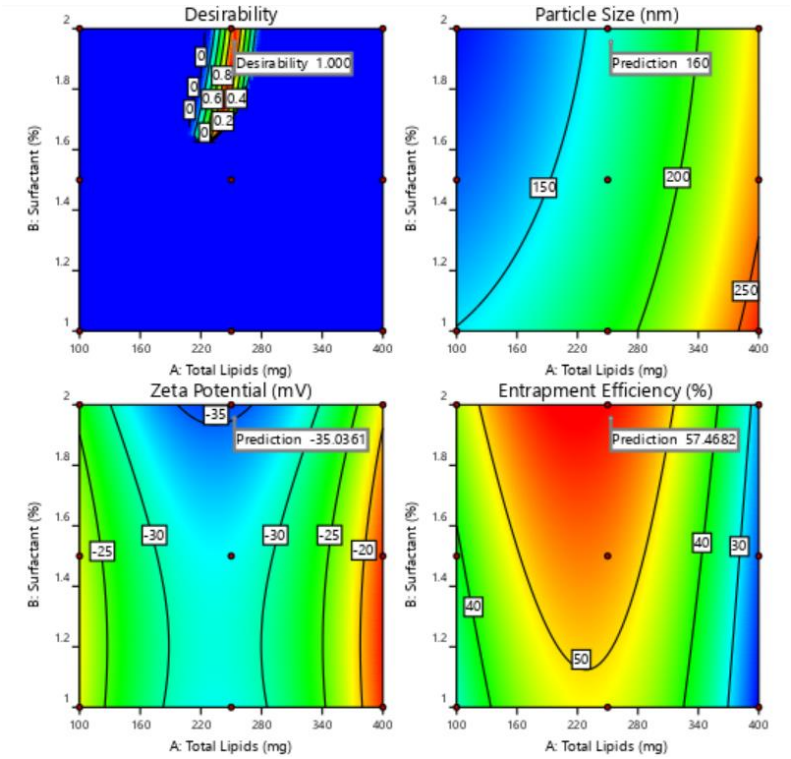


Figure 10: Contour plot for Optimization of NLCs

Table 10: Predicted Factors for Optimized Formulation

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	Total Lipids	253.17	100.00	400.00	0.0000	Actual
B	Surfactant	1.96	1.0000	2.00	0.0000	Actual

Table 11: Point Prediction & Confirmation

Solution 29 of 43 Response	Predicted Mean	Predicted Median	Observed	Std Dev	SE Mean	95% CI low for Mean	95% CI high for Mean	95% TI low for 99% Pop	95% TI high for 99% Pop
Particle Size	160	160	159.3	4.24743	2.96016	150.579	169.421	121.087	198.913
Zeta Potential	-35.0361	-35.0361	37	2.0272	1.41282	-39.5323	-30.5399	-53.6085	-16.4638
Entrapment Efficiency	57.4682	57.4682	58.14	0.551678	0.384481	56.2446	58.6918	52.4139	62.5224

Predicted values of total lipids (mg), surfactant (%) were 253.17 mg, 1.96% respectively showing great similarity to observed values of 250 mg and 2% of the same for AF8 NLC (Table 7). Literature validates the results of **optimized formulation AF8** (Table 12). Particle size of less than 200 nm falls in the acceptable range for NLCs for intranasal delivery from nose-to-brain[35]. Zeta potential in range of ±30 mV prevents aggregation of particles[36]. Higher the zeta potential, higher the physical stability of NLCs for intranasal delivery[37]. However, entrapment efficiency was lower than observed in literature[36] which can be attributed to drug leakage at high homogenization speeds during preparation.

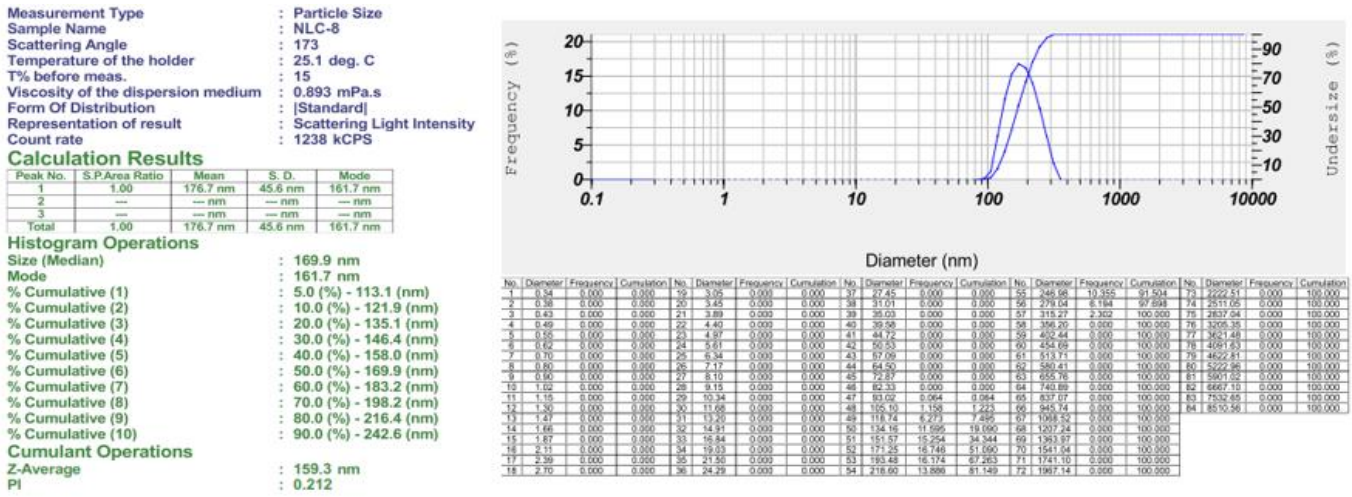


Figure 11: Particle Size & Polydispersity Index for Optimized AF8

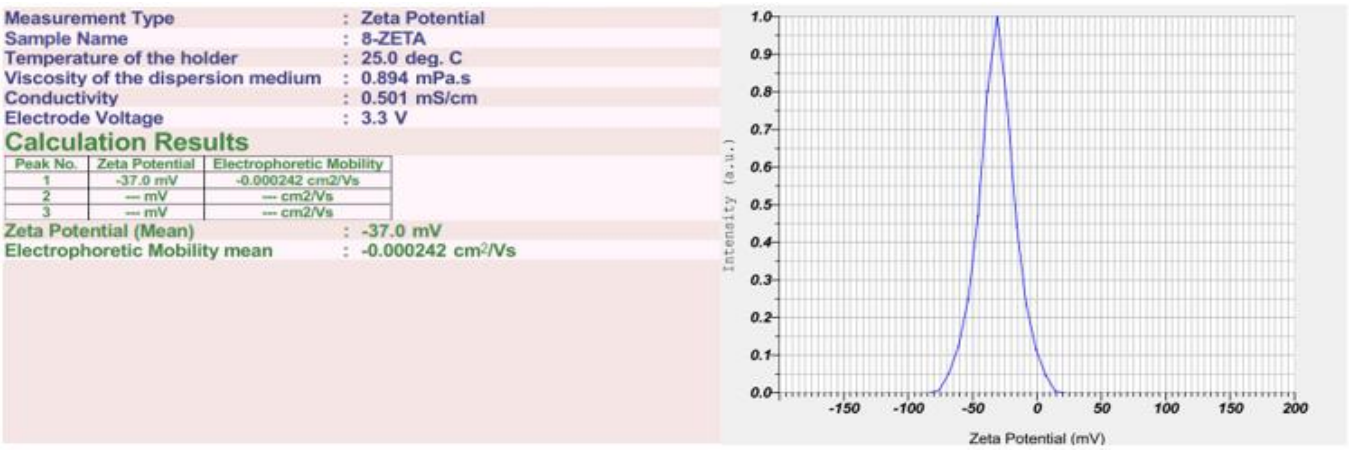


Figure 12: Zeta Potential for Optimized AF8

PDI values were obtained for all nine formulations in the range of 0.181-0.354, among which AF1-AF3, AF5-AF8 exhibited polydispersity (Table 12). For lipid-based NLCs, PDI less than 0.3 is acceptable and affirms monodisperse, narrow size distribution[38]. Optimized AF8 showed polydispersity index of 0.212 indicating homogenous nanosize population.

Table 12: Characterization of NLCs for Formulations AF1-AF9

Formulation	Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index	Entrapment Efficiency (%)
AF1	130.2	-23.2	0.181	39.23
AF2	172.3	-29.3	0.231	53.08
AF3	235.3	-20.1	0.312	27.65
AF4	241.1	-18.1	0.354	23.28
AF5	152.1	-21.3	0.201	32.31
AF6	184.0	-32.2	0.240	48.41
AF7	115.9	-25.4	0.164	45.05
AF8	**159.3**	**-37.0**	**0.212**	**58.14**
AF9	265.0	-16.1	0.392	21.11

Preparation and Evaluation of Thermosensitive AG-NLC In-situ Gel

Optimized AG-NLC formulation AF8 was integrated into thermosensitive poloxamer 407 of different conc. (2-10%) and sodium alginate at a level that was fixed, 0.3% to give in-situ gels[39] (Table 5).

Viscosity, pH, and Drug content

In reported literature, pH of nasal mucosa ranges from 5.5-6.5[40] with baseline pH of 6.3[41] which can vary depending on physiological condition. Formulated AG-NLC in-situ gels (NLCG1-NLCG5) were found to have pH in the range of 6.10±0.54 – 6.34±0.73 (Table 13) showing that no mucosal irritation is expected.

For NLCG to be easily injected into the nose by a syringe and undergo an immediate sol-to-gel transition, its formulation must have an appropriate viscosity. Instead of breaking down or eroding quickly, the prepared gel should remain intact enough to provide localized, persistent drug release. It was noted that the polymer solutions had a viscosity which was thixotropic with respect to temperature change. (Table 13)[25,42]. In an aqueous solution of poloxamer 407, the poly (propylene oxide) core dehydrates as the temperature increases gradually. Conversely, solid and transparent gels are created when the hydrophilic poly (ethylene oxide) shell gets hydrated and inflated[43].

Drug content was found from 92.17±0.12 – 97.48±0.38% (Table 13) which is within an acceptable range of 90-97% which means agomelatine was evenly dispersed.

Gelation temperature & Gelation time

Gelling temperature for intranasal in-situ gels has a range of 30-36°C[44] corresponding to internal nasal physiological temperature from 29-34 °C[45]. The gelation temperature of all in-situ gels was measured (Table 13). NLCG3-NLCG5 gelation temperatures corresponded to accepted range. With increasing poloxamer 407 conc., gelation temperature decreases. Forming of gel at temperature less than 30°C causes difficulty in administering the formulation, and gelling at more than 36°C results in leakage and loss of in-situ gel solution from nasal cavity. If gelation does not occur at nasal mucosal site instantly within required time at required temperature, immediate nasal clearance of formulation occurs, hence gelation time is considered, it is the time required for in vitro gelation of polymeric liquid solution into gel phase. For AG-NLC in-situ gels, gelation time was measured from 9.4±0.96 to 23.2±0.46 seconds[44] (Table 13). NLCG1 and NLCG2 with high gelation temperatures and gelation times signify the formulations cannot be opted for intranasal delivery.

Table 13: Viscosity, pH, Gelation Temperature, Gelation Time, and Drug Content of Formulations NLCG1-NLCG5.

Formulation Code	*pH*	*Gelation Temperature (℃)*	*Gelation Time (Sec)*	*Viscosity (cP)*		*Drug Content (%)*
				at 25 ℃	at 37 ℃	
NLCG-1	6.12 ±0.70	41.58 ±0.57	23.2 ±0.46	50.34 ±0.54	222.29 ±0.88	92.17 ±0.12
NLCG-2	6.24 ±0.22	38.20 ±0.61	21.0 ±0.07	67.18 ±0.52	670.33 ±0.40	97.48 ±0.38
NLCG-3	6.10 ±0.54	35.67 ±0.98	14.7 ±0.13	88.20 ±0.02	905.52 ±0.23	90.36 ±0.21
NLCG-4	**6.34 ±0.73**	**33.24 ±0.11**	**10.3 ±0.38**	**103.75 ±0.17**	**1690.65 ±0.40**	**95.16 ±0.90**
NLCG-5	6.18 ±0.09	30.12 ±0.72	9.4 ±0.96	187.41 ±0.34	2113.14 ±0.63	94.29 ±0.06

Results are expressed as mean ± SD, n=3

From the above results NLCG-4 formulation (Table 13) was chosen as optimized development. All values were within optimal range of literature.

In-Vitro Drug Release Study

For NLCG1–NLCG5, release behavior was assessed using dialysis-based in-vitro drug release method. The in-situ gels presented enhanced sustained agomelatine release over a period of 6 hours in pH 6.8 phosphate buffer, which acted as simulated nasal fluid (pH 6.5). It has been observed from the results depicted in Table 14 and Figure 13 that NLCG-1, NLCG-2, NLCG-3 initially showed burst release in first 30 mins of 43.77%, 27.07% and 21.21% respectively as compared to regular stable controlled release of agomelatine from NLCG-4 and NLCG-5. Formulation NLCG-1 achieved complete drug release within 2.5 hours. This is due to lower concentrations of poloxamer-407 polymer in the formulation (2%) indicating low viscosity, which is not sufficient to achieve sustained drug release.

NLCG containing Poloxamer 8% (NLCG-4) showed sustained release till the 6th hour with a release of 100.01% and it is considered as optimized formulation based on preliminary characterization of AG-NLC in-situ gels depicted in sections 4.1 and 4.2, along with in-vitro drug release profile. Increase in polymer concentration resulted in a prolonged release rate (Figure 13). Saturation of concentration was observed in case of formulation prepared with poloxamer 10% solution (i.e NLCG-5) as the complete drug release was not achieved at the end of 6th hour.

Table 14: In-Vitro Release study for Formulations NLCG-1-NLCG-5

Time (minutes)	*% Cumulative Drug Release (%CDR)*				
	NLCG-1	*NLCG-2*	*NLCG-3*	*NLCG-4*	*NLCG-5*
0	0	0	0	0	0
30	43.77 ±0.03	27.07 ±0.29	21.21 ±0.98	10.60 ±0.05	6.77 ±0.27
60	55.06 ±0.21	36.61 ±0.61	30.90 ±0.02	17.48 ±0.68	11.35 ±0.61
90	74.58 ±0.11	49.62 ±0.91	41.83 ±0.89	22.85 ±0.62	15.08 ±0.82
120	95.20 ±0.48	61.41 ±0.98	52.19 ±0.56	28.73 ±0.84	21.33 ±0.98
150	100.23 ±0.07	72.64 ±0.71	64.23 ±0.71	36.02 ±0.02	27.64 ±0.51
180	-	87.09 ±0.44	71.88 ±0.87	42.59 ±0.93	30.78 ±0.56
210	-	96.16 ±0.35	80.18 ±0.02	49.61 ±0.99	36.62 ±0.75
240	-	99.56 ±0.24	87.78 ±0.16	57.32 ±0.09	40.89 ±0.15
270	-	-	93.33 ±0.21	69.79 ±0.49	47.18 ±0.33
300	-	-	101.06 ±0.04	78.93 ±0.19	52.56 ±0.58
330	-	-	-	90.13 ±0.17	64.06 ±0.80
360	-	-	-	100.01 ±0.25	69.98 ±0.90

Results are expressed as mean ± SD, n=3

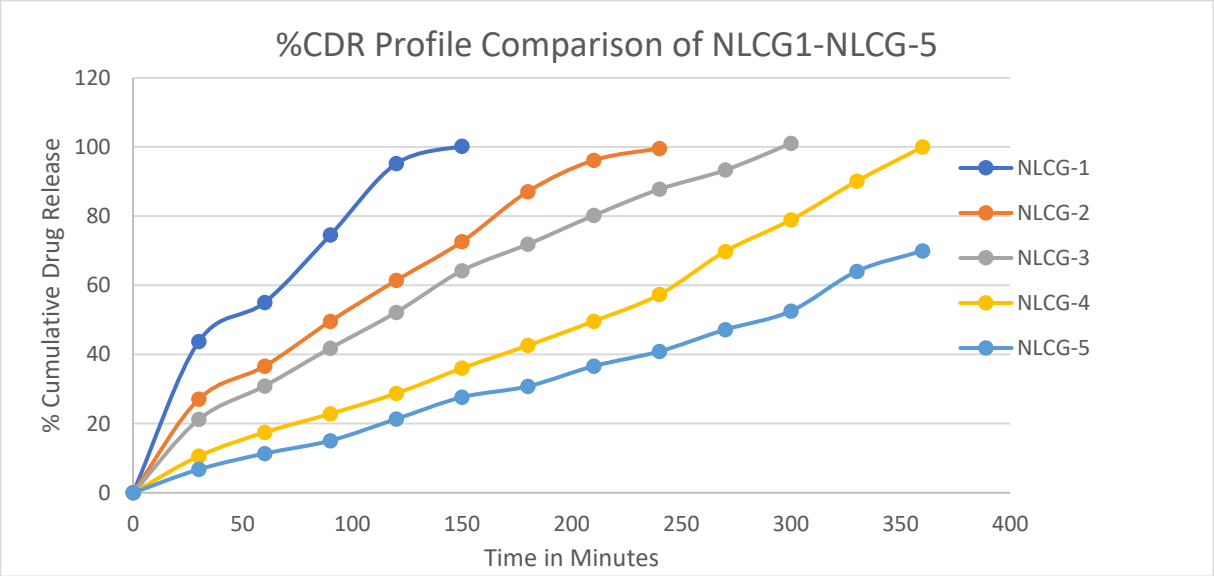


Figure 13: Drug release profile comparison of formulation NLCG-1-NLCG-5

Release Kinetics

The estimations of k (release constant), R^2 (correlation coefficient) & n (release exponent) values are shown in Table 15. On interpreting data, it is concluded that with highest correlation coefficient R^2 0.9859 with n 0.7722, kinetic analysis of the drug release profile of optimized NLCG-4 showed drug release data best fits the Korsmeyer model (Figure 14); which states that $0.45<n<1$ suggests non-Fickian transport mechanism[46]. NLCG-4 follows non-Fickian diffusion/anomalous diffusion; indicating diffusion is not the only drug release mechanism involved, but could also be contributed by erosion at a controlled rate[47] due to slight alterations in the formulation matrix at elevated temperatures (37°C). Liquid lipid present in the matrix gives an arrangement that is less organized which averts drug purging.

Table 15: Kinetic Data for Optimized Formulation NLCG-4

Kinetic Models	*K*	*R^2*	*n*
Zero Order	-1.5042	0.9775	0.2665
First Order	2.2517	0.6670	-0.0038
Korsmeyer-Peppas	-0.0747	0.9859	0.7722
Higuchi	-19.204	0.8816	5.329

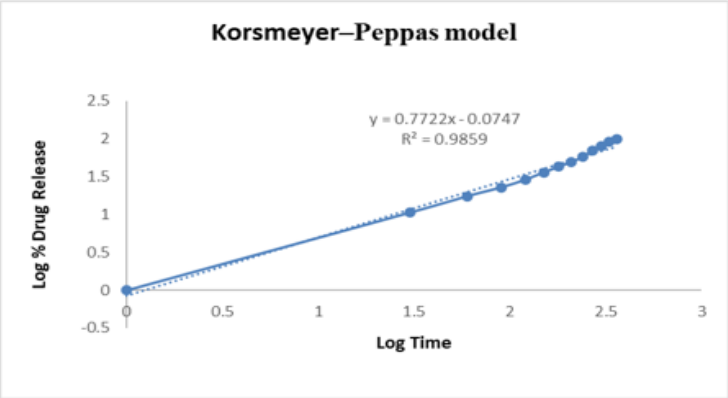


Figure 14: Korsmeyer Model Graph for Optimized NLCG-4

Scanning Electron Microscopy

The optimized NLCG-4 showed particles as distinct, with uniform, almost spherical, and smooth surfaces without aggregation at different magnifications (Figure 15). Turbidity in the background indicates lipid matrix.

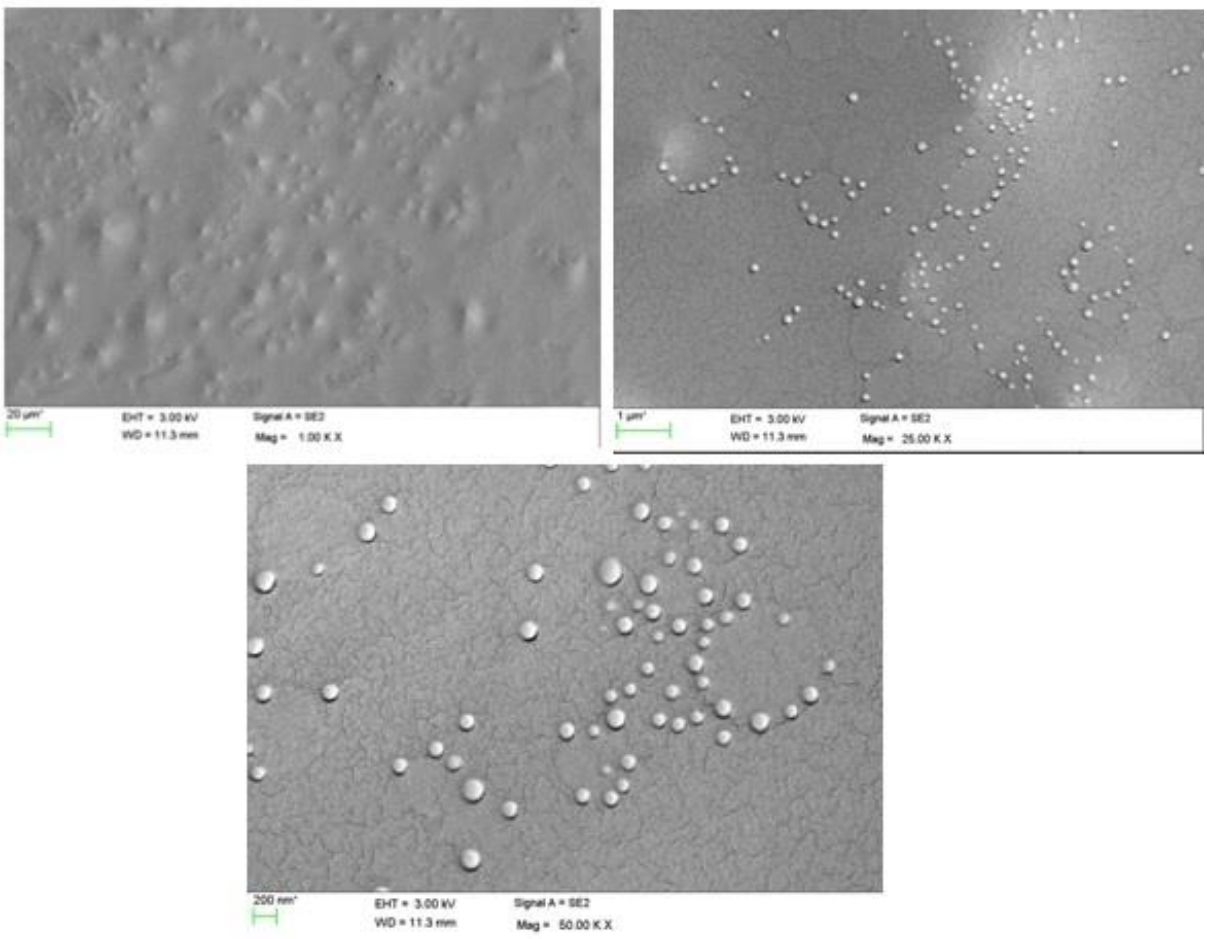


Figure 15: SEM images of NLCG-4 at different magnifications – 1.00 KX, 25.00 KX, 50.00 KX

Stability Studies

Short term accelerated stability data (Table 16) for NLCG-4 showed almost no loss of drug at 4°C, a loss of 0.009% drug content at 25°C after 30 days which was considered negligible. There was minimal change in gelation temperature and pH. This concludes the changes in characterization data after stability studies were not significant as compared to the data procured on day 0 before starting stability studies, indicating the formulation retained its properties hence, confirming its stability.

Table 16: Stability studies of Thermosensitive AG-NLC In-situ Gel Optimized Formulation NLCG-4

Short-Term Stability Study: Optimized Trial (NLCG-4)			
Formulation		**4°C±1°C**	**25°C±1°C**
	Day 0	**Day 30**	**Day 30**
Time (Minutes)	**% Cumulative Drug Release**		
0	0	0	0
30	10.60	9.70	11.06
60	17.48	17.92	16.59
90	22.85	23.30	22.40
120	28.73	28.05	29.40
150	36.02	35.33	35.79
180	42.59	43.26	43.03
210	49.61	49.16	49.16
240	57.32	56.86	56.88
270	69.79	70.24	69.33
300	78.93	78.48	74.47
330	90.13	91.25	85.56
360	100.01	100.80	98.68
pH	6.34	6.29	6.10
Drug Content (%)	95.16	95.08	94.17
Gelation Temperature (°C)	33.24	33.21	32.24

CONCLUSION AND SCOPE

Due to agomelatine's low oral bioavailability (<5%), the intranasal route was investigated. FTIR analysis confirmed compatibility with excipients such as lipids, surfactants, and gelling polymers. Agomelatine was successfully incorporated into NLCs and suspended in an in-situ gel using poloxamer 407 for controlled release. A 3^2-factorial design optimized the NLC formulation, resulting in nanosized particles with good zeta potential and acceptable entrapment efficiency for AF8. Optimized AG-NLC gel (NLCG-4) had suitable pH, viscosity for nasal administration, and enhanced mucosal residence time. *In vitro*, the optimized NLCG-4 maintained 100% agomelatine release prolonged over 6 hours. Stability studies at 4°C and 25°C showed no degradation, confirming the formulation's stability which can be achieved with proper choice of excipients and optimization parameters. Future research should explore higher poloxamer concentrations with various mucoadhesive combinations for AG-NLC in-situ gels. In conclusion, this intranasal AG-NLC *in-situ* gel holds promise for enhanced bioavailability and antidepressant activity, warranting further pharmacokinetic and cytotoxicity studies to expand its therapeutic potential.

Abbreviations

1. 2FI – Two-Factor Interaction
2. AF1-AF9 – Agomelatine-loaded Nanostructured Lipid Carrier Formulations
3. AG – Agomelatine
4. ANOVA – Analysis of variance
5. ATR – Attenuated Total Reflectance
6. BBB – Blood-Brain Barrier
7. BCS – Biopharmaceutical Classification System
8. EE – Entrapment Efficiency
9. GI – Gastro Intestinal
10. HLB – Hydrophilic Lipophilic Balance
11. ICH – International Council for Harmonization
12. NLCG1-NLCG5 – AG-loaded Nanostructured Lipid Carrier Gel Formulations
13. NLCs – Nanostructured Lipid Carriers
14. PBS – Phosphate Buffer Saline
15. PDI – Polydispersity Index
16. PS – Particle Size
17. SEM – Scanning Electron Microscopy
18. SLN – Solid Lipid Nanoparticles
19. UV – Ultraviolet
20. ZP – Zeta Potential

Conflict of Interest: The authors declare no conflict of interest.

Ethical Approval: No ethical approval was necessary for this study.

Funding/Grant Support: None

Informed Consent Statement: Not applicable.

Data Availability Statement: The data supporting in this paper are available on the request from corresponding author.

Acknowledgements: We owe our profound gratitude to Dr. M Srujan Kumar, Director, SS Pharma Labs Pvt. Ltd., Hyderabad, India for providing support in completing experimental design and formulation for current research work.

REFERENCES:

1. Briley M, Lépine JP. "The increasing burden of depression." Neuropsychiatric Disease and Treatment. 2011 May; 7(1):3. https://doi.org/10.2147/NDT.S19617
2. Prasitlumkum N, Cheungpasitporn W, Ding K, Tokavanich N, Chokesuwattanaskul R. "Antidepressants and Risk of Sudden Cardiac Death: A Network Meta-Analysis and Systematic Review." Journal of the American College of Cardiology. 2021 May; 77(18):290. http://dx.doi.org/10.1016/S0735-1097(21)01649-1
3. De Berardis D, Fornaro M, Serroni N, Campanella D, Rapini G, Olivieri L, et al. "Agomelatine beyond Borders: Current Evidences of Its Efficacy in Disorders Other than Major Depression." International Journal of Molecular Sciences. 2015 Jan 5; 16(1):1111–30. https://doi.org/10.3390/ijms16011111
4. Dolder CR, Nelson M, Snider M. "Agomelatine Treatment of Major Depressive Disorder." Annals of Pharmacotherapy. 2008 Nov 18; 42(12):1822–31. https://doi.org/10.1345/aph.1l296
5. Freiesleben SD, Furczyk K. "A systematic review of agomelatine-induced liver injury." Journal of Molecular Psychiatry. 2015 Apr 21; 3(1). https://doi.org/10.1186/s40303-015-0011-7
6. Prajapati SK, Maurya SD, Das MK, Tilak VK, Verma KK, Dhakar RC, Dendrimers in drug delivery, diagnosis and therapy: basics and potential applications, Journal of Drug Delivery and Therapeutics. 2016;6(1):67-92 https://doi.org/10.22270/jddt.v6i1.1190
7. Vintiloiu A, Lafleur M, Bastiat G, Leroux JC. "In-situ-Forming Oleogel Implant for Rivastigmine Delivery." Pharmaceutical Research. 2007 Aug 12; 25(4):845–52. https://doi.org/10.1007/s11095-007-9384-3
8. Batrakova EV, Kabanov AV. "Pluronic block copolymers: Evolution of drug delivery concept from inert nanocarriers to biological response modifiers." Journal of Controlled Release. 2008 Sep; 130(2):98–106. https://doi.org/10.1016/j.jconrel.2008.04.013
9. Akash MSH, Rehman K. "Recent progress in biomedical applications of Pluronic (PF127): Pharmaceutical perspectives." Journal of Controlled Release. 2015 Jul; 209:120–38. https://doi.org/10.1016/j.jconrel.2015.04.032
10. Grassi G, A. Crevatin, Farra R, Guarnieri G, Pascotto A, B. Rehimers, et al. "Rheological properties of aqueous Pluronic–alginate systems containing liposomes." 2006 Sep 1; 301(1):282–90. https://doi.org/10.1016/j.jcis.2006.04.068
11. Garcês A, Amaral MH, Sousa Lobo JM, Silva AC. "Formulations based on solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for cutaneous use: A review." European Journal of Pharmaceutical Sciences. 2018 Jan; 112:159–67. https://doi.org/10.1016/j.ejps.2017.11.023
12. Agrawal M, Saraf S, Pradhan M, Patel RJ, Singhvi G, Ajazuddin, et al. "Design and optimization of curcumin loaded nano lipid carrier system using Box-Behnken design." Biomedicine & Pharmacotherapy. 2021 Sep; 141:111919. https://doi.org/10.1016/j.biopha.2021.111919
13. Dash SK, Patra CN, Acharjya SK, Jena GK, Panigrahi KC, Kumar NK, et al. "Development and Characterization of Paliperidone Loaded Nanostructured Lipid Carrier." Indian Journal of Pharmaceutical Education. 2022 Sep 23; 56(4):1003–12. http://dx.doi.org/10.5530/ijper.56.4.181
14. Cunha S, Costa CP, Loureiro JA, Alves J, Peixoto AF, Forbes B, et al. "Double Optimization of Rivastigmine-Loaded Nanostructured Lipid Carriers (NLC) for Nose-to-Brain Delivery Using the Quality by Design (QbD) Approach: Formulation Variables and Instrumental Parameters." Pharmaceutics. 2020 Jun 28; 12(7):599. https://doi.org/10.3390/pharmaceutics12070599
15. Tichota DM, Silva AC, Sousa Lobo JM, Amaral MH. "Design, characterization, and clinical evaluation of argan oil nanostructured lipid carriers to improve skin hydration." International journal of nanomedicine. 2014; 9:3855–64. https://doi.org/10.2147/ijn.s64008
16. Anwar W, Dawaba HM, Afouna MI, Samy AM. "Screening Study for Formulation Variables in Preparation and Characterization of Candesartan Cilexetil Loaded Nanostructured Lipid Carriers." Universal Journal of Pharmaceutical Research. 2020 Jan 15; http://dx.doi.org/10.22270/ujpr.v4i6.330
17. Rajput AP, Butani SB. "Resveratrol anchored nanostructured lipid carrier loaded in-situ gel via nasal route: Formulation, optimization and in vivo characterization." Journal of Drug Delivery Science and Technology. 2019 Jun; 51:214–23. http://dx.doi.org/10.1016/j.jddst.2019.01.040
18. Eleraky NE, Omar MM, Mahmoud HA, Abou-Taleb HA. "Nanostructured Lipid Carriers to Mediate Brain Delivery of Temazepam: Design and In Vivo Study." Pharmaceutics. 2020 May 14; 12(5):451. https://doi.org/10.3390/pharmaceutics12050451
19. Kassem AA, Asfour MH, Abd El-Alim SH, Khattab MA, Salama A. "Topical caffeine-loaded nanostructured lipid carriers for enhanced treatment of cellulite: A 3^2 full factorial design optimization and in vivo evaluation in rats." International Journal of Pharmaceutics. 2023 Aug 1; 643:123271–1. https://doi.org/10.1016/j.ijpharm.2023.123271
20. Khan A, Mudassir J, Akhtar S, Murugaiyah V, Darwis Y. "Freeze-Dried Lopinavir-Loaded Nanostructured Lipid Carriers for Enhanced Cellular Uptake and Bioavailability: Statistical Optimization, in Vitro and in Vivo Evaluations." Pharmaceutics. 2019 Feb 25; 11(2):97. https://doi.org/10.3390/pharmaceutics11020097

Ajiboye AL, Nandi U, Galli M, Trivedi V. "Olanzapine Loaded Nanostructured Lipid Carriers via High Shear Homogenization and Ultrasonication." Scientia Pharmaceutica. 2021 May 27; 89(2):25. https://doi.org/10.3390/scipharm89020025

21. Mendes A, Silva AM, Catita JAM, Cerqueira F, Gabriel C, Lopes C. "Miconazole-loaded nanostructured lipid carriers (NLC) for local delivery to the oral mucosa: Improving antifungal activity." Colloids and Surfaces B: Biointerfaces. 2013 Nov 1; 111:755–63. https://doi.org/10.1016/j.colsurfb.2013.05.041
22. Anand A, Arya M, Kaithwas G, Singh G, Saraf SA. "Sucrose stearate as a biosurfactant for development of rivastigmine containing nanostructured lipid carriers and assessment of its activity against dementia in C. elegans model." Journal of Drug Delivery Science and Technology. 2019 Feb 1; 49:219–26. http://dx.doi.org/10.1016/j.jddst.2018.11.021
23. Shelke S, Shahi S, Jalalpure S, Dhamecha D, Shengule S. "Formulation and evaluation of thermoreversible mucoadhesive in-situ gel for intranasal delivery of naratriptan hydrochloride." Journal of Drug Delivery Science and Technology. 2015 Oct; 29:238–44. https://doi.org/10.1016/j.jddst.2015.08.003
24. Jeswani G, Paul SD, Ajazuddin, Deshmukh R. "Design of vincristine sulfate loaded poloxamer in-situ nanogel: Formulation and in vitro evaluation." Journal of Drug Delivery Science and Technology. 2021 Feb; 61:102246. https://doi.org/10.1016/j.jddst.2020.102246
25. Mohanty D, Alsaidan OA, Zafar A, Dodle T, Gupta JK, Yasir M, et al. "Development of Atomoxetine-Loaded NLC In-situ Gel for Nose-to-Brain Delivery: Optimization, In Vitro, and Preclinical Evaluation." Pharmaceutics. 2023 Jul 20; 15(7):1985–5. https://doi.org/10.3390/pharmaceutics15071985
26. Amiji MM, Lai PK, Shenoy DB, Rao M. "Intratumoral Administration of Paclitaxel in an In-situ Gelling Poloxamer 407 Formulation." Pharmaceutical Development and Technology. 2002 Jan 1; 7(2):195–202. https://doi.org/10.1081/pdt-120003487
27. Verekar RR, Gurav SS, Bolmal U. "Thermosensitive mucoadhesive in-situ gel for intranasal delivery of Almotriptan malate:

Formulation, characterization, and evaluation." Journal of Drug Delivery Science and Technology. 2020 Aug; 58:101778. https://doi.org/10.1016/j.jddst.2020.101778

28. Marzouk MA, Osman DM, Amany A. Abd El-fattah. "Formulation and in vitro evaluation of a thermoreversible mucoadhesive nasal gel of itopride hydrochloride." 2018 Aug 24; 44(11):1857–67. https://doi.org/10.1080/03639045.2018.1504059

29. Uppuluri CT, Ravi PR, Dalvi AV, Shaikh SS, Kale SR. "Piribedil loaded thermo-responsive nasal in-situ gelling system for enhanced delivery to the brain: formulation optimization, physical characterization, and in vitro and in vivo evaluation." Drug Delivery and Translational Research. 2020 Jun 8; 11(3):909–26. https://doi.org/10.1007/s13346-020-00800-w

30. Cunha S, M Swedrowska, Y Bellahnid, Xu Z, Sousa M, Forbes B, et al. "Thermosensitive in-situ hydrogels of rivastigmine-loaded lipid-based nanosystems for nose-to-brain delivery: characterisation, biocompatibility, and drug deposition studies." International Journal of Pharmaceutics. 2022 May 1; 620:121720–0. https://doi.org/10.1016/j.ijpharm.2022.121720

31. Khalil RM, Abd-Elbary A, Kassem MA, Ghorab MM, Basha M. "Nanostructured lipid carriers (NLCs) versus solid lipid nanoparticles (SLNs) for topical delivery of meloxicam." Pharmaceutical Development and Technology. 2013 Mar 26;19(3):304–14. http://dx.doi.org/10.3109/10837450.2013.778872

32. Youssef A, Dudhipala N, Majumdar S. "Ciprofloxacin Loaded Nanostructured Lipid Carriers Incorporated into In-Situ Gels to Improve Management of Bacterial Endophthalmitis." Pharmaceutics. 2020 Jun 19; 12(6):572. https://doi.org/10.3390/pharmaceutics12060572

33. Kharwade RS, Mahajan NM. "Formulation And Evaluation Of Nanostructured Lipid Carriers Based Anti-Inflammatory Gel For Topical Drug Delivery System." Asian Journal of Pharmaceutical and Clinical Research. 2019 Mar 18; 286–91. https://doi.org/10.22159/ajpcr.2019.v12i4.32000

34. Lee MJ, Chun NH, Kim HC, Kim MJ, Kim P, Cho MY, et al. "Agomelatine co-crystals with resorcinol and hydroquinone: Preparation and characterization." Korean Journal of Chemical Engineering. 2018 Apr;35(4):984–93. https://doi.org/10.1007/s11814-017-0347-z

35. Gandhi S, Shastri DH, Shah J, Nair AB, Jacob S. "Nasal Delivery to the Brain: Harnessing Nanoparticles for Effective Drug Transport." Pharmaceutics. 2024 Apr 1; 16(4):481–1. https://doi.org/10.3390/pharmaceutics16040481

36. Costa CP, Nodilo LN, Silva R, Martins E, Zadravec D, Kalogjera L, et al. "In-situ hydrogel containing diazepam-loaded nanostructured lipid carriers (DZP-NLC) for nose-to-brain delivery: development, characterization and deposition studies in a 3D-printed human nasal cavity model." International journal of pharmaceutics. 2023 Sep 1; 644:123345–5. https://doi.org/10.1016/j.ijpharm.2023.123345

37. Formica ML, Real DA, Picchio ML, Catlin E, Donnelly RF, Paredes AJ. "On a highway to the brain: A review on nose-to-brain drug delivery using nanoparticles." Applied Materials Today. 2022 Dec 1; 29:101631. https://doi.org/10.1016/j.apmt.2022.101631

38. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. "Impact of Particle Size and Polydispersity Index on the Clinical Applications of Lipidic Nanocarrier Systems." Pharmaceutics. 2018 May 18; 10(2):57. https://doi.org/10.3390/pharmaceutics10020057

39. Youssef NAHA, Kassem AA, Farid RM, Ismail FA, EL-Massik MAE, Boraie NA. "A novel nasal almotriptan loaded solid lipid nanoparticles in mucoadhesive in-situ gel formulation for brain targeting: Preparation, characterization and in vivo evaluation." International Journal of Pharmaceutics. 2018 Sep; 548(1):609–24. https://doi.org/10.1016/j.ijpharm.2018.07.014

40. England RJA, Homer JJ, Knight LC, Ell SR. "Nasal pH measurement: a reliable and repeatable parameter." Clinical Otolaryngology and Allied Sciences. 1999 Feb; 24(1):67–8. https://doi.org/10.1046/j.1365-2273.1999.00223.x

41. Washington N, Steele RJC, Jackson SJ, Bush D, Mason J, Gill DA, et al. "Determination of baseline human nasal pH and the effect of intranasally administered buffers." International Journal of Pharmaceutics. 2000 Apr; 198(2):139–46. https://doi.org/10.1016/s0378-5173(99)00442-1

42. Harish NM, Prabhu P, Charyulu RN, Gulzar MA, Subrahmanyam EVS. "Formulation and Evaluation of In-situ Gels Containing Clotrimazole for Oral Candidiasis." Indian Journal of Pharmaceutical Sciences. 2009; 71(4):421–7.https://doi.org/10.4103/0250-474X.57291

43. Giuliano E, Paolino D, Fresta M, Cosco D. "Mucosal Applications of Poloxamer 407-Based Hydrogels: An Overview." Pharmaceutics. 2018 Sep 12; 10(3):159. https://doi.org/10.3390/pharmaceutics10030159

44. Sherafudeen SP, Vasantha PV. "Development and evaluation of in-situ nasal gel formulations of loratadine." PubMed. 2016 Jan 19; 10(6):466–76. PMID: 26779266; PMCID: PMC4698857.

45. Chung SK, Na Y. "Dynamic characteristics of heat capacity of the human nasal cavity during a respiratory cycle." Respiratory Physiology & Neurobiology. 2021 Aug; 290:103674. https://doi.org/10.1016/j.resp.2021.103674

46. Dash S, Murthy PN, Nath L, Chowdhury P. "Kinetic modeling on drug release from controlled drug delivery systems." Acta Pol Pharm. 2010 May-Jun; 67(3):217-23. PMID: 20524422.

47. Fosca M, Rau JV, Uskoković V. "Factors influencing the drug release from calcium phosphate cements." Bioactive Materials. 2022 Jan; 7:341–63. http://dx.doi.org/10.1016/j.bioactmat.2021.05.032

48. Ajiboye AL, Nandi U, Galli M, Trivedi V. "Olanzapine Loaded Nanostructured Lipid Carriers via High Shear Homogenization and Ultrasonication." Scientia Pharmaceutica. 2021 May 27; 89(2):25. https://doi.org/10.3390/scipharm89020025