

# THE DEVELOPMENT OF A BRAIN TARGETED MUCOADHESIVE AMISULPRIDE LOADED NANOSTRUCTURED LIPID CARRIER

MANAR ADNAN TAMER \*, HANAN JALAL KASSAB

Department of Pharmaceutics, University of Baghdad, College of Pharmacy, Iraq

\*corresponding author: [mannar.adnan@copharm.uobaghdad.edu.iq](mailto:mannar.adnan@copharm.uobaghdad.edu.iq)

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## Abstract

This research aimed to create and refine intranasal mucoadhesive amisulpride-loaded nanostructured lipid carriers (AMS-MNLCs) to increase efficacy and safety by increasing nasal-mucosal adhesion and facilitating direct brain targetability. Using HPMC K4M, Carbopol 934, and Hyaluronic acid (HA), AMS-MNLCs were made by applying modified melt-emulsification and ultra-sonication procedures. The results of the pH, osmolarity, and mucoadhesive tests on the optimized formulation were  $6.3 \pm 0.02$ ,  $304.53 \pm 2.15$ , and  $26.07 \pm 0.37$ , respectively. Rheological investigation, *in-vitro* release and *ex-vivo* permeation study of the optimized formulation revealed a shear-thinning behaviour, significantly higher release, and the flux of the drug from the formula was  $975.2 \pm 12.45$ . Based on the pharmacokinetic analyses in rats, intranasal AMS-MNLCs were reported to have a brain  $C_{max}$  about 14 times higher than intravenous AMS-solution. In addition, two weeks of intranasal administration of formulation did not affect haematological parameters in the *in vivo* research. The histopathological investigation showed that there was no disruption of the naso-mucosal membrane structure. Therefore, the created and optimized intranasal MNLCs showed a promised venue for the efficient and secure delivery of AMS as an antiemetic drug for the prevention and treatment of preoperative nausea and vomiting (PONV).

## Rezumat

Studiul prezintă dezvoltarea unor nano-purtători lipidici mucoadezivi intranazali (AMS-MNLC) încărcăți cu amisulprid, cu scopul creșterii eficacității și siguranței prin mărirea aderenței mucoasei nazale și facilitarea țintirii directe a creierului. Folosind HPMC K4M, Carbopol 934 și acid hialuronic (HA), purtătorii nanostructurați au fost obținuți prin proceduri modificate de emulsionare în topitură și ultrasunete. Rezultatele testelor de pH, osmolaritate și mucoadezivitate pentru formularea optimizată au fost  $6,3 \pm 0,02$ ,  $304,53 \pm 2,15$  și, respectiv,  $26,07 \pm 0,37$ . Investigația reologică, cedarea *in vitro* și studiul de permeație *ex-vivo* ale formulării optimizate au evidențiat un comportament de subțiere prin forfecare, eliberare semnificativ mai mare, iar fluxul medicamentului din formulă a fost de  $975,2 \pm 12,45$ . Pe baza analizelor farmacocinetice la șobolani, s-a observat că AMS-MNLC intranasal au o  $C_{max}$  cerebrală de aproximativ 14 ori mai mare decât soluția intravenoasă de AMS. În plus, două săptămâni de administrare intranasală a formulării nu au afectat parametrii hematologici *in vivo*. Investigația histopatologică a arătat că nu a existat nici o perturbare a structurii membranei nazale. Prin urmare, purtătorii nanolipidici cu administrare intranasală obținuți au permis o cedare eficientă a AMS, folosit ca medicament antiemetic pentru prevenirea și tratamentul sindromului de greață și vomă preoperatorie (PONV).

**Keywords:** amisulpride, nanostructured lipid carrier, hyaluronic acid, intranasal delivery, brain targeting

## Introduction

Preoperative nausea and vomiting (PONV) is a common adverse consequence of anaesthesia. The current incidence of PONV is predicted even after taking two or three preventative antiemetic drugs, to be 30 - 40%, and in patients who meet all four PONV risk factors identified by Apfel [1].

Women are more likely to experience PONV than men, as are non-smokers, younger patients, volatile anaesthetics (either nitrous oxide or not), and opiate use. Significant postoperative discomfort and dissatisfaction are only the first issues that PONV brings about [2]. Since its introduction to clinical practice in 1970 as an atypical antipsychotic, amisulpride (AMS) has proven to be a very effective and selective antagonist of dopaminergic D2 and D3 receptors. The lower

frequency of extrapyramidal symptoms at prescribed doses (50 - 1200 mg) contributed to its rapid rise in popularity. Recently, the US-FDA authorized the use of intravenous (IV) AMS for the treatment of PONV [3].

Less invasive than other routes of drug administration, intranasal dosing is readily available for both adult and paediatric patients. In most cases, the intranasal administration of a drug is more effective than the subcutaneous or intramuscular administration of the same drug. When the intravenous route is unavailable, the intranasal route can be helpful in an emergency. Therapeutic systemic concentrations of drugs can be obtained *via* the intranasal route, making it a useful delivery method for a wide range of purposes [4].

Drug molecules and therapeutic agents can be delivered to the brain non-invasively through the nasal passages *via* intranasal drug delivery [5, 6].

Interestingly, the intranasal cavity has two different routes for drug delivery, the olfactory and trigeminal pathways, allowing for immediate access to the brain [7].

Olfactory receptors enable neurons and their axons from the olfactory area to reach the olfactory bulb, which communicates with the central nervous system directly. The olfactory system is the sole portion of the body where the central nervous system (CNS) has direct touch with the outside world. Olfactory and trigeminal nerve routes are particularly effective at transporting active compounds from the olfactory region to the brain [8].

However, nanoparticles facilitate the blood-brain barrier (BBB) crossing for CNS disease treatment. Lipid-based nanocarriers, such as solid lipid nanoparticles (SLNs), nanostructured lipid carriers (NLCs), liposomes, nano-emulsions, and nanoliposomes, have been developed by researchers from physiological lipids to reduce the neurotoxicity of their delivery systems. Due to its utilization of physiological lipids such as mono-, di-, and triglycerides, fatty acids, and waxes, NLCs are significantly more stable than other nanocarriers and can encapsulate both hydrophilic and lipophilic medicinal compounds. They can traverse the BBB because they are lipophilic, and the irregular structure of NLCs allows for a greater concentration of the drug to be stored within it [9].

Liquid lipids are combined with a solid lipid to generate a matrix of nanostructured solid particles that we call NLCs [10].

The intranasal route does have certain disadvantages, though, such as poor absorption of hydrophilic and bulky molecules, a small formulation amount that may be injected into the nasal cavity, and a brief residence period due to rapid mucociliary clearance. This limits the formulation's nasal mucosal absorption by decreasing its nasal residential time. Intranasal formulations using various mucoadhesive polymers improve the retention time of the formulations in the nasal cavity as compared to conventional formulations, increasing the bioavailability in the brain [11-13].

## Materials and Methods

### Materials

Amisulpride (AMS), mucoadhesive polymers; hyaluronic acid, HPMC K4M (hydroxypropyl methylcellulose with medium-molecular weight), Carbopol 934 (C934) (cross-linked polyacrylic acid polymer) was supplied from Hyperchem LTD CO, United Kingdom. Solid lipid; glyceryl monostearate (GMS) (glycerol ester of stearic acid) was supplied from Hyperchem LTD CO, United Kingdom. Liquid lipid; Imwitor® 988 (I-988) (partial ester of caprylic acid and glycerol), was kindly gifted by IOI Oleochemical, GmbH, Germany. Permeation enhancer; E-TPGS (D- $\alpha$ -Tocopherol polyethylene glycol 1000 succinate) was supplied from Hyperchem LTD CO, United Kingdom. Surfactant; Cremophor®EL® (C-EL) (Polyethoxylated castor oil) from International Laboratory, USA. Co-surfactant; Transcutol HP (TR-HP) (Mono-ethyl ether of diethylene glycol) from Gattefosse Co., St. Priest, France. All other chemicals and solvents were of analytical grade and used without further purification.

### Methods

#### Preparation of mucoadhesive amisulpride loaded nanostructured lipid carrier

Melt-emulsification and ultrasonication were used to create the NLC. The lipid phase (GMS and I-988) was placed on a hot plate magnetic stirrer (ISOLAB Laborgerate GmbH, Germany), and the mixture was heated to about 5°C higher than GMS melting point while being continuously stirred. E-TPG and the medication (AMS) were also added to the melted lipid phase. The surfactant and co-surfactant were dissolved in *qs.* of D.W. up to 10 gm, then also heated to about 5°C above the melting point of GMS. The primary emulsion was made by adding the hot aqueous phase to the melted oily phase while stirring for 15 minutes at 600 rpm using a magnetic stirrer maintained at 78°C, the primary emulsion was then homogenized by probe-ultrasonic (Ultrasonic processor UP200Ht Hielscher, Germany) with pulse amplitudes that were maintained at specific on-off intervals for a duration of five minutes. The created O/W nano-emulsion was then cooled to 4 ± 0.5°C by immersing the container in an ice bath for 15 - 20 minutes after homogenization. This was followed by the formation of the AMS-NLC dispersions [14, 15].

**Table I**

Formulations of mucoadhesive amisulpride loaded nanostructured lipid carriers. All ingredients were expressed as % w/w

Form. No.	AMS	GMS	I-988	C-EL	TR-HP	E-TPGS	HA	HPMC K4M	C934	D.W. up to*
F1	1	1.8	1.2	5	2.5	1		0.5		100
F2	1	1.8	1.2	5	2.5	1			0.5	100
F3	1	1.8	1.2	5	2.5	1		0.5		100
F4	1	1.8	1.2	5	2.5	1	0.25			100
F5	1	1.8	1.2	5	2.5	1	1			100

\*Where, the final weight of each formula was 20 g using enough D.W.

The polymers that promote mucoadhesion (hyaluronic acid, HPMC K4M and Carbopol 934) were added to the second part of the aqueous component (D.W. up to 10 gm). After storing this dispersion overnight, the mixture was continuously stirred. The AMS-NLCs formula was then mixed with the aqueous phase containing the mucoadhesive polymer at 300 rpm for 30 minutes to ensure homogeneous dispersion of the mucoadhesive formulation (MNLCs) (Table I).

#### *In-vitro evaluation of mucoadhesive amisulpride loaded nanostructured lipid carrier*

##### *pH and osmolarity investigation*

A pH-meter (Mettler Toledo, Milan, Italy) was used to determine each formulation's pH level. In addition, an Osmometer (Osmomat 030, Germany) was used to measure the osmolarity of each formulation after being calibrated with ultra-purified water and physiological solution [16].

##### *Rheological investigation of optimized mucoadhesive formulation*

The NDJ-5S digital viscometer from the United Kingdom was used to measure the viscosity using spindle 4. The temperature range for the viscosity tests was  $25 \pm 0.5^\circ\text{C}$ . Before recording the viscosity measurements, the mucoadhesive formulations were allowed to spin for 1 minute [17].

##### *Mucoadhesive test*

All five formulations (F1-F5) underwent mucoadhesive studies to guarantee the longest feasible contact period between the pharmaceutical formulation and the mucosal layer of the nasal cavity. Muco-adhesion properties of AMS-NLC formulations were analysed to determine mucin adsorption capacity, 5 mL of the formula was mixed with 5 mL of the mucin solution (1 mg/mL). After 2 hours incubation at  $37 \pm 0.5^\circ\text{C}$ , the mixture was centrifuged at 3000 revolutions per minute for 60 minutes. The concentration of free mucin was determined by taking the supernatant, diluting it, and analysing it spectrophotometrically. The following formula can be used to determine the efficiency of muco-adhesion [18]:

$$\text{Mucoadhesive efficiency \%} = \frac{C_0 - C_f}{C_0} \times 100,$$

where,  $C_0$  = initial mucin content and  $C_f$  = free mucin content.

##### *AMS in-vitro release profile from NLCS*

Dialysis bag diffusion was performed to examine drug release over the course of 8 hours using a phosphate-buffered solution (pH 7.4) as the release medium. Researchers compared the AMS release patterns of five different formulations of AMS-MNLCs and AMS-Solution. The molecular weight cut-off of the dialysis membrane is between 8000 and 14000, containing 0.5 mL of AMS-MNLCs formulations and 0.5 mL AMS-Solution (5 mg of AMS) was clamped, immersed in a glass vial containing 200 mL of release medium at  $37 \pm 0.5^\circ\text{C}$ , and then stirred at 100 rpm using dissolution apparatus type

II. Samples of 5 mL were obtained at 0.083, 0.167, 0.25, 0.33, 0.5, 1, 2, 3, 4, 5, 6 and 8 hour intervals. To maintain sink conditions, an identical volume of new milieu was added in place of the original release medium. Following collection, samples were filtered through a 0.45 mm syringe filter before being analysed for AMS concentration using a UV visible spectrometer (UV-19001 Shimadzu, Tokyo, Japan) set to a detection wavelength of 280 nm. Accuracy and precision (relative standard deviation, %) of employed tests were also assessed, and mean values from three independent experiments ( $n = 3$ ) were reported. It was determined what percentage of AMS was released over time [19, 20].

##### *Lyophilization of MNLCs optimized formulation*

Lyophilization of AMS-MNLCs was performed using 5% (w/v) mannitol as a cryoprotectant and then lyophilized for 48 h. Pressure was set at 0.4 bar in Labconco FreeZone Benchtop freeze dryer (USA) [21].

##### *Ex-vivo permeation studies of optimized mucoadhesive formulation*

The MNLC's capacity to enhance medication permeation across the nasal mucosa and successful brain targeting was determined using an *ex vivo* permeation study.

Using recently isolated goat nasal mucosa from a slaughterhouse, the penetration of AMS from the optimized MNLCs was compared to the AMS suspension. The mucosal membranes were frozen and then diluted with phosphate buffer saline (pH 7.4). After isolating the intact nasal membrane, washing it thoroughly, and placing it in a Franz diffusion cell containing 11 mL of phosphate-buffered saline (pH 7.4), the mucosa was stabilized. For 8 hours, the cell was incubated at  $37 \pm 0.5^\circ\text{C}$  with 50 rpm and an aliquot of 0.5 mL of the optimum formulation equal to 5 mg AMS. All media was withdrawn at various time intervals (0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 and 8 hours), and new media is added every time. The samples were analysed by UV spectrophotometer at lambda max of AMS in pH 7.4 (280 nm). Three separate measurements were used to calculate the results, and their standard deviations were shown. The amount of the drug permeated was calculated as a function of the surface area of the utilized nasal membranes ( $\mu\text{g}/\text{cm}^2$ ) [22]. Permeability coefficient (cm/hr.) was calculated by using the following method [23]:

$$\text{Permeability coefficient} = \left( \frac{DC}{DT} \right)_{ss} \times \frac{V}{a \times CD}$$

Where  $(DC/DT)_{ss}$  is the alteration of concentration under steady state ( $\mu\text{g}/\text{mL} \times \text{hr.}$ );  $a$  is the permeation area equal ( $1.76 \text{ cm}^2$ );  $V$  (mL) is the volume of the receiver section (11 mL); and  $CD$  is the original donor concentration ( $10000 \mu\text{g}/\text{mL}$ ).

The permeability coefficient was derived from the flux ( $J$ ) ( $\text{g}/\text{mL} \times \text{h}$ ) that was originally determined from the slope of concentration *versus* time in the permeation study [24].

The optimized formulation of the medicine and the drug suspension were statistically compared by one-way ANOVA.

#### *Differential scanning calorimetry (DSC)*

The formulation was subjected to a DSC (DSC-60 plus, Shimadzu, Japan) thermal analysis to see if any interactions occurred between the AMS and the excipients. Lyophilized optimal mucoadhesive AMS-MNLCs (formula F3) were analysed with pure Amisulpride, GMS, hyaluronic acid and other ingredients. Under a blanket of nitrogen gas, we carefully weighed and stored each sample in aluminium pans, then heated them at a pace of 10°C/min and cooled them at a rate of 40°C/min. In the research, an empty aluminium baking pan served as a control [25].

#### *Fourier transform infrared spectroscopy (FTIR)*

Using an FTIR spectrometer (FTIR-8400S Shimadzu, Japan), the FTIR spectra of AMS, GMS, Cremophor® EL, Imwitor 988, E-TPGS, Transcutol, hyaluronic acid, the physical mixture of optimized mucoadhesive AMS-MNLCs formula, and the optimized formula F3 were recorded. FTIR compatibility tests were performed to look for potential interactions in the formulation [26].

#### *X-ray powder diffraction (XRPD)*

Using an X-ray diffractometer (model XD-610, Shimadzu, Kyoto, Japan) set up with Cu as the tube node, X-ray diffraction patterns of AMS, GMS, HA, physical mixture, and lyophilized optimum AMS-MNLCs formula F3 were obtained. Diffractograms were captured at room temperature with a voltage of 45 kV, a current of 30 mA, a step size of 0.02°, and a counting rate of 0.5 s/step. Scattering angle (2θ) was taken from 4° to 50° [27].

#### *In vivo biodistribution study*

##### *Pharmacokinetics study*

As a first step, three animals were taken as control (negative control) to get plasma and brain samples that can be used for HPLC analysis.

Then the animals (male Wistar rats weighed 200 ± 50 g) were separated into two groups (n = 60 in each category):

Group I: IN administration of mucoadhesive AMS-NLCs (5 mg/kg bw).

Group II: IV administration of AMS-solution (5 mg/kg bw).

The dose is equally related to body weight although it is not the lone factor that influences the scaling for dose calculation. The correction factor ( $K_m$ ) is calculated by dividing a species' mean body weight (in kilograms) by its mean body area (in square centimetres). For rats, 6 is the equivalent of the  $K_m$ . To calculate an approximate animal dose, the following formula is utilized:

$$\text{HED mg/kg} = \text{Animal dose mg/kg} \times \frac{\text{Animal } K_m}{\text{Human } K_m}$$

Calculations are made simpler by using the  $K_m$  ratio because the  $K_m$  factor for each species is constant. As a result, the above equation is changed to:

$$\text{HED (mg/kg)} = \text{Animal dose (mg/kg)} \times K_m \text{ ratio.}$$

It is simple to calculate the  $K_m$  ratio values by dividing the human  $K_m$  factor by the animal  $K_m$  factor, or *vice versa* [28].

When the therapeutics' toxicities can be monitored, reversed, predicted, and show a moderate-to-shallow dose-response relationship, and when these toxicities are consistent across the tested species (both qualitatively and with respect to appropriately scaled dose and exposure), a smaller safety factor may be used. Toxicology investigations in healthy volunteers should be longer than the anticipated clinical schedule to identify the no observed adverse effect level (NOAEL), which would allow for a safety factor lower than 10 [29].

In group I, ether was used as an anaesthetic to gently sedate the animals prior to intranasal administration. Each group of animals was given 100 µL of mucoadhesive AMS-NLCs (50 µL/nostril) under general anaesthesia using a yellow cannula (24 gauge) linked to a syringe of 1 mL (the needles were removed). AMS-Solution was injected intravenously into the submandibular facial veins of the rats in group II. Therefore, at 0, 0.25, 0.5, 0.75, 1, 2.0, 3.0, 5, 12 and 24 hours, in the microcentrifuge tubes (precoated with EDTA to avoid clotting), 0.5 mL of blood samples were collected. After that, a clear supernatant was obtained by centrifuging the blood samples at 4000 rpm for 10 minutes, and then separating the components with a micropipette. Concurrently, six animals were sacrificed from each group at each time point, and their brains were extracted, washed with normal saline, homogenized with normal saline in a ratio of 1:9 (w/w), and blotted for the brain kinetics investigation. Brain and plasma samples were taken and frozen at -70°C for later examination.

#### *Analysis of AMS-concentration in brain and a blood plasma samples*

The HPLC system S600- Sykam GmbH (Germany) equipped with a Nucleosil -18 (10 × 50 mm I.D., particle size 2 µm) column was used. AMS was separated chromatographically at a flow rate of 0.5 mL/min using a mobile phase of methanol: 0.2% formic acid (70:30). The photodiode array (PDA) detector was used for chromatographic detection at a wavelength of 274 nm. Samples from the brain and blood plasma can be bioanalyzed for AMS, the drug metoclopramide hydrochloride (4-amino-5-chloro-N-[(2-diethylamino)ethyl]-2-methoxybenzamide hydrochloride; purity 99.73%) is employed as an internal standard (IS) [30].

Homogenates were used for drug concentration analyses after being prepared by homogenizing brain tissue with normal saline at a 1:9 (w/w) ratio using a

tissue homogenizer. The protein precipitating agent, methanol, was applied to a volume of 100 L (to each treated plasma and brain homogenate), along with 10 L of (50 g/mL) internal standard. The materials were blended in a vortex for 15 minutes, and centrifuged at 4000 rpm for 10 minutes.

After collecting the supernatant, we filtered and injected 100 L of the resulting filtrate into an RP-HPLC system for AMS analysis. Parameters of pharmacokinetics  $C_{max}$ , AUC, and  $T_{max}$ , as well as the time required to reach  $C_{max}$  in the brain or plasma, were all examined using the PK-Solver software program [31].

Drug targeting index (DTI), targeting efficiency (%), (DTE), and direct transport percentage (%), (DTP), were calculated for intranasally administered AMS-MNLCs based on their AUC values in the brain and plasma, and the results were compared with those obtained from intravenous injections [32]:

$$DTI = \frac{[AUC_{Brain}/AUC_{Blood}]_{Intranasal}}{[AUC_{Brain}/AUC_{Blood}]_{Intravenous}}$$

$$\%DTE = \frac{[AUC_{Brain}/AUC_{Blood}]_{Intranasal}}{[AUC_{Brain}/AUC_{Blood}]_{Intravenous}} \times 100$$

$$\%DTP = \frac{[B_{Intranasal} - Bx.]}{[B_{Intranasal.}]} \times 100$$

$$Bx. = \frac{[B_{Intranasal.}]}{[P_{Intravenous.}]} \times P_{Intranasal}$$

where,  $B_{Intranasal} = AUC_{0-24}$  of brain resulting through intranasal administration;  $B_{Intravenous} = AUC_{0-24}$  of brain resulting through intravenous administration;  $P_{Intranasal} = AUC_{0-24}$  of plasma resulting through intranasal administration;  $P_{Intravenous} = AUC_{0-24}$  of plasma resulting through intravenous administration.

#### *In vivo safety assessment*

The *in vivo* safety assessment was carried using two groups of Male Wistar rats (six animals in each group) which weighed  $200 \pm 50$  g; Group I was a negative control and to Group II was administered IN-AMS-MNLCs in a dose of 5 mg/kg bw. Group II received the treatments daily for two weeks. The collected blood samples were then analysed to determine the extent to which AMS had induced agranulocytosis. At the same time, the animals were examined for histological and gross pathological consequences as well as haematological toxicity from the optimized formulation [33]. In accordance with the principles of the Office International des Epizooties (OIE) on animal ethics, the *in vivo* study was approved by the University of Baghdad- College

of Pharmacy, Iraq's institutional animal Research Ethics Committee.

#### *Gross pathology*

Throughout the whole two-week experimental protocol, animals were visually inspected, and differences between untreated (negative control) and treated (positive control) mice were noted. Blinking, nose bleeding, eye irritation, nasal secretions, erythema, nostril irritation, and other odd moments inside or outside the cage were recorded as undesirable indicators before and after intranasal administration. Toxic effects and mortality rates observed in the different animal groups throughout the experiment were eventually linked together [34].

#### *Histopathological evaluation*

A 10% formalin solution was used to preserve the isolated nasal mucosal pieces. Each sample was cleaned to eliminate any connective tissue before this analysis was performed. Haematoxylin and eosin solution were used to stain individual sections. Finally, a light microscope was used to inspect the slices for necrosis and structural damage [35].

#### *Statistical Analysis*

The results of the experiments are presented as the mean SD of triplicate models, and the significance of the observed changes in the applied components was determined using one-way analysis of variance (ANOVA) at the 0.05 level of significance [36].

## Results and Discussion

#### *pH and osmolarity investigation*

The pH of MNLCs was in the range from  $4.4 \pm 0.05$  to  $7.63 \pm 0.077$ . The typical range for the pH of nasal secretions is 4.5 to 6.4 [37]. Based on the data in Table II, it appears that hyaluronic acid-based mucoadhesive polymer formulations may be non-irritating to the nasal mucosa because their pH values are within the range of nasal secretions. On the other hand, the nasal mucosa could be irritated by formulations made with HPMC K4M and Carbopol 934 because their pH values were out of the nasal secretion pH comfort range. Maintaining a healthy pH level in the nose helps with drug absorption and prevent bacterial growth [38]. Also to prevent irritating the nasal mucosa and achieve the desired tonic effects, formulations for nasal medication delivery should have an osmolarity of between 285 and 310 mOsmol/L [39]. F3 had the most accepted value of osmolarity for nasal drug delivery (Table II).

**Table II**

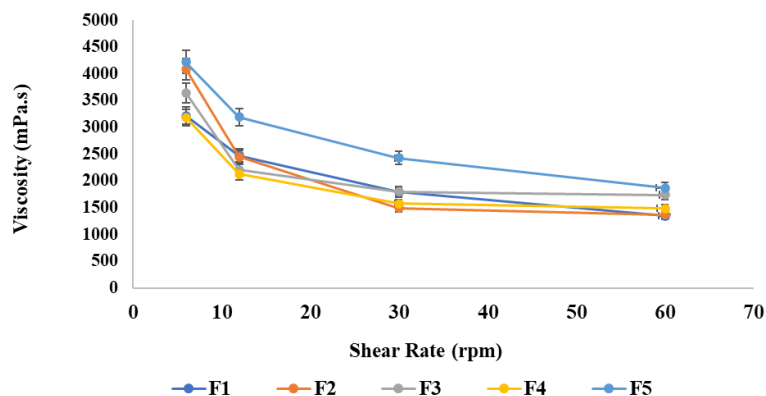
pH values, osmolarity and mucoadhesive efficiency of MNLCs formulations

Formulations	pH	Osmolarity (mOsmol/L)	Mucoadhesive efficiency
F1	$7.63 \pm 0.077$	$268.05 \pm 1.55$	$20.47 \pm 0.15$
F2	$4.4 \pm 0.05$	$439.36 \pm 2.06$	$8.43 \pm 0.24$
F3	$6.3 \pm 0.02$	$304.53 \pm 2.15$	$26.07 \pm 0.37$
F4	$6.35 \pm 0.025$	$342.24 \pm 2.65$	$22.15 \pm 0.27$
F5	$6.33 \pm 0.025$	$405.66 \pm 3.2$	$28.41 \pm 0.12$

### Rheological investigation of the optimized muco-adhesive formulation

Different shear rates were used to test viscosity in order to learn more about the rheological behaviour of the mucoadhesive NLC formulations. Shear-thinning behaviour was observed in all formulations as the

shear rate was increased from 6 rpm to 60 rpm, and this behaviour was consistent with that of a pseudo-plastic system [40]. Formula F5 with 1% hyaluronic acid showed significantly higher viscosity than the others, as presented in Figure 1.



**Figure 1.**

Viscosities of MNLCS formulations

### Mucoadhesive test

Converting NLCs to MNLCS with mucoadhesive polymers could solve the problem of the nasal mucociliary clearance, as it is the rate-limiting factor in intranasal formulations. The formulations can stay in contact with the nasal mucosa for a longer period with the aid of the mucoadhesive polymer. The mucoadhesive strengths for each formula were evaluated by determining the nanoparticle binding efficiency to mucin. To study the mucoadhesive properties of polymers, samples of mucin with mucoadhesive formulation F1 - F5 were prepared.

The results showed there are indeed interactions between mucin and HPMC K<sub>4</sub>M (F1), Carbopol 934 (F2) and HA (F3, F4 and F5) (Table II). The mucoadhesive efficiency was also determined and it demonstrated high mucoadhesive efficiency of F1, F3, F4 and F5 with a significant lower ( $p < 0.05$ ) mucoadhesive efficiency by F2.

The HA containing formulations in general showed increasing of muco-adhesion and this increase suggests that a strong interaction between mucin and HA occurred. Since F5 presents more concentration of HA (1%), more interactions with the mucin were possible. Hydrogen bonding between the polymer's hydroxyl and carboxyl groups and mucin's amino groups may be responsible for the resulting increase in viscosity caused by these interactions. Since HA is linear, it can readily inter-penetrate a mucin random coil, which can boost the likelihood of interfacial interactions with mucin and ultimately lead to a more robust bond. Hassan and Gallo were assumed the same conclusions [41]. Not just electrostatic bonding, which showed to be ambiguous, but also

other types of binding and interactions contributed to the adhesive capacity [42].

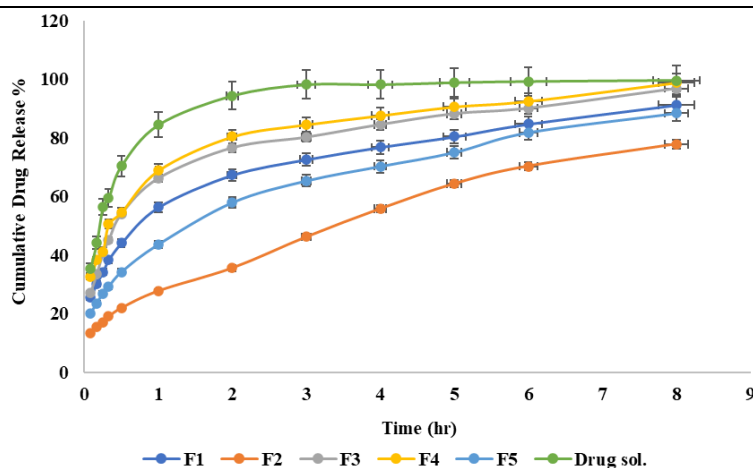
### In vitro drug release studies

The purpose of this investigation was to investigate how the manufactured nano formulations released drugs in comparison to AMS-Solution. The dissolution was carried out on MNLCS (F1, F2, F3, F4 and F5) and drug solution. It was carried out for 8 hours at 37°C using phosphate buffer solution of pH 7.4, utilizing the Type II Dissolution Apparatus. The cumulative % drug release was plotted against time, as shown in Figure 2.

The results of this study's experiments showed that the formulations F3 and F4 (0.5%, 0.25% HA, respectively) revealed a quick release significantly higher ( $p < 0.05$ ) than that of the other formulations prepared with HPMC K<sub>4</sub>M (F1) and Carbopol 934 (F2) and 1% HA (F5), they were the most closer release profile to the drug solution. The drug's dispersion and hydrophilicity would likely improve at these concentrations of the hydrophilic carrier HA [43].

### Optimized formulation selection

According to the previous evaluation of MNLCS formulations (F1-F5), it was found that formulations with (0.25 and 0.5%) hyaluronic acid as a mucoadhesive polymer F3 and F4 had the most preferred characteristics which were represented by pH values, release profile of drug and mucoadhesive characteristics with slight preference for F3 formulation by the more accepted value of osmolarity and higher mucoadhesive efficiency than F4. So, more evaluation was done on this formula to prove that it is the optimized formula.



**Figure 2.**

Release profile of AMS (F1-F5) and drug solution in phosphate buffer saline solution pH 7.4.

*Ex-vivo permeation studies of optimized muco-adhesive formulation*

Because it resembles the human nasal mucosa, goat nasal mucosa was chosen to assess the *ex-vivo* permeability of the suggested formulations. Figure 3 depicts the diffusion pattern of the formulations *via* the mucosal membrane.

Table III displays the steady-state flux ( $J_{ss}$ ), permeation coefficient ( $P_{app}$ ), and the amount of medication penetrated. Compared to the spread of NE and the discontinuation of medication, the permeation coefficient of MNLCs-F3 through the goat nasal mucosa was observed to be substantially higher ( $p < 0.05$ ). It was discovered that the steady-state flow of AMS-MNLCs is considerably ( $p < 0.01$ ) greater than the

drug suspension. Hyaluronic acid, a naturally occurring anionic polysaccharide, is used in the formulation of MNLCs because of its great mucoadhesive capacity and several keys use it in the design of bio adhesive drug delivery systems. It was discovered that this biopolymer, in addition to its mucoadhesive qualities, may improve the absorption of medicines and proteins *via* mucosal tissues [44].

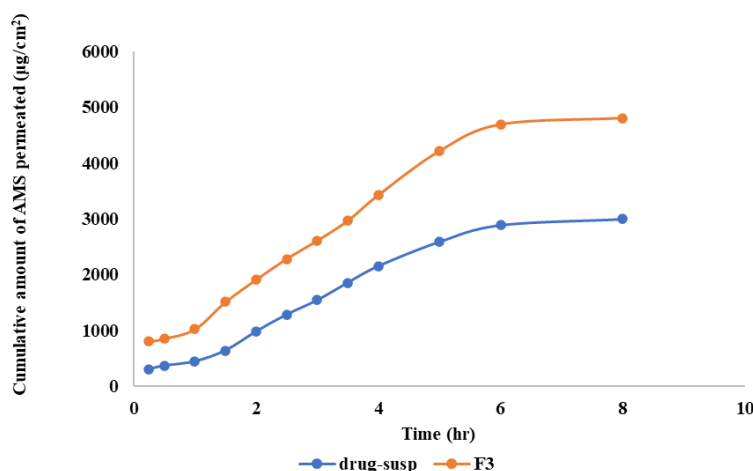
Because vitamin E is present, E-TPGS may also contribute as a permeability enhancer and absorption booster, which causes internalization of cells *via* membrane receptors, and PEG, which lessens clearance of nanoparticles so they can be transported to their intended location [45].

**Table III**

Representations of *ex vivo* permeation of AMS solution and AMS-MNLCs through sheep nasal mucosa

<i>Ex-vivo</i> permeation parameters	Formulation	
	ASP solution	AMS-MNLCs
$J_{ss}$ ( $\mu\text{g}/\text{cm}^2/\text{h}$ )	$628.75 \pm 10.16$	$975.29 \pm 12.45$
$P_{app}$ ( $\text{cm}/\text{h} \times 10^{-3}$ )	$62.87 \pm 1.54$	$97.52 \pm 2.82$

$P_{app}$  = Permeability co-efficient,  $J_{ss}$  = Steady-state flux.



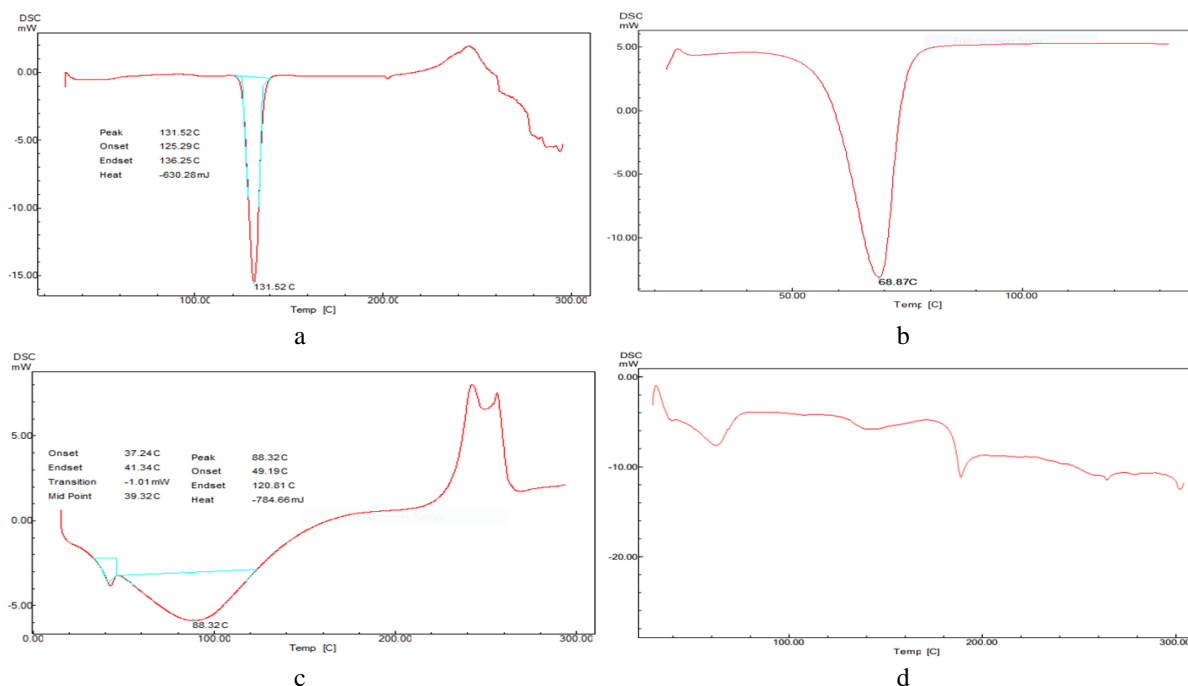
**Figure 3.**

Cumulative amount of AMS permeated ( $\mu\text{g}/\text{cm}^2$ ) from optimum formula F3 and drug suspension through goat nasal mucosa at  $37 \pm 0.5^\circ\text{C}$

*Differential scanning calorimetry (DSC)*

The thermograms of lyophilized NLCs demonstrate that the endothermic peak of AMS (131.52°C) has

vanished. AMS has been homogeneously disseminated in the lipid matrix, indicating that the crystalline form has been converted to the amorphous form [46].



**Figure 4.**

DSC thermograms of (a) amisulpride, (b) GMS, (c) hyaluronic acid and (d) lyophilized NLCs F3

*Fourier-transform infrared spectroscopy (FTIR)*

An important guideline in choosing the optimal excipient is whether the medicine is compatible with the others. The FTIR study is useful for determining whether the drug's molecular structure has changed because of the extreme conditions under which it was developed [47].

AMS's FTIR spectra displayed obvious bands at 3417.86, 3305.99 and 3217.27  $\text{cm}^{-1}$  corresponding to N-H stretching of primary amine, pyrrole, and secondary amide, respectively. Furthermore, a strong absorption peak was identified at 1627.92  $\text{cm}^{-1}$  due to the stretching vibration of the C=O group in the secondary amide. The stretching vibrations of the O=S=O group appeared at 1122.57  $\text{cm}^{-1}$  and 1361.74  $\text{cm}^{-1}$ . Except for a modest reduction in the intensity of the bands, which might be caused by the medicine being diluted when mixed with other excipients, there was no significant change in the position of the AMS characteristic bands in the FTIR spectra of F3. These findings backed up the claim that AMS did not react chemically with the excipients that were employed [48].

*X-ray powder diffraction (XRPD)*

The diffractogram of AMS showed sharp peaks with high intensity at  $2\theta$  scattered angles of 12.19, 15.98, 18.81, 20.35, 23.35, 26.13, 26.91 and 29.11° indicating the highly crystalline nature of the drug. Moderately intense peaks were observed for the solid lipid glyceryl

monostearate. The diffractogram of physical mixture and lyophilized selected formula F3 showed that the crystalline peaks of AMS at 20.35° were less intense, supporting the transformation of AMS into an amorphous form that had been uniformly distributed throughout the lipid matrix [49]. The lyophilized NLCs diffractograms also showed a drop in the strength of the typical lipid peaks, indicating a reduction in the crystallinity of the lipids in the F3 formula.

*In-vivo biodistribution study**Pharmacokinetics study**Analysis of AMS-concentration in brain and blood plasma samples*

The calibration curve was obtained by using the developed method for the spiked plasma, brain, and a standard solution of known concentration of AMS and IS.  $R^2$  was set at a value of 0.9996. The method was precise, specific, and sensitive.

These data obtained from the chromatograms (AMS, blank plasma sample, blank brain sample and plasma, AMS and internal standard metoclopramide) demonstrated that when compared to intravenous administration of AMS-Solution, intranasal administration of MNLC's intranasal formulation had a greater brain concentration. Intranasal MNLCs formulation achieves greatest brain concentration of AMS and least plasma concentration, while intravenously delivered AMS-Solution proved the opposite, according to the pharmacokinetic results



shown in Table IV. When compared to the brain concentrations in animals given intravenous AMS-Solution, it was discovered that the brain concentration

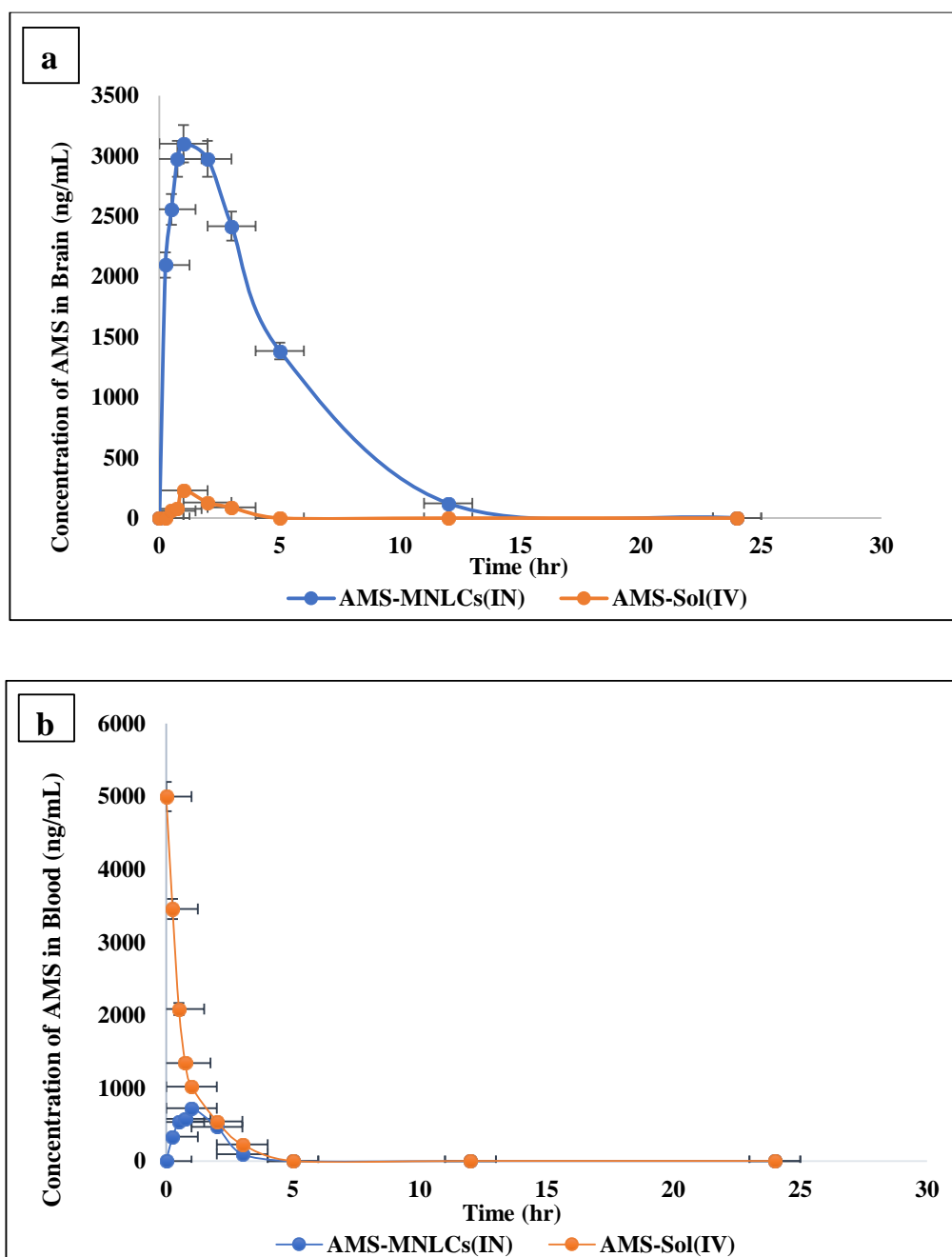
of AMS following intranasal delivery of AMS nano formulations was considerably greater (\*p was set at 0.05) (Figure 5).

**Table IV**

Results of pharmacokinetics parameters of IN-MNLCs and IV-AMS solution

Formulations	Brain			Plasma			Brain targeting parameters		
	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0-∞</sub> (h*ng/mL)	C <sub>max</sub> (ng/mL)	T <sub>max</sub> (h)	AUC <sub>0-∞</sub> (h*ng/mL)	DTI	%DTE	% DTP
AMS-NLCs Intranasal	3101.84 ± 156.28	1	17474.83 ± 466.75	725.23 ± 82.45	1	1423.73 ± 148.21	87.05	8704.96	63.84
AMS-Sol Intravenous	228.04 ± 22.35	1	555.66 ± 35.6	5000 ± 0.0	0	3937.67 ± 135.42	-	-	-

DTI: Drug targeting index, DTE (%): Drug targeting efficiency, DTP (%): Direct transport percentage, AUC: Area under curve.



**Figure 5.**

Concentration–time curve for MNLCs (Intranasal), AMS-Solution (Intravenous), (a) Brain and (b) Plasma

The pharmacokinetic studies showed that the direct intranasal distribution of AMS to the brain by intranasal MNLCs formulation may occur *via* the trigeminal or olfactory pathway. On the other hand, direct drug delivery from the nose to the brain was assessed using DTI, DTE, and % DTP. As shown in Table IV, the intranasal MNLC formulation in this case displayed the greatest values for DTI, % DTE, and % DTP. The DTE and DTP values range between 0 to +∞ and -∞ to 100, respectively. DTP values above 0 indicate the occurrence of drug brain targeting through the olfactory and/or trigeminal pathways, whereas DTE values above 100 indicate that intranasal administration of the drug is more successful than intravenous administration. After intranasal administration, a DTP score of 100 indicates that no medication was taken up by the body's circulatory system *via* the indirect route [50].

*Effect of MNLCs formulation on haematological parameters*

To identify any potential harmful consequences of intranasal delivery of MNLCs formulation, a haematological study was performed. Two weeks of therapy with MNLCs formulation generated haemolysis and agranulocytosis in the study's animal subjects. Direct systemic exposure to AMS has long been linked to agranulocytosis and neutropenia [7].

Figures 6 (a), (b) and (c) represent the differential erythrocyte parameters, the differential leukocyte parameters, and the differential platelet parameters of the control and intranasal MNLCs formulation treated groups respectively. Each variable was found to be within a physiologic range. All haematological parameters are listed in Table V, for the control and

intranasal MNLCs formulations. They are all within normal ranges and show no significant physiological changes after delivery. Blood films of the control and intranasal MNLCs formulations treated groups reveal normocytic normochromic type of RBCs and no abnormal cells can be seen with adequate platelets.

*Gross pathology*

Results from the *in vivo* safety tests showed and validated that mice given intranasal administration did not show major differences from the control group in terms of blinking, nasal secretion, nasal haemorrhage, or eye irritation. However, intranasal delivery did not result in any cases of abnormal behaviour or difficulty breathing. The proposed formulation is therefore considered to be risk-free for intranasal use in the management of PONV.

*Histopathological evaluation*

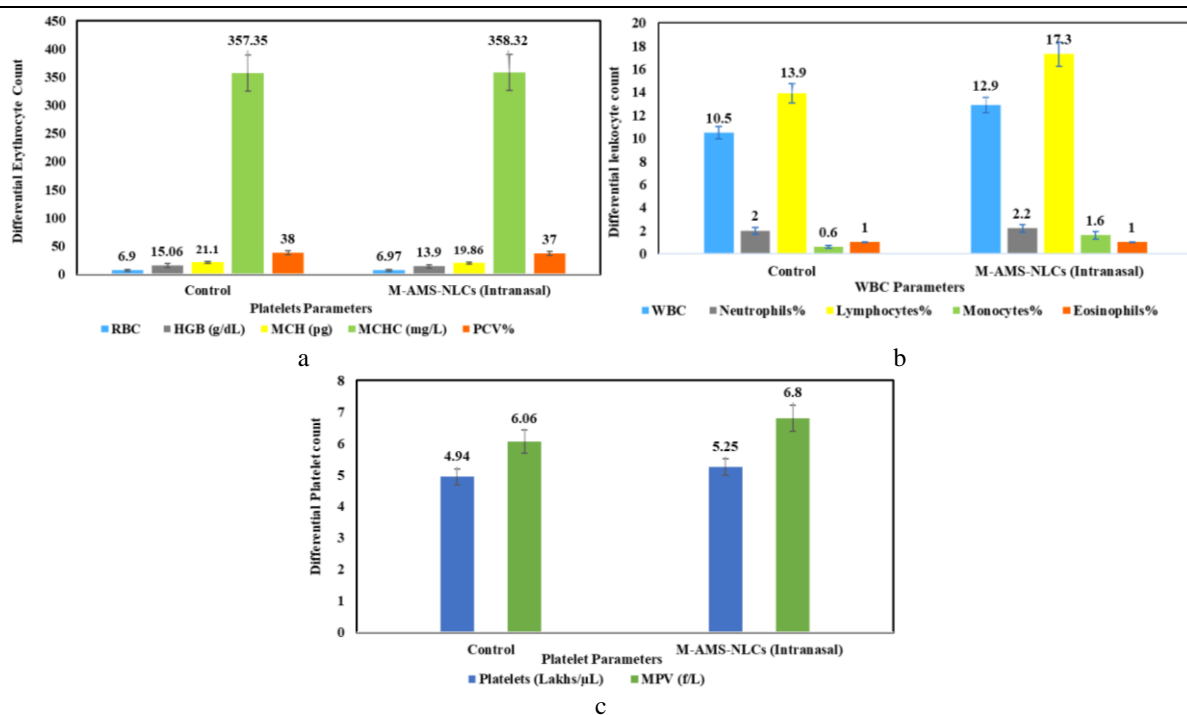
After two weeks, nasal mucosa from control and intranasal MNLCs formulation-treated rats was isolated to test for ciliotoxicity. Histological studies, which look for changes in tissue structure and inflammation as indicators of toxicity, were used to conduct a thorough analysis. A normal-looking respiratory epithelium with some minor inflammatory cells beneath was seen in the control segment, as seen in the pathological micrographs of nasal tissues shown in Figure 7 (a). In contrast, Figure 7 (b) draws the conclusion that, other than a few lacerations, there were no notable alterations in the treated portions. The treated and control groups showed similar results in terms of outward appearance. Therefore, the produced formulations are benign and well-suited for intranasal use. Epithelial cells, cilia, and glands in the mucosa were not harmed.

**Table V**

Complete blood count parameters of control and IN-MNLCs (n = 6, mean ± SD)

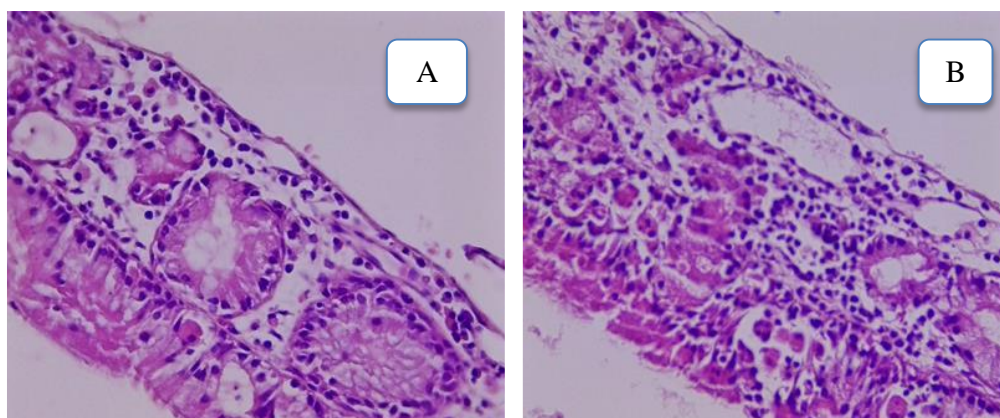
Complete Blood Count Parameters	Physiological Values	Control	AMS-MNLCs (Intranasal)
HGB (g/dL)	12 - 18	15.06 ± 0.37	13.9 ± 0.22
PCV %	35 - 55	38 ± 0.05	37 ± 0.12
RBC (x 10 <sup>6</sup> / μL)	6.5 - 9.5	6.9 ± 0.16	6.97 ± 0.11
WBC (x 10 <sup>3</sup> / μL)	4.5 - 12.5	10.5 ± 0.29	12.9 ± 0.45
MCV (fL)	55 - 78	54.53 ± 0.35	52.6 ± 0.18
MCH (pg)	16 - 22	21.1 ± 0.24	19.86 ± 0.45
MCHC (mg/L)	320 - 360	357.35 ± 0.64	358.32 ± 0.52
MPV (fL)	6 - 12	6.06 ± 0.23	6.8 ± 0.3
Platelets (Lakhs/μL)	2 - 10.5	4.94 ± 0.12	5.25 ± 0.05
Neutrophils %	0 - 3	2 ± 0.0	2.2 ± 0.01
Lymphocytes %	11 - 22	13.9 ± 0.01	17.3 ± 0.02
Monocytes %	0 - 3	0.6 ± 0.1	1.6 ± 0.01
Eosinophils %	0 - 1	1 ± 0.0	1 ± 0.0
RBC morphology	Normocytic Normochromic	Normocytic Normochromic	Normocytic Normochromic
WBC morphology	Normocytic Normochromic	Normocytic Normochromic	Normocytic Normochromic

HGB: Haemoglobin, HCT: Haematocrit, PCV: Packed cell volume, RBC: Total red blood cells count, WBC: Total white blood cells count, MCV: Mean corpuscular volume, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, MPV: Mean platelet volume



**Figure 6.**

Effect of intranasal MNLCS and AMS-Solution on haematological parameters for control, group I (IN-MNLCS) and group II (IV-AMS solution): (a) Differential Erythrocyte count, (b) Differential leukocyte count and (c) Differential Platelet count.



**Figure 7.**

Histopathological examinations (x 40) (H & E) of nasal mucosal sections (a) control mucosa, (b) IN-MNLCS.

**Conclusions**

Successful incorporation of mucoadhesive polymer, HA, into the development of the AMS-MNLCS formulation, appropriate for intranasal delivery, has improved the drug's brain targetability. This was accomplished by the invention of ASP-NE, a low-energy, low-cost self-emulsification approach. Improved *in vitro* release profile of the drug from the formulation and *ex vivo* permeation across the sheep nasal mucosa were observed with the optimized MNLCS, demonstrating the desirable pH, osmolarity, viscosity and mucoadhesive strength of this promising intranasal delivery system.

The physico-chemical evaluation by DSC, X-ray and FTIR exhibited information about the drug and other ingredients in the formulation including the purity, crystallinity and entity. On the other hand, the data registered in these tests has been proved that the selected formula contains the amorphous form of the drug without any interaction between the components of the formulation.

In a study of the *in vivo* biodistribution of AMS in Wistar rats, the AUC and  $C_{max}$  values of the medication in the brains of the animals treated with intranasal AMS-MNLCS were considerably higher than those of the intravenous AMS-Solution group. Therapeutics can enter the brain *via* this novel drug delivery method and pathway by avoiding the tight connections of the

BBB capillary endothelial cells. In addition, high DTE%, DTI and DTP% confirmed the intranasal AMS-MNLCs' enhanced potential to transport AMS in the brain. Gross pathology, haematological, and histopathological studies confirmed that the formulation was safe for administration on animals, as the components of the optimized formulations had no harmful effect on haematological parameters or the nasal mucosa of the animals.

So, it follows that the discovered mucoadhesive AMS-NLCs have promise as a formulation for safe and efficient brain targeting of the medication as an antiemetic for the prevention and treatment of PONV.

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### Conflict of interest

The authors declare no conflict of interest.

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