https://doi.org/10.31925/farmacia.2023.6.15 **ORIGINAL ARTICLE**

LASMIDITAN NANOEMULSION BASED *IN SITU* GEL INTRANASAL DOSAGE FORM: FORMULATION, CHARACTERIZATION AND *IN VIVO* STUDY

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Abstract

This study aimed to formulate lasmiditan (LAS) as a nanoemulsion *in situ* gel (NEIG) by utilising nanotechnology in order to escape the problems associated with the poor oral bioavailability of the drug. A study regarding the LAS solubility in different oils, surfactants and co-surfactants, was determined. Different nanoemulsion (NE) formulations were prepared depending on the pseudo-ternary phase diagrams, which were first constructed. Visual characterization, thermodynamic stability study and droplet size were determined for the prepared NEs. Muco-ciliary clearance can be overcome with the *in situ* gelling agent carbopol 934, a pH-sensitive polymer that was selected to increase the residence time on the nasal mucosa. The gel strength, pH, gelation time and viscosity were predicted for the prepared NEIG. *In vitro* release and *ex vivo* nasal permeation were measured for both NE and NEIG formulations. Formula coded-15 (F15) with a droplet size of 58.4 nm provided a maximum *in vitro* as well as *ex vivo* permeation to be considered as a selected one, to which 0.5% carbopol 934 was added for the preparation of NEIG2 that exerts comparable release and permeation values as F15 with more residence time in order to overcome the normal nasal physiological clearance. Finally, LAS as NEIG2 was subjected to an *in vivo* study as a promising intranasal novel formula that exerts rapid onset of action (T_{max} 0.75 hours) accompanied by higher bioavailability (2.5 folds) and tissue permeation (4 folds) relative to the oral dosage form (8% aqueous LAS suspension) was considered.

Rezumat

Studiul a avut ca scop formularea lasmiditanului (LAS) sub forma unui gel de tip nanoemulsie *in situ* (NEIG) prin utilizarea nanotehnologiei, pentru a evita problemele asociate cu biodisponibilitatea orală scăzută a substanței. S-au preparat diferite nanoemulsii (NE) în funcție de diagramele de fază pseudoternare. Caracterizarea vizuală, studiul stabilității termodinamice și dimensiunea picăturilor au fost determinate pentru NE-urile preparate. *Clearance*-ul mucociliar poate fi redus cu ajutorul agentului de gelificare *in situ* carbopol 934, un polimer sensibil la pH care a fost selectat pentru a crește timpul de rezidență pe mucoasa nazală. Rezistența gelului, pH-ul, timpul de gelifiere și vâscozitatea au fost estimate pentru NEIG preparate. Cedarea *in vitro* și permeabilitatea nazală *ex vivo* au fost măsurate pentru ambele formulări NE și NEIG. Formula F15, cu o dimensiune a picăturilor de 58,4 nm, a prezentat o permeabilitate maximă *in vitro*, precum și *ex vivo*; la aceasta s-a adăugat 0,5% carbopol 934, rezultând NEIG2, care prezintă valori de cedare și permeabilitate comparabile cu F15, cu un timp de rezidență mai mare, ce depășește *clearance*-ul fiziologic nazal normal. În cele din urmă, LAS sub formă de NEIG2 a fost supus unui studiu *in vivo*, fiind considerat o formulă nouă intranazală promițătoare, care exercită un debut rapid al acțiunii (T_{max} de 0,75 ore) însoțit de o biodisponibilitate mai mare (de 2,5 ori) și o permeabilitate tisulară (de 4 ori) în comparație cu forma farmaceutică orală (suspensie apoasă de LAS 8%).

Keywords: pseudo-ternary phase diagram, *in situ* gel, nanoemulsion, lasmiditan

Introduction

The FDA authorised the oral drug lasmiditan (LAS) for the treatment of migraine headaches in October 2019 [1].

In contrast, LAS is a highly selective agonist of 5-HT_{1F} receptors, carrying virtually no affinity for other receptors, which appears to be largely responsible for the adverse effect profile of its predecessors. This selectivity allows for the successful termination of migraines without causing vasoconstriction [2].

LAS is the first and sole member of a novel family of anti-migraine drugs called the neurally acting anti-migraine medications (NAAMAs), which are distinguished by their selectivity for 5-HT_{1F}, lack of vasoconstrictive action and capacity to terminate migraines through neuronal inhibition [3].

The oral bioavailability of LAS has been found to be around 40%. It is classified as a Class I medication according to the Biopharmaceutical Classification System (BCS). When LAS was given after a high-fat meal, its maximum drug concentration (C_{max}) and area under the concentration-time curve (AUC) were raised by 22 and 19 percent, respectively, and the time to reach C-max (T_{max}) was pushed out by around an hour [4].

The reasons for the LAS's poor oral bioavailability include its substantial hepatic and extra-hepatic metabolism in humans, predominantly by non-CYP mediated ketone reduction, and its status as a substrate for P-gp [5, 6].

Drugs are typically administered orally since it is the most convenient and least invasive method. Nevertheless, the drug's poor solubility, low permeability, instability and quick metabolism restrict its oral bioavailability, limiting its use in clinical practice. One of the major difficulties in medication formulation design is increasing the bioavailability of medicines with such limiting physicochemical features [7].

Formulation scientists have found the development of nasally administered compounds appealing due to their many potential benefits, including the avoidance of hepatic first-pass metabolism and gastrointestinal degradation, increased bioavailability at a reduced dose, simplified administration and rapid uptake into the body's bloodstream [8]. The main obstacle to medication administration *via* this route is mucociliary clearance, so increasing the drug's duration spent in the nasal cavity might be the answer to this issue.

Recently, lipid-based formulations have shown promise as a means of increasing medication bioavailability [9]. Medication delivery techniques include the use of oils or surfactant dispersions, liposomes, lipid nano-carriers, microemulsions and NEs [10].

NEs are isotropic mixtures of oils, surfactants and co-surfactants that create oil in water (O/W) when exposed to aqueous fluids. Droplet sizes of NEs between 20 and 200 nm have several benefits, such as high solvent capacity, tiny particle size and great stability [11].

Moreover, a droplet-sized formulation that promotes greater transmembrane transport can increase the drug's bioavailability. Based *in situ* systems, which are liquid aqueous solutions before administration but transform into gel under physiological circumstances, are regarded as contemporary advances in hydrogel drug delivery research [12]. The most popular pH-induced gelling agent is carbopol 934, which prolongs the drug's persistence on the nasal mucosa and slows down the body's natural elimination [13].

By using nanotechnology to formulate LAS as a nasal nano-emulsion and by avoiding mucociliary clearance, which is regarded as the main barrier to drug delivery, by including a pH-sensitive polymer like carbopol 934 in the formulas, this study aims to solve the issues associated with poor oral bioavailability of LAS as an oral tablet. It also aims to achieve *in situ* gelling properties [14].

Materials and Methods

Materials

Lasmiditan was purchased from Yongchi Chemical Technology Co., LTD., China.

Labrafil M 1944, Labrafact PG, Labrasol ALF, isopropyl myristate, Transcutol and Cremophor® EL were purchased from Gattefosse (France). Imwitor® 988 and Miglyol 812 N were purchased from IOI Oleochemical GmbH, Germany. Oleic acid (OA), triacetin, propylene glycol (PG) and carbopole 934 were purchased from Central Drug House (CDH®). Castor, olive and sesame oils were purchased from Now Food, USA. Potassium dihydrogen phosphate and disodium hydrogen phosphate, Tween 20, 40, 60, 80, Span 20, 80, Polyethylene glycol (PEG) 200, 300, 400, 600 were all purchased from Hi Media Laboratories Pvt. Ltd., India. Methanol was purchased from Loba Chemie Pvt. Ltd., India. A Millipore filter syringe was purchased from Chm Lab, Spain. Dialysis Bag MD34-5M, Wide flat: 34 MM, Mw: 8000 - 14000 D was purchased from MYM Biomedical Technology Company Limited, USA. Deionized water (DD), the rest of the chemicals and reagents were of analytical grade.

Research on solubility

Labrafil M 1944, Labrafac PG, isopropyl myristate, Miglyol 812N, Imwitor 988, triacetin, oleic acid (OA), castor oil, sesame oil and olive oil were among the oils studied in order to determine the LAS solubility. Surfactants studied were Span 20, 80, Tween 20, 60, 40, 80, Cremophor EL (CE) and Labrasol ALF. Co-surfactants included Transcutol (TC), PEG 200, 300, 400, 600 and PG. In a water bath, each 2 mL of the upward listed substances with an excess amount of drug (LAS) was shaken for 72 hours (h) at 25 ± 0.5°C. After the mixture had reached equilibrium, it was centrifuged for 20 minutes (min) at 3000 rpm. For the dilution of the supernatant, methanol was utilized. At 256 nm, the LAS concentration was measured spectrophotometrically [15].

Productive of pseudo-ternary phase diagrams

Depending on the solubility studies, OA, CE and TC were used to prepare the NEs as the selected oil, surfactant and co-surfactant, respectively. To identify NE regions and choose the optimal combination, pseudo-ternary phase diagrams were built up using the water titration method. Surfactant and co-surfactant, which are both represented as S-mix, were mixed at various weight ratios (1:1, 2:1, 3:1 and 1:2 w/w) to determine the phase diagram's productivity. For each ratio, oil was added and blended using a vortex mixer to form a mixture with nine different S-mix ratios (ranging from 1:9 to 9:1 w/w). Then, a homogenous, yellowish-transparent mixture of S-mix was titrated by adding DD slowly, drop by drop, while being magnetically stirred continuously and gently. In order to verify the phase clarity, the mixture was visually inspected after each water addition. At the point at which the system became turbid or viscous gel-like, titration was stopped. The percent weight of oil, S-mix and water in the mixture was calculated to delineate the phase boundaries in each diagram, then plotted using the Original Lab software. The NE's area, which is

considered to be visually clear, was the shaded one in a triangle plot with one apex representing the oil, the second one representing water and the third representing the S-mix at a fixed weight ratio [16-18].

The method used for the preparation of NE

Scientists in a variety of sectors, including pharmaceutical sciences, have paid close attention to the phase inversion composition method (also known as the self-nano emulsification method), which produces NEs at ambient temperature without the need for organic solvents or heat. By gradually adding water to an oil and surfactant solution while gently stirring and maintaining a steady temperature, kinetically stable NEs with tiny droplet sizes (50 nm) may be produced [19].

Formulation of LAS NE

As listed in Table I, numerous O/W NE formulations have been created by employing a water titration strategy in accordance with pseudo-ternary phase diagrams. Ten g of 8% LAS NE was prepared by dissolving 800 mg of LAS in a mixture of OA (the selected oil according to the solubility study) with an appropriate ratio of S-mix (CE and TC), both of which were previously weighed in a beaker and stirred continuously on the magnetic stirrer at room temperature (~ 500 rpm) for ten minutes until a clear solution was achieved. DD was then added to the clear solution with continuous stirring until a clear NE was formed [20]. The produced NEs were exposed to ultrasonication using a 25 kHz probe sonicator for two minutes in order to achieve extremely tiny droplet sizes. Heat is produced when ultrasonication is used repeatedly. This problem has been fixed by placing the NE formula in the icebreaker [21]. The NEs were transferred to the new vial and kept at 4°C in the refrigerator. The derived final formula (**F1 - F18**) underwent optimisation and then characterization.

Table I

Composition of LAS NE

Formula Code	OA% (w/w)	Surfactant	Co-Surfactant	S-Mix ratio	S-Mix% (w/w)	DD% (w/w)
F1	7	CE	TC	1:1	58	35
F2	10	CE	TC	1:1	55	35
F3	20	CE	TC	1:1	52	28
F4	25	CE	TC	1:1	55	20
F5	7	CE	TC	2:1	58	35
F6	15	CE	TC	2:1	55	30
F7	20	CE	TC	2:1	55	25
F8	25	CE	TC	2:1	55	20
F9	7	CE	TC	3:1	56	37
F10	10	CE	TC	3:1	57	33
F11	15	CE	TC	3:1	55	30
F12	20	CE	TC	3:1	55	25
F13	25	CE	TC	3:1	55	20
F14	7	CE	TC	1:2	50	43
F15	10	CE	TC	1:2	50	40
F16	15	CE	TC	1:2	50	35
F17	20	CE	TC	1:2	50	30
F18	25	CE	TC	1:2	50	25

*For all NE formulations (**F1-F18**), 8% LAS was included

Optimization of NE

To be optimized as stable NE formulations, the generated NEs must pass thermodynamic stability experiments. After centrifuging all of the prepared NEs for 30 minutes at 3500 rpm, all of the formulations that did not exhibit any phase separations underwent heating and cooling tests. On these formulations, three freeze-thaw cycles (-20°C and 25°C) were carried out. After passing the preceding test, NE formulations were subjected to physical characterization [22, 23].

Characterization of NEs

Particle size, particle size distribution (PDI), % transmittance, dilution test, drug content, morphological investigations, drug release and permeation study are considered only a few of the physicochemical criteria used to define or characterize the NE.

Particle size and PDI measurement

Using a Malvern Zeta sizer (UK), dynamic light scattering was used to assess the globule size and PDI of NE formulations. 10 mL of DD was used to dilute 0.5 mL of each mixture. For each formulation, the mean droplet size and PDI were projected [24, 25].

Percentage transmittance (T%)

A Shimadzu UV/VIS (UV-1700 Pharma Spec, Shimadzu, Japan) spectrophotometer was used to calculate the percentage transmittance. Using DD, one millilitre of the formulation was diluted 100 times before being examined at 650 nm with DD as the reference material [26].

Dilution test

This test was performed to observe the NE's phase inversion. Only one mL of the optimized NE was diluted with 10 mL of DD in a test tube and observed for phase inversion [27].

Drug content measurement

In a volumetric flask, the NE's formulation was diluted with methanol before being filtered through a 0.45 μm filter syringe, using a UV-VIS spectrophotometer to measure the LAS NEs' content at the chosen λ max (256 nm) [28].

In vitro release study

A dialysis bag (Mw: 8000 - 14000 D) made of cellulose and a quarter-gram sample of the NE were combined together and then linked to the basket immersed in the jar with 100 mL of phosphate buffer (pH 7.4) at a temperature of 37°C. The mixture was agitated at 50 rpm for up to two hours. Several time intervals were used to collect 2 mL of samples from the jar, which were then analysed spectrophotometrically at 256 nm [14].

Ex vivo drug permeation studies

From the nasal cavities of goats supplied by the neighbourhood butcher, fresh nasal tissue was carefully extracted and washed with phosphate buffer pH 7.4, then mounted on the Franz diffusion cells with a 1.76 cm^2 permeation area. The receptor chamber was filled with 11 mL of 7.4 phosphate buffer. A 20 minute pre-incubation period was followed by the addition of 0.25 g of NE, NEIG and 8% LAS as aqueous suspension (the medication is pure and suspended in DD) separately. During the course of three hours, 0.5 mL samples were taken at predefined time intervals, and the concentration of LAS was determined at 256 nm spectrophotometrically using Shimadzu UV/VIS. The quantity that entered the receptor chamber was used to express the results. The steady-state flux (Jss) was calculated from the slope of the line obtained by plotting the amount of drug permeated through a certain surface area (mg/cm^2) *versus* different time intervals (min). The permeability coefficient (p) was calculated by dividing the obtained slope value (Jss) over the initial LAS concentration (C_0) in the donor compartment [29].

Formulation of in situ gel

The selected NE that passed all the above tests was subjected being formulated as 0.2, 0.5 and 0.7% *in situ* gel. A calculated weight of carbopol 934 (pH-induced *in situ* gelling polymer) was sprinkled over the water content of the NE, allowed to hydrate overnight then dropped gradually with continuous stirring at ~ 500 rpm over a homogeneous transparent yellow previously weighed mixture of OA, CE and TC till it got the NEIG 1, 2 and 3 respectively [30].

Evaluation of in situ gels

pH of the in situ gel. Using a pH meter, the pH of each formulation (NEIG1, NEIG2 and NEIG3) was ascertained.

Studies on gelation. The various NEIGs were combined in a glass cylindrical tube at a 1:1 ratio with artificial nasal fluid (phosphate buffer pH 6.4), and the tube was then submerged in a water bath maintained at 37 ± 1°C. The tubes were flipped over after 20 seconds. A "+" gelation was given to the formulation if the gel stuck to the tube wall and did not slip down [31].

Viscosity measurement. The Brookfield digital viscometer spindle number 4 was utilized in order to acquire the data for the apparent viscosity. Before and after gelation, the viscosity of the NEIGs was tested at 6, 12, 30 and 60 rpm for a period of 30 seconds [32].

Gel strength measurement. A five-gram sample of each NEIG was neutralized with artificial nasal fluid (phosphate buffer pH 6.4) to form a gel. The gel was then covered with a 3.5 g weight. The time span was assessed in seconds, at which the weight was able to reach a depth of three centimetres into the nasal *in situ* gel [33].

In vitro release study. Using dissolution apparatus no. 1 (basket type), a quarter-gram sample of the NEIGs was put in a dialysis bag (Mw: 8000 - 14000 D) made of cellulose bound with the basket immersed in the jar that contained 100 mL of phosphate buffer (pH 7.4) at a temperature of 37°C. During the period of two hours, the mixture was stirred at 50 rpm. Two mL of samples were taken from the jar at various time intervals, and they were then examined spectrophotometrically at a wavelength of 256 nm after dilution if required [28].

Ex vivo drug permeation experiments. The selected NEIGs that passed all the above tests were subjected to *ex vivo* studies as listed previously [29].

In vivo studies and determination of pharmacokinetic parameters

Research plan

The *in vivo* drug pharmacokinetics study was conducted according to the institutional guidelines of the Animal Ethics Committee of the College of Pharmacy at the University of Baghdad, Iraq. During the collection of blood samples, six male, white rabbits weighing 2.0 - 2.5 kg were captured in a rabbit hutch. Afterward, a table was held in a horizontal position with the rabbits placed on it. The oral and nasal dosages for rabbits were estimated, and the animal received a comparable dose of LAS. The body surface area (BSA) normalisation method and the human equivalent dose (HED) of pharmaceuticals, including the species (Km) factor (body weight in kg divided by BSA in m^2), were used to calculate the animal nasal and oral doses for the rabbit as follows [34]:

$$\text{HED (mg)} = (\text{Animal Km}/\text{Human Km}) \times \text{Animal Dose (mg)}, \quad \text{Eq. 1.}$$

The daily single dose of LAS for adults is advised to be 0.28 mg/kg, and the values of Km were 37 and 12 for adult humans and rabbits, respectively [35]. Hence, the LAS dose for rabbits based on the BSA normalisation method was 1.72 mg and reached 17.26 mg after multiplying by 10 (safety factor).

Twelve albino male rabbits weighing between 2 and 2.5 kg were employed in the *in vivo* study. Based on the results of the *in vitro* and *ex vivo* studies,

NEIG2 was selected for the *in vivo* examination. Before the experiment began, the rabbits fasted for 24 hours. They were then divided into two groups, each with six rabbits. At a dosage of 0.863 mg/kg, Group I received LAS as an aqueous oral suspension (reference). Group II received NEIG2 intranasally at a dosage of 0.863 mg/kg. 2 mL blood samples were collected in EDTA tubes before the medication was administered (control sample), and then again at 0.25, 0.5, 0.75, 1.0, 2.0, 3.0, 5.0, 7.0, 12 and 19 hours after the drug was administered, then centrifuged [35]. Prior to analysis utilizing the HPLC technique previously established and confirmed by L. Santosh Kumar, samples were kept at -20°C [36].

Parameters involved in pharmacokinetics

The pharmacokinetics of LAS were analysed in a non-compartmental fashion. Using the plasma concentration-time curve, we were able to calculate the maximum plasma concentration (C_{max}) and the time needed to attain C_{max} following oral and intranasal delivery. The elimination half-life time ($t_{1/2}$) is calculated as 0.693/Ke [37], and the elimination rate constant (Ke) is calculated from the slope of the straight section of the terminal phase (where the slope is equal to Ke/2.303) [16]. Extrapolation of the area to infinity (AUC t_0-α) was estimated by adding the last measured plasma concentration divided by the elimination rate constant (Ke) to AUC $t_{0\text{-}19}$ according to the following equation no. 2 [38]. This was done for each individual rabbit by calculating the area under the plasma concentration-time curve from time 0 - 19 hours:

$$(AUC\ t_{0\text{-}19}).AUC_{0-\alpha} = AUC_{0-19} + [C_{last} / Ke], \quad \text{Eq. 2.}$$

where, C19 is the concentration of LAS after 19 hs. The relative bioavailability was calculated from the following equation no. 3 [39]:

$$F_{relative} = (AUC_{NEIG2}/AUC_{oral} \times Dose_{oral}/Dose_{NEIG2}) \times 100, \quad \text{Eq. 3.}$$

Field emission scanning electron microscope (FESEM)

The NE of LAS was also examined using a field emission scanning electron microscope (FESEM). It is a device for analysing the surface roughness that is used to explain the size and shape of the droplets in the LAS NE [40].

Statistical Analysis

The experiments were repeated three times, and the results were expressed as means and standard deviations (mean ± SD). One-way analysis of variance (ANOVA) was used to determine whether or not there were statistically significant differences between groups when comparing means; p-values were considered significant at a level of ($p < 0.05$) and non-significant at a level of ($p > 0.05$).

Results and Discussion

Studies on Solubility

Figure 1 displays the amount of LAS soluble in various oils, surfactants and co-surfactants. The results showed that OA and CE solubilize 32 and 62.4 mg/mL of LAS as an ideal oil and surfactant, respectively. The addition of a co-surfactant aimed at increasing the mixture's ability to load drugs; TC was chosen as a co-surfactant because of its effective solubilizing action (62.3 mg/mL).

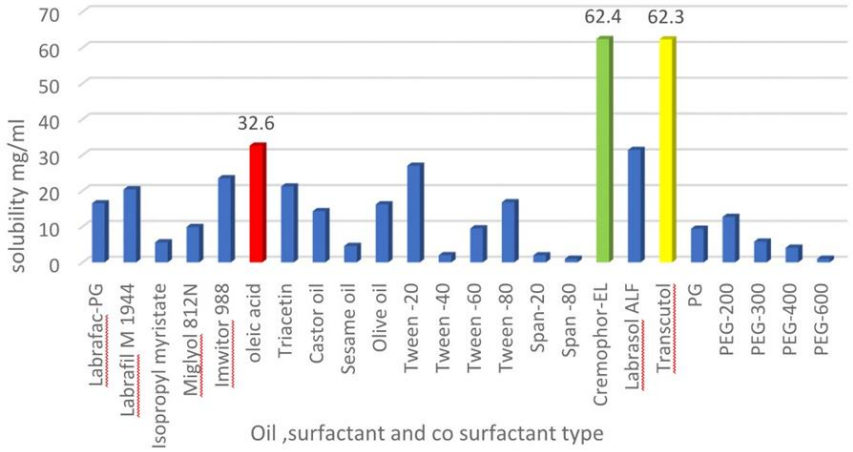

Figure 1.
A graphic representation of LAS solubility in various oils, surfactants and co-surfactants

Phase diagram of a pseudo-ternary system

A phase diagram can be used to investigate the connection between the phase behaviour of a mixture and its constituents. As can be seen in Figure 2, four ternary phase systems were built by preparing the S-mix with varying mass ratios (1:1, 2:1, 3:1 and 1:2). In phase diagrams, the shaded sections represent the NE, while the unshaded zone represents the multiphase emulsion.

NEs are systems that are thermodynamically stable, and they are produced when a precise concentration of oil, S-mix and water are mixed. When there was a greater amount of surfactant in the S-mix (2:1 or 3:1), researchers saw that the NE areas were elevated.

This might be a result of a further decrease in the interfacial tension, which would increase the fluidity of the interface and allow the oil phase to more thoroughly penetrate the hydrophobic area of the CE [41]. From each phase diagram, five different formulas with fixed OA% (7, 10, 15, 20 and 25) were extrapolated on the shaded area with corresponding S-mix (CE and TC) percentages and water, then subjected to optimisation and then characterization.

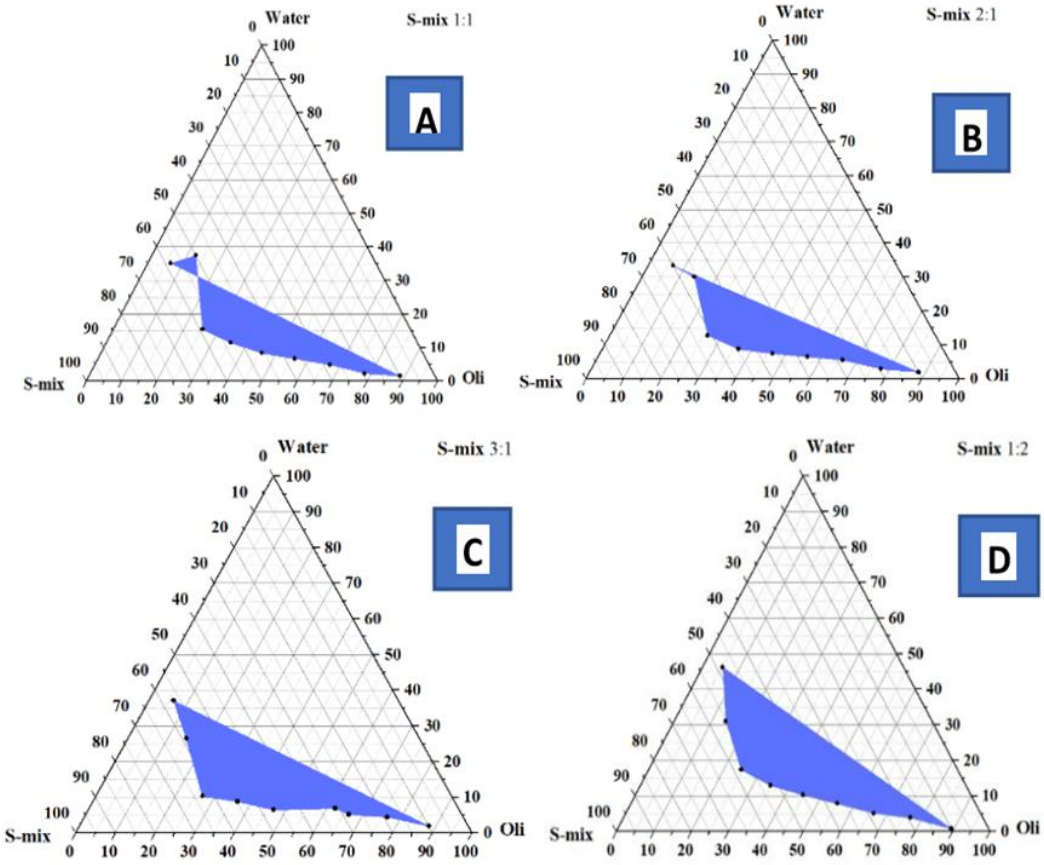

Figure 2.

Pseudo ternary phase diagrams of the OA, CE, TC (S-mix) and water system at the 1:1 (**A**), 2:1 (**B**), 3:1 (**C**) and 1:2 (**D**) weight ratios of S-mix at ambient temperature

Thermodynamic stability studies

The thermodynamic stability studies were accomplished on all of the developed NE formulations. Table II results showed that, with the exception of F9, F10 and F13, phase separation was not observable, and there was no sign of phase separation, cracking, or a change in odour or colour for all the prepared Nes, which were then subjected to further characterization [42].

Table II

Thermodynamic Stability Study Results

F-code	Centrifugation test	Heating-cooling cycle	Freeze-thawing cycle	F-code	Centrifugation test	Heating-cooling cycle	Freeze-thawing cycle
F1	pass	pass	pass	**F10**	pass	fail (phase separation)	-----
F2	pass	pass	pass	**F11**	pass	pass	pass
F3	pass	pass	pass	**F12**	pass	pass	pass
F4	pass	pass	pass	**F13**	pass	fail (phase separation)	-----
F5	pass	pass	pass	**F14**	pass	pass	pass
F6	pass	pass	pass	**F15**	pass	pass	pass
F7	pass	pass	pass	**F16**	pass	pass	pass
F8	pass	pass	pass	**F17**	pass	pass	pass
F9	pass	fail (phase separation)	-----	**F18**	pass	pass	pass

Characterization of NEs

Droplet size measurement and polydispersity index (PDI)

The NE created all of the nanosizes. The results listed in Table III showed that globule size increases with the oil concentration across all the S-mix ratios. The interfacial film was stabilized and condensed at high surfactant ratios, and it will grow with an increase in co-surfactant concentration. The tiny mean droplet size may be due to the co-surfactant molecules penetrating the surfactant coating. This may decrease the surface of the interfacial layer, resulting in a smaller radius of curvature for the droplets and the formation of transparent systems. Consequently, the proportional quantity of a surfactant to a co-surfactant affects droplet size differently [43]. The polydispersity index for each formulation was less than 0.5, indicating a uniform and constrained globule size distribution [44].

Table III

The average droplet size, PDI, %T and drug content (mean ± SD, n = 3)

Formula code	OA%	S-mix Ratio	S-mix%	Particle size (nm)	Polydispersity (PDI)	Light transmittance (T%)	Drug% Content
F1	7	1:1	58	49.75 ± 0.02	0.275 ± 0.01	99 ± 0.001	95 ± 0.6
F2	10	1:1	55	51.77 ± 0.01	0.3775 ± 0.0	96.7 ± 0.003	96 ± 0.5
F3	20	1:1	52	53.17 ± 0.012	0.374 ± 0.0	98.1 ± 0.002	95 ± 0.45
F4	25	1:1	55	57.3 ± 0.02	0.3 ± 0.01	97.9 ± 0.002	95 ± 0.5
F5	7	2:1	58	45.1 ± 0.01	0.38 ± 0.0	98.1 ± 0.001	96 ± 0.4
F6	15	2:1	55	57.97 ± 0.02	0.417 ± 0.01	98.7 ± 0.002	99.8 ± 0.2
F7	20	2:1	55	64.060 ± 0.21	0.291 ± 0.01	99 ± 0.001	96 ± 0.4
F8	25	2:1	55	64.78 ± 0.01	0.408 ± 0.0	98.2 ± 0.002	100 ± 0.4
F11	15	3:1	55	52.66 ± 0.02	0.3943 ± 0.01	97.4 ± 0.003	95 ± 0.45
F12	20	3:1	55	58.2 ± 0.03	0.441 ± 0.012	92.6 ± 0.004	98 ± 0.2
F14	7	1:2	50	62.99 ± 0.012	0.406 ± 0.01	96.8 ± 0.003	98 ± 0.32
F15	10	1:2	50	58.4 ± 0.01	0.2811 ± 0.0	95.4 ± 0.001	100 ± 0.1
F16	15	1:2	50	77.19 ± 0.02	0.285 ± 0.01	94.5 ± 0.002	98 ± 0.2
F17	20	1:2	50	91.93 ± 0.021	0.2081 ± 0.01	92 ± 0.006	96 ± 0.4
F18	25	1:2	50	66.29 ± 0.01	0.245 ± 0.0	94.6 ± 0.001	100 ± 0.2

Measuring light transmission (T%)

Table III shows that all formulations have transmission percentages close to 100%, indicating that they are transparent, clear and efficient light transmitters [45].

Dilution test

The LAS NE has been shown to be physically stable even when diluted with water. All NE formulations (F1 - F18) showed clear and fine blue NE indicating O/W type in less than a minute, confirming that they may be diluted in nasal fluids without drug precipitation and maintaining their nanosized nature [46].

Estimate of drug content

All the prepared LAS NEs contained more than 95% of the drug as shown in Table III previously listed and did not significantly differ from one another ($p > 0.05$). These formulations all met British pharmacopoeia requirements and fell within a tolerable range (95.0% - 105.0%), indicating that none of the prepared formulations had any drug precipitate [43].

In vitro drug release study

Owing to its low degree of water solubility (1.0 - 3.56 mg/mL at 25 and 37°C, respectively), the pure drug (LAS) released 36% at the end of 35 minutes, while the drug released from numerous NEs (F1, F7, F14, F15 and F18) was close to 100%. Droplet size affects the drug's quantitative release from a NE formulation [47]. As the concentration of CE increases, the viscosity increases and the drug release decreases from the formulas (F5, F6, F8, F11 and F12). The higher release rate was achieved for NEs (F14, F15 and F18) prepared with increasing the TC content (1:2) ratio due to a decline in the viscosity values accompanied by the good solubilizing activity of TC that aided the dissolving process of the pure medication (LAS). The co-surfactant's function in the pseudo-ternary system was able to reduce interfacial tension and boost the fluidity of the interface. Moreover, it makes the hydrocarbon tail more mobile, allowing more oil to penetrate the NE zone [43, 48]. The optimum formula (F15) produces 100% release in 15 minutes, as seen in Figure 3, which depicts the release profile of NE formulations.

Selection of optimum formulas for LAS NEs

Based on the characterization studies findings of the prepared NEs, F1, F7, F14, F15 and F18 were chosen as the best formulas because of their good droplet size, PDI, T% and drug content as declared previously in Table III. All the optimized selected NEs exert high exertion with rapid release of LAS within 35 minutes, except for F15, which shows 100% release within only 15 minutes. Further studies on the optimized formulas would be provided.

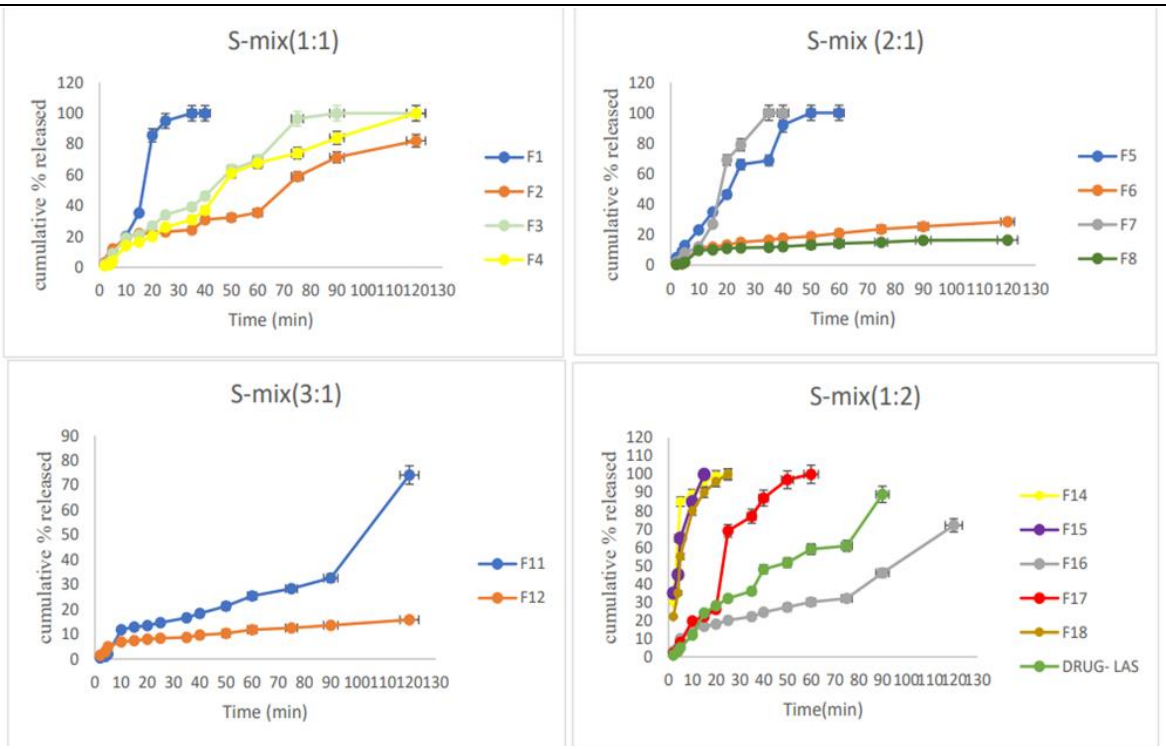

Figure 3.
The effect of the S-mix: oil ratio on the release profile of LAS NEs and the pure drug

Analysing permeation in an ex vivo model

Using the fresh nasal mucosa of sheep, *ex vivo* permeation research was conducted on the receptor part of the Franz diffusion cell. F1, F7, F14, F15, F18 and pure drug (LAS 8%) as aqueous drug suspension were subjected to the experiment separately.

Figure 4a and Figure 4b declared that although all the optimised NEs (F1, F7, F14 and F18) approximately 100% permeated through sheep's nasal mucosa at 3, 0.5 and 0.5 hours respectively, only F15 achieved 100% permeation within 15 minutes while the pure drug exerts only 30%.

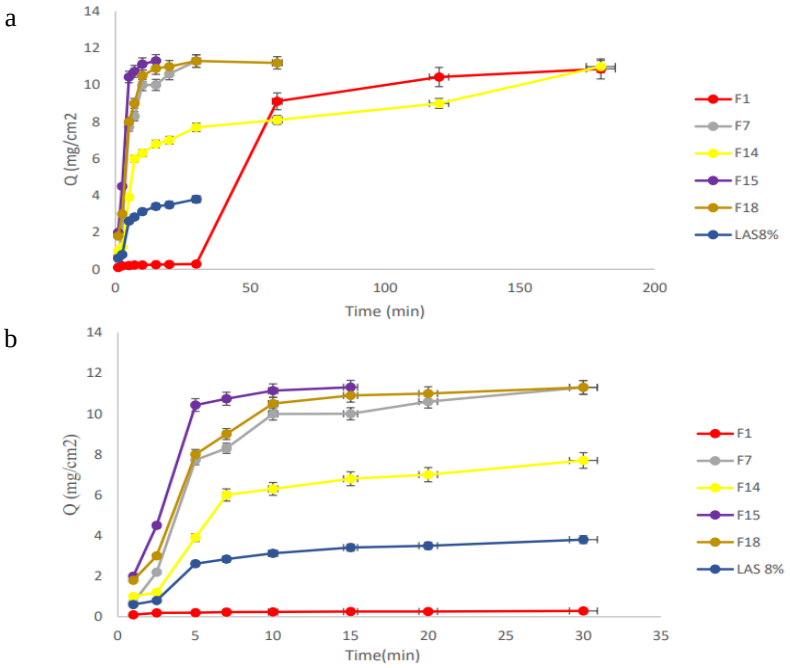

Figure 4.
Ex vivo permeation of the optimised NEs formulas with the pure drug
(a) within 180 minutes; (b) within 30 minutes

The findings suggested that LAS from NEs-optimised formulas would permeate significantly better ($p < 0.05$) than pure drug (aqueous LAS suspension). This may be because NEs remain intact in nasal tissue, affecting both lipid and polar permeation pathways. Moreover, the hydrophilic domain of NEs can more thoroughly moisten the nasal tissue's outer layer, which can facilitate drug uptake in the tissue. The inter-lamellar volume of the tissue lipid bilayer grows as the NEs aqueous fluid travels down the polar pathway, disrupting the interfacial structure [49]. Also, the formulation's reduced globule size contributes to quicker penetration [50]. Moreover, oleic acid acts as an activator of lipophilic permeation, which can improve membrane permeability [51].

The permeation characteristics of F1, F7, F14, F15, F18 and control (LAS 8% as aqueous suspension) are shown in Table IV, and the findings demonstrate that the F15 NE formula has greater permeation and flux than the control, though it is subjected being formulated as NEIG with three different strengths 0.2, 0.5 and 0.7% of carbopol 934.

Table IV

Ex vivo Permeation parameters

Formula code	Lag time (min)	Flux (J)- (mg/cm^2 * min)	P = J/Cd * 10^{-2} (cm/min)
F1	30	0.294	0.368
F7	1.6	2.21	2.76
F14	1.5	1.05	1.31
F15	0.5	2.37	2.97
F18	1.1	2	2.5
Drug-LAS	2	0.724	0.91

In vitro evaluation of NEIGs

All the prepared NEIG-1, -2 and -3 that contain 0.2, 0.5 and 0.7% carbopol934, respectively, were subjected to the *in vitro* evaluation (pH, gelling capacity, viscosities and gel strength) and then compared to the results obtained with NE (F15) and emulgel *in situ* (EMI), as shown in Table V.

The obtained results indicated that all the prepared NEIGs were clear and had a pH value between 5.84 and 6.1 which is compatible with nasal mucosal pH. The results of the viscosity tests for the NEIGs in solution and in gel form revealed a significant increase ($p < 0.05$) in viscosity values (2573 - 3483 mPa*s) at the nasal pH in comparison to the NEIGs in solution (2129 - 2605 mPa*s); this change was exclusively the result of the gel conversion, indicating the *in situ* nature of all the prepared gels.

Table V

The pH, gelling capacity, gel strength and viscosities (using spindel no. 4 at 30 rpm) of NE (F15), NEIGs (1, 2 and 3) and EMI of LAS (mean ± SD, n = 3)

Formula code	pH	Gelling capacity	Viscosity before gelation (mPa*s)	Viscosity after gelation (mPa*s)	Gel strength (s)	Particle size (nm)	PDI
NEIG1	6.1 ± 0.01	+	2129 ± 1.2	2573 ± 1.5	18 ± 0.2	68.64 ± 0.02	0.357 ± 0.021
NEIG2	6.05 ± 0.002	+++	2547 ± 2.0	3102 ± 2.5	27 ± 0.1	100.3 ± 0.01	0.38 ± 0.001
NEIG3	5.84 ± 0.005	++	2605 ± 1.6	3483 ± 2.1	52 ± 0.4	149 ± 0.03	0.74 ± 0.02
EMI	5.5 ± 0.02	+	549 ± 1	737 ± 1.5	16 ± 0.5	252.9 ± 0.02	0.448 ± 0.005
NE (F15)	6.5 ± 0.01	-----	2100 ± 2.5	-------	------	58.4 ± 0.01	0.281 ± 0.0

*Where: +: immediate gelation (10 seconds) remains for several minutes; ++: immediate gelation but for few extended periods; +++: immediate gelation, harder gel but for more extended periods

The gel strength is a crucial consideration when creating a NEIG; the optimal gel strength enables simple nasal administration of the gel while preventing gel leakage from the nose. The formulation of the nasal gel must have the proper gel strength, which is critical. Gels with a strength > 50 seconds (s) may be excessively stiff and uncomfortable, whereas gels with a strength of less than 25 seconds may not have enough structural integrity and may degrade quickly [14]. Because of this, NEIG1 and NEIG3 with gel strengths of 18 and 52 seconds, respectively, were excluded from further evaluations except for NEIG2.

In vitro release and ex vivo permeation studies

Only NEIG2 was subjected to *in vitro* release and *ex vivo* permeation, then comparing the results with F15, EMI and the control (8% LAS as an aqueous suspension), as shown in Figure 5a and Figure 5b.

The permeation parameters of NEIG2, F15, EMI and control are shown in Table VI.

The results showed that NEIG2 achieved 100% permeation and was then released within 20 and 25 minutes, respectively. Although NE (F15) exerts faster permeation and release, the leakage problem can be overcome by the inclusion of 0.5% carbopol 934 which is commonly employed as a pH-induced gelling polymer to lengthen the medication's residence period [52]. The *ex vivo* permeation results showed that NEIG2 significantly increased the nasal permeation of LAS ($p < 0.05$), so it achieved a 4-folds higher permeation percentage compared with an aqueous drug suspension

(8% LAS) and about 1.5 folds compared with EMI, which may be due to the smallest droplet size and the higher viscosity value of NEIG2 as listed previously in Table V. All these characteristics make NEIG2 an excellent carrier for the nasal administration of LAS and subjected to an *in vivo* study [14].

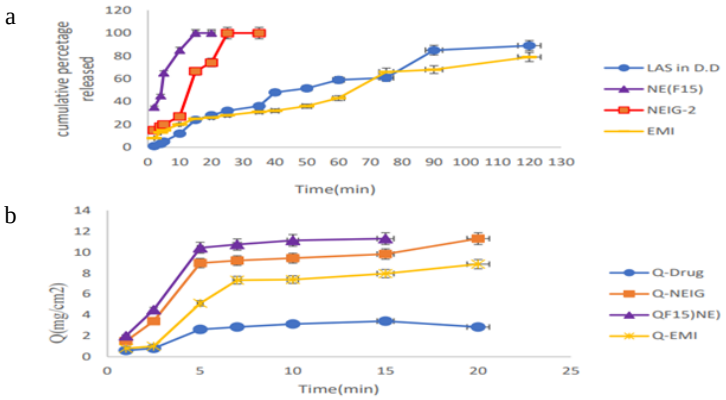

Figure 5.
In vivo release (a) and *ex vivo* permeation (b) studies

Table VI
Ex vivo permeation parameters

Formula code	Lag-time (min)	Flux (J) (mg/cm^2 * min)	P = J/Cd * 10^{-2} (cm/min)
NE (F15)	0.5	2.37	2.97
NEIG2	1	2.2	2.87
EMI	1.5	1.644	2.055
Drug-LAS	2	0.724	0.91

Field emission scan electron microscope (FESEM)
Figure 6a demonstrates that the optimised improved formula is spherical in shape and with nano-sized particles, compared with aqueous LAS suspension or LAS powder that appears in Figure 6b as irregular macro-sized particles. The shape and size of the oil droplets did not significantly alter as they accumulated [40].

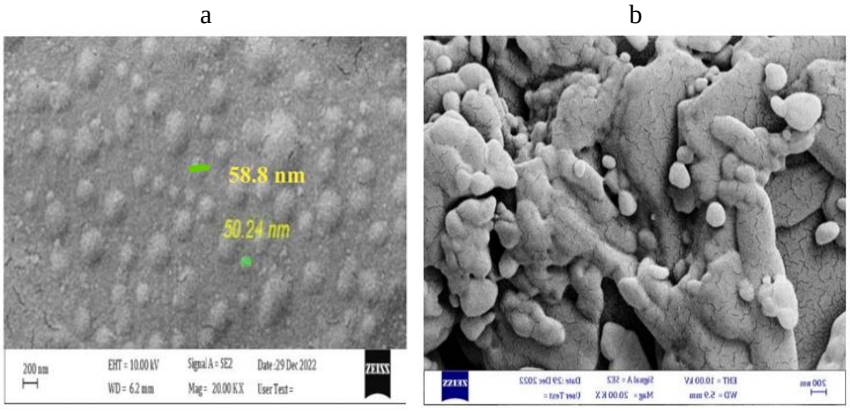

Figure 6.
FESEM of optimised formula (NEIG-2) (a) and aqueous LAS suspension (b)

In vivo pharmacokinetics study
The concentration of LAS in rabbit's blood was determined using a reproducible, sensitive and rapid HPLC method. The retention time and peak chromatograms of LAS (standard solution), hydrochlorothiazide (internal standard solution) and plasma sample (control) to be equal to 2.8, 6.08 and 4.33 minutes respectively. The chromatographic conditions were previously developed and validated [36].

Regarding the calibration curve of LAS in plasma a straight line with a high regression coefficient (R^2 = 0.9999) was obtained by plotting the area under the peak of the standard or internal standard *versus* the concentration; thus indicating that the calibration curve obeys Beer's law within the range of concentration used.

The pharmacokinetic study results revealed that intra-nasal application of LAS as NEIG2 can significantly

($p < 0.05$) modify its pharmacokinetic profile and can increase its bioavailability by more than 2.5 folds in comparison with the oral LAS aqueous suspension (control), as shown in Table VII and Figure 7.

Table VII

Pharmacokinetics parameters of a single intranasal dose of LAS as optimised NEIG2 and a single oral aqueous suspension (8% LAS)

Kinetic parameters	Oral - LAS (control)	Intranasal LAS (NEIG-2)
C max (mg/mL)	4181.09 ± 125	8066 ± 242
T max (h)	2 ± 0.2	0.75 ± 0.05
AUC0-∞ (ng * h/mL)	8852.67 ± 266	19616.86 ± 589
Ke (h-)	0.125 ± 0.002	0.116 ± 0.001

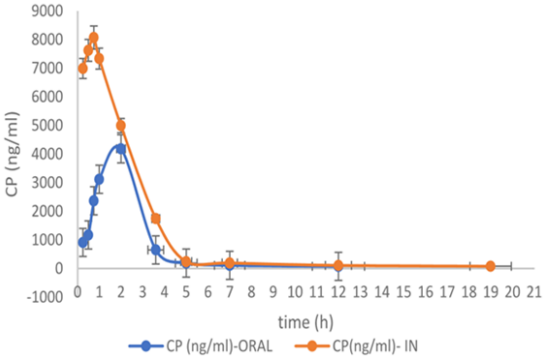

Figure 7.

Mean plasma drug concentration profile *versus* time obtained during the *in vivo* pharmacokinetic studies carried out in six rabbits receiving a single dose of LAS as NEIG2 (given intranasally) and the other six rabbits receiving aqueous drug suspension (given orally)

The illustrated results fixed that formulation of LAS as NEIG achieves rapid onset of action (lower T_{max} value = 0.75 hours) with higher extent of LAS absorption (C_{max} = 8066 ng/mL) accompanied by higher value of total AUC (19616.86 ng * h/mL), so the new intranasal formulation gains the goal of increasing the bioavailability of LAS compared with a lower F value when administered orally.

Increased permeability is a result of the physico-chemical characteristics of the drug molecule known as NEIG, which include reduced particle size, high lipoidal characteristics and the presence of CE and TC. Oligosaccharide chains in the mucus membrane include a high proportion of carboxyl groups that form hydrogen bonds with them, creating a dense network between the polymer and mucus membrane, increasing the residence time of drug in the nasal mucosa when *in situ* gelling agents are present [53]. Additionally, switching the route of administration helps to eliminate the hepatic and extrahepatic metabolism.

Conclusions

The most effective *in vitro* and *ex vivo* tissue permeation was achieved using the formulation of LAS as a nasal NEIG, which is a new drug delivery strategy. The LAS's bioavailability increased in comparison to the aqueous drug suspension (8% LAS) by more than two and a half times. The modified nasal NEIG formula contained 10% OA, 50% CE and TC combined in a (1:2) S-mix ratio.

0.5% carbopol was used as an *in situ* gelling polymer to gel the chosen NE formula. The main flaws of the commonly used orally-marketed pill might be fixed by using this mixture. In fact, it won't eliminate the necessity for future clinical testing of this innovative recipe, which might provide other crucial information to physicians.

Conflict of interest

The authors declare no conflict of interest.

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