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Original Article

AGOMELATINE ETHOSOMES FOR ENHANCED TRANSDERMAL DRUG DELIVERY

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ABSTRACT

Objective: The current study aimed to prepare and optimize Agomelatine (AMN) ethosomes for enhanced transdermal drug delivery.

Methods: In this study cold method was employed to manufacture the AMN-loaded ethosomes with dissimilar quantities of Phosphatidyl Choline (PC): Cholesterol: Ethanol. Transmission Electron Microscopy (TEM) was employed to evaluate the appearance of the formed ethosomes. Other formulation parameters like vesicle size and zeta potential, polydispersity index, transition temperature, and entrapment efficiency were also investigated.

Results: The microscopy results showed that AMN ethosomes have a smooth surface. It was discovered that the AMN-3 formulation of transdermal ethosomes had 92.15±1.3 entrapment efficiency with good vesicle diameter. The release of agomelatine adhered to the zero-order release model. The polydispersity Index (PI) and zeta potential of the optimized formulation were found to be 0.209 and-14.09±1.95 mV, respectively. The maximum flux for the ethosome formulation (AMN-3) was 34.29 µg. h/cm^2. A 10.71 fold increment was observed in the bioavailability of optimized formulation than control (oral suspension). A higher drug concentration in the blood suggested better systemic absorption of ethosomes. The optimized formula has a Tmax of 4.0±0.08h and 73.38±1.37 of Cmax. The AMN ethosomes were found to be more stable when stored at 4 °C.

Conclusion: The current study suggests that ethosomal vesicles may improve transdermal dispersion without causing skin irritation. Agomelatine-loaded ethosome has the potential to be one of the most important transdermal application techniques for the treatment of depression.

Keywords: Ethosomes, Agomelatine, Vesicle, Transdermal, Permeation, TEM, Vesicle

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INTRODUCTION

AMN was well known as a BCS II class drug where it possesses less water solubility [1]. Which had chosen antidepressant therapy gets rapidly absorbed (>75%) upon oral administration, but due to poor water solubility AMN resulting they declined the rate of absolute bioavailability (4%) [2]. In comparison to other antidepressants, this AMN exhibits good efficacy and tolerability; this drug was granted in 2009 for major depression therapy in Europe as the first antidepressant with no monoaminergic mechanism of drug [3]. The drug faces extensive first-pass metabolism, resulting in low bioavailability [4]. The study focuses on developing ethosomes of AMN to improve transdermal drug delivery.

In recent years, researchers have notified us that drug delivery via transdermal route has owed avid importance in the field of drug delivery. Stretching the drawbacks over the conventional dosage forms colloidal particulate carrier systems like ethosomes, liposomes, transfersomes, and niosomes have a good advantage [5-7]. The type drug carriers are also known as drug reservoirs. Ethosome offers versatile drug delivery for transdermal application upon hydration and agitation in hot water over a short period [8]. Ethosomes can adhere to occlusive circumstances after being applied and hydrated by skin moisture. This study aims to determine whether ethosomes may be applied to the field of transdermal medicine administration. "Ethanolic liposomes are ethosomes." Ethosomes are non-invasive delivery systems that enable drugs to deeply penetrate the layers of the skin or enter the systemic circulation. These are pliable, soft vesicles designed to improve the delivery of active agents. For a long time, the vesicles' significance in particle (drug-loaded vesicles) transportation and cellular communication has been well established. Additionally, by protecting the drug from immune responses and other removal mechanisms, vesicles would enable prolonged drug release rate control [9-12]. The discovery of a vesicle derivative known as an ethosome and the Trans Dermal Drug Delivery system (TDDS) is most feasible in terms of patient compliance, especially for patients who are in a coma and unsupportive for parenteral medication delivery.

MATERIALS AND METHODS

Materials

The Propylene Glycol (PG) was supplied by Merck (Mumbai); Lipoid, Germany, sponsored Soya Phosphatidyl Choline and Dr. Reddy's Laboratories in Hyderabad gave a sample of AMN. Every other chemical used in the investigation was analytical grade, including the solvents used in High-Performance Liquid Chromatography (HPLC). For the duration of the experiment, fresh double-distilled water was utilised.

Animals

Rats weighing between 200 and 300 gs, male albino twisters were used for the animal examination. The institutional animal ethics committee of Chaitanya Deemed to be University, Hyderabad, approved the study.

Method

Preparation of AMN-loaded ethosomes

Ethosomes were prepared by adopting the previously proposed cold method [13-15]. A simple and convenient process. Precisely, various quantities of Lecithin with the drug and other lipid materials mixtures solubilized in ethanol upon vigorous stirring and added propylene glycol in different ratios (20:1, 30:1, and 40:1) maintain the temperature at 40 °C under continuous stirring and formulation details mentioned in table 1 [16]. Then add the aqueous phase 7.4 pH Phosphate Buffer Solution (PBS) to the above mixture and stir well until a homogenous ethosomal mixture is formed kept the preparation vials at refrigerator temperature by sonication for 5 min [21].

As indicated in table 1, various ratios of PC were combined with an alcohol mixture (ethanol and PG). The AMN of (10 mg) is entrapped in every ethosomal formulation. In a tightly closed container, AMN was dissolved in the mixture while being stirred magnetically at 700 rpm and room temperature. A microinjection (160µl*) was used to add the required amount of water. After the water was added, magnetic stirring was maintained for an additional five minutes. Five ethosomal formulations (AMN1–AMN5) were obtained by subjecting the system to sonication for a minimum of ten minutes.

Table 1: Formulae of agomelatine-loaded ethosomes

Formulation code	AMN (mg)	PG (%)	Ethanol (%)	PC (%)
AMN1	10	0	20	1
AMN2	10	0.5	30	1.5
AMN3	10	1.0	40	2.0
AMN4	10	1.5	50	2.5
AMN5	10	2.0	60	3.0

PC, PG-indicates soy phosphatidylcholine and Propylene Glycol, respectively.

Characterization of AMN ethosomes

Fourier Transform Infrared Spectroscopy (FTIR) studies of rat abdominal skin

The optimized ethosomal formulation equivalent to 5 mg of agomelatine was treated on the prepared rat skin for 6 h. Later treatment, the skin sample was washed with water and blotted dry where untreated skin was used as control. The FTIR spectrum of the above-treated skin sample using an FTIR-multipurpose spectrophotometer (Shimadzu, Japan) against untreated normal rat skin, which served as the control, was recorded in the range of 4000-400 cm^{-1} [17].

Differential scanning calorimetry (DSC)

The thermal properties and phase transition behaviour of optimized ethosomes (AMNPL4), soy phosphatidylcholine, propylene glycol, and AMN were examined using a DSC (Mettler DSC 821e, Mettler-Toledo, Switzerland). An aluminum pan that was hermetically sealed was used to heat a 5±2 mg sample to a temperature range of 20 °C to 300 °C on average. Both a steady nitrogen gas flow of 30 ml/min and a heating rate of 10 °C/min were maintained.

Vesicle size and surface charge

The ethosomes were soaked in PBS (pH 7.4) for three minutes to hydrate them. After sonication, the resultant ethosomal dispersion was used to determine the vesicle size using the dynamic light scattering approach. After appropriate dilution of each sample, size analysis was carried out under 25 °C with a 90 °C detection angle. Characteristics were analysed by using an instrument Zeta sizer, nano ZS 90 (Malven instruments, Malven, UK) [16].

Entrapment efficiency % (% EE)

After centrifugation, the amount of unentrapped drug was subtracted from the total amount of drug injected to determine the entrapment efficiency (% EE) of the generated ethosomes. The drug's concentration in the aqueous phase was measured using HPLC. The following formula, shown below [17], was used to determine the proportion of AMN entrapment in ethosomes.

$$\% \text{ Drug entrapment } = \frac{\text{Total amount of drug added } - \text{ Unentrapped drug}}{\text{Total amount of drug added}} \times 100$$

Determination of spreadability

Precisely 0.5 g of ready-to-use ethosomes were positioned inside the designated 0.5 cm radius circle on the glass plate, and another glass plate with the same measurements was placed on top of the first one. Around 500g of weight was placed on the glass plate and allowed for 5 min. The weight was removed from the glass plate the increased gel diameter was noted as its spreadability.

Determination of rheological behavior

Using a controlled stress rheometer (Brookfield Programmable DVIII+Digital Rheometer, MA, USA) with cone (24 mm) and plate geometry, the rheological behavior of ethosomal formulations was investigated. Before measuring, the sample was given five minutes to acclimatize to a torque sweep between 10 and 110%. The Rheocalc 32 programming was used to determine the rheological properties after making multiple observations at room temperature. The Ostwald power equation was used to study the rheological properties of a thixotropic fluid.

$$\eta = KS\text{-}n \ldots\ldots (1)$$

After logarithmically transforming the equation, we obtain: $Ln\ \eta = ln - n\ ln\ S$ (2), where η is the apparent viscosity, K is the constant, S is the shear rate, and n is the thixotropic degree ascertained from the slope of the curve when ln η is plotted against ln S [17].

***In vitro* drug release**

A synthetic cellophane membrane was used in the *in vitro* release tests. The effective diffusion surface area was 4.59 cm^2, while the receptor cell volume was 17 ml. There was filling in the vertical Franz diffusion cells. Before the trial, the dialysis membrane was soaked, and the donor compartment was filled with the ethosome formulation or Control (AMN suspended in the same solvent as the formulations). PBS (pH 7.4) was added to the receptor compartment, which was continuously swirling and kept at 37±2 °C for the day. Parafilm was used to cover the sample ports and donor chamber to stop the diffusion medium from evaporating. The samples were regularly removed and replaced with an equivalent volume to maintain the receptor phase volume constant. After the study was over, the samples were appropriately diluted, and HPLC was used to measure the drug's concentration Release. The ethosomal formulations data mathematically fitted into first and second-order kinetics along with the Higuchi model to determine the drug release pattern [18].

HPLC assay of AMN

HPLC assembled (Shimadzu Corporation, Japan) equipped with an LC-10 AT VP Liquid Chromatograph pump, UV-detector, spinchrom software, and an injector (Rheodyne) with a 20 µl** capacity loop was applied for the analysis. An octadecylsilane (C-18) reverse-phase stainless steel analytical column (250 x 4.6 mm) with a 5 µm particle size (Lichrospher 100) was used for the chromatographic separation. The mobile phase consists of 0.02M potassium dihydrogen phosphate, pH 3.5, and 95:5 acetonitrile. The parameters were as follows: UV detection at 254 nm, ambient temperature separation, one milliliter per minute flow rate, and sensitivity of 0.005 AUFS [18].

***Ex vivo* permeation study**

Preparation of the skin

The study used male Wistar rats weighing 180–200 gm, that were purchased from Sainath Agencies 1656/PO/BT/S12/CPCSEA in Hyderabad, India. The rats were allowed to have food and water until they were murdered for their skins. The animals were housed in individual cages with temperature controls. The Institutional Animal Ethical Committee at Chaitanya, Deemed to be a University in Hyderabad, Telangana, India gave its prior approval before the study was carried out. PO/Re/S/17/CPCSEA; 0105/2024/1963). The carcass was disposed of and euthanized in compliance with the rules. An excessive amount of ether was inhaled to sacrifice the rats. The hair was gently cut out of the exposed flesh of the abdomen so as not to rip it. The rat's abdomen skin was divided and removed, and its adherent subcutaneous fat, tissue, and capillaries were extracted. The epidermis was prepared by involving the heat separation method where the entire abdomen was submerged in the water under 60 °C for 45 sec. This is immersing the entire abdominal skin for 45 sec in 60 °C water. The prepared epidermis was kept for two weeks before being cleaned, wrapped in aluminum foil, and stored at-200 °C to be used later [18].

Permeation study

Unjacketed vertical Franz diffusion cells with an effective diffusion surface area of 4.59 cm2 and a 17 ml receptor cell volume were used for the *in vitro* permeation investigations. The skin was placed

between the donor and receiver compartments of the franz diffusion cell, its stratum corneum side facing the donor compartment, as soon as it reached room temperature. The donor compartment was filled with limited dosages of the formulations and the control, which was a medication equivalent to 5 mg AMN suspended in the same solvent after the skin had equilibrated for 30 min. For an entire day, the PBS pH 7.4 receptor compartment was continuously stirred and maintained at 37±2 °C. To stop evaporation during the study, Para film was placed over the sampling port and donor chamber. At predefined intervals of time (0.25, 0.5, 1) [18].

Drug content retained in the skin

The skin was removed from the cell and manually cleaned to get rid of the formulation deposits after the ex vivo permeation investigation. After a brief 15 sec methanol wash, the material was allowed to air dry for 10 min. The skin was separated into sections, homogenized, and sonicated for 30 min using a bath sonicator (Sonica, Italy) following the addition of PBS. The centrifuged samples were filtered through a membrane filter with an effective pore size of 0.45µm, and the drug concentration was determined using HPLC.

Treatment of permeation data and statistical analysis

A graph was created by plotting the Cumulative Amount Penetrated (CAP) per unit area against time. The CAP vs. time plot's terminal linear segment slope was used to compute the steady-state flux (Jss). To calculate the permeability coefficient (Kp), divide the Jss by the drug's initial concentration in the donor compartment. Divide the Jss of the test formulation by the Jss of the control to get the enhancement ratio. A one-way Analysis of Variance (ANOVA) was performed on the permeation parameters to ascertain the statistical significance. Using Instant Graph pad prism software, the student-Newman-Keuls (compare all pairings) method was used to determine the significance of the variations between the formulations. At $p<0.05$, the difference was deemed statistically significant [18].

Skin irritation test

The male Wistar rats were subjected to the skin irritation test to look for any signs of reddening or irritation. The rats were kept in individual cages with the prepared ethosome formulations applied to their dorsal sides, and they were observed every day for a week [19, 20].

Stability analysis

To test the durability of the ethosome formulations, they were carefully packed in glass vials and kept in the refrigerator and at room temperature. The formulation was hydrated with PBS at predetermined intervals of 1, 3, 6, 9, and 12 mo, and any indication of phase separation or aggregation was checked under optical microscopy. The samples were assessed for drug entrapment as well as vesicle size [19, 20].

RESULTS AND DISCUSSION

Vesicles' size and zeta potential

The vesicle size and zeta potential of ethosomes were examined using a zeta sizer, and the results provided in table 2 indicate that the ethosomal formulations range in size from 326.11±0.8 to 623.20±0.3 nm. This was comparatively less than the ethosomes made by T. S. Saraswathi *et al.*, which had a vesicle size of 706.1±0.94 to 1433.6±0.54 nm [21]. The vesicles' size rose linearly with the amount of propylene glycol in the formulations. When propylene glycol (AMN-2) was added to the ethosomes formulation, the zeta potential increased significantly to-8.81±1.2 mV from-4.26±2.06 mV. Further raising the PC and PG concentration, table 2's zeta potential dramatically rose (-14.09±1.95). However, even with a further increase in PG or PC concentration, we were unable to observe a discernible change in ZP.

PI

A population is homogenous if its polydispersity index (PI) value is less than 0.1, and more heterogeneous if it is more than 0.3. The PI of the optimized formulation was 0.209, which was shorter than that of the ethosomes made by Bora *et al.*, which had a PDI of 0.493±0.021 [22]. Every formulation's PI falls within permitted limits.

Entrapment efficiency

The entrapment efficiency was discovered to vary between 82% and 94% It was found that the formulation without propylene glycol (AMN-1) had an entrapment efficiency of 78.6±2.6%. Propylene glycol has been found to significantly increase the entrapment efficiency of AMN-2 (88.84±2.6) and AMN-3 (92.15±1.3) this was comparatively higher than the ethosomes made by Mustofa *et al.*, which had an entrapment efficiency 0.71±0.11% [23]. All the formulation's entrapment efficiency was depicted in table 2. Our findings are consistent with the theory presented in the literature reports [EL-Samaligy MS]. The reason for the increased entrapment efficiency is the bilayer formed in the presence of PC and PG, which has increased hydrophobicity and stability, which in turn reduces drug leakage. Conversely, in both forms of formulations, the entrapment efficiency decreased as PC and PG concentrations increased. This could be because of the drug's strong competition for embedding in the bilayer, which is PC. When ethanol was added to formulations AMN-1 to AMN-3, the drug entrapment efficiency increased. This is because the addition of ethanol causes the lipid bilayer to fluidize excessively, and formulations containing more than 40% ethanol leach the drug (AMN-4 and AMN-5). The fluidization of the bilayer caused a disturbance of its regular linear structure, which hindered the accommodation of the medication. PG also demonstrated a similar effect in the formulations AMN-4 and AMN-5.

Table 2: Size, zeta potential, %EE, thixotropic degree, and spreadability of agomelatine ethosomal formulations

Formulation code	Size (nm)	Zeta potential (mV)	PI	EE (%)	n	Spreadability (cm)
AMN-1	326.11±0.8	-4.26±2.06	0.198	78.6±2.6	0.957	5.92±0.16
AMN-2	419.26±0.4	-6.81±1.21	0.205	88.84±2.6[1]	0.962	7.02±0.52
AMN-3	429.13±0.3	-14.09±1.95[1,2]	0.209	92.15±1.3[1,2,4][,5]	0.969	7.26±0.68
AMN-4	489.18±0.4	-16.04±2.06[1,2]	0.211	83.41±2.9	0.971	6.23±0.33
AMN-5	623.20±0.3	-17.32±2.14[1,2]	0.223	80.2±3.1	0.981	6.06±0.22

n and PI indicates thixotropic degree, Poly Dispersity Index, respectively.[1,2,3]·[4,5]represents control, mAMN-1, AMN-2, AMN3, AMN4, and AMN5 respectively. Data is expressed as Mean±SD, n=6.

Morphology of agomelatine

The TEM indicates the morphology of vesicles, and the TEM images for AMN3 were found to be spherical with a uniform surface. The vesicles were spherical with very flawless borders. The uniform surface and shape of vesicles have proved that there won't be any drug leakage happening from the formulations.

FTIR analysis

The bands at 2919 and 2851 cm^{-1} present in the functional group region of FTIR spectra can be attributed to the asymmetric CH_2 and symmetric CH_2 vibrations of long-chain hydrocarbons of lipids. The peak height and area are proportional to the amount of lipids present gets reduced if any extraction of lipids from stratum corneum results. The reduction in peak area suggests that the fluidization of the stratum corneum due to the fusion of the vesicle bilayer with the skin membrane.

DSC thermogram

The agomelatine and cholesterol have shown sharp endothermic peaks at 109.70 °C and 148.42 °C, respectively. The DSC thermogram clearly shows the disappearance of the sharp endothermic peak of the cholesterol is due to the change in phase transition behavior of

the pure lipids to liquid crystalline state in the ethosomal formulation shown in fig. 3.

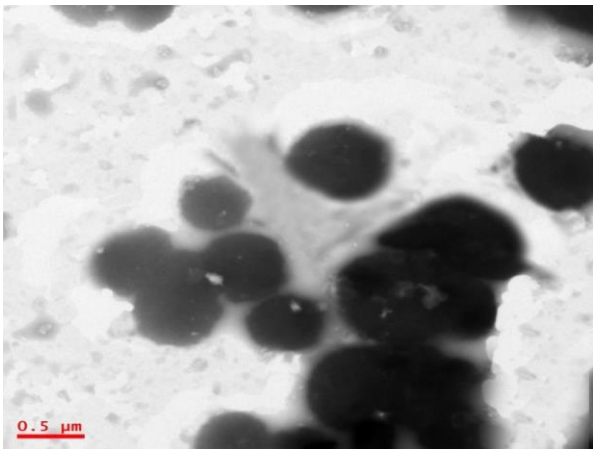

Fig. 1: Morphology of agomelatine ethosomal vesicles (x 500)

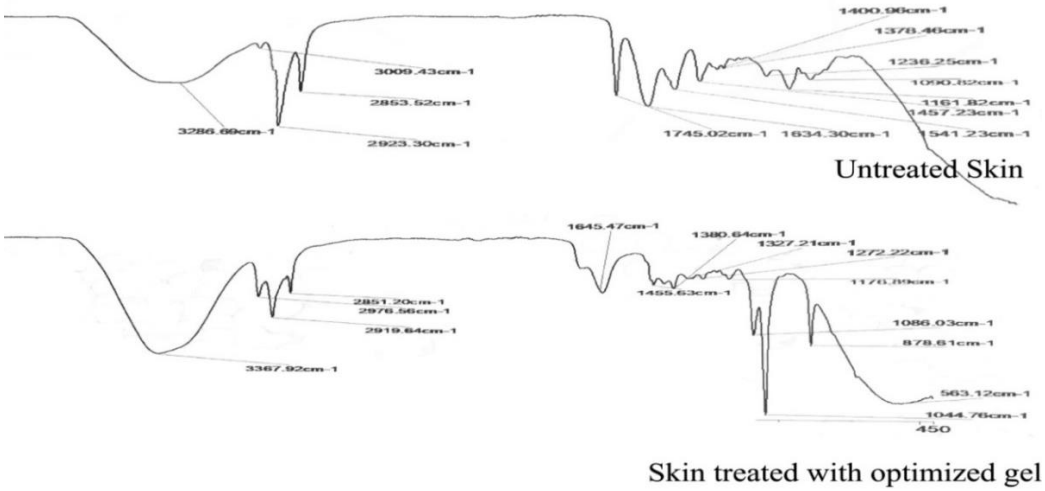

Fig. 2: FTIR spectra of A) Untreated rat skin (Control) B) Rat skin treated with optimized AMN containing ethosomal formulation (AMN-3)

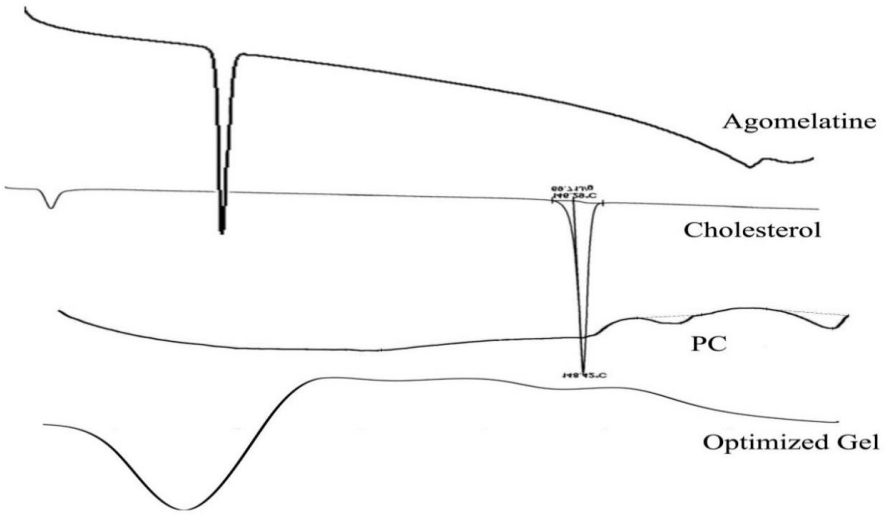

Fig. 3: DSC thermograms of A) Agomelatine, B) Cholesterol, C) PC, D) optimized ethosomal formulation (AMN-3)

Spreadability and rheological behavior

One of the most important factors to consider for excellent patient compliance is spreadability, which increases the gel's absorptivity and penetration by increasing the contact area. All

ethosomal formulations were found to have spreadability between 5.9 and 7.26 cm. A significant reduction was observed in the spreadability of the ethosome systems with higher Propylene glycol content. All the formulations exhibited good skin feel and spreadability (fig. 4).

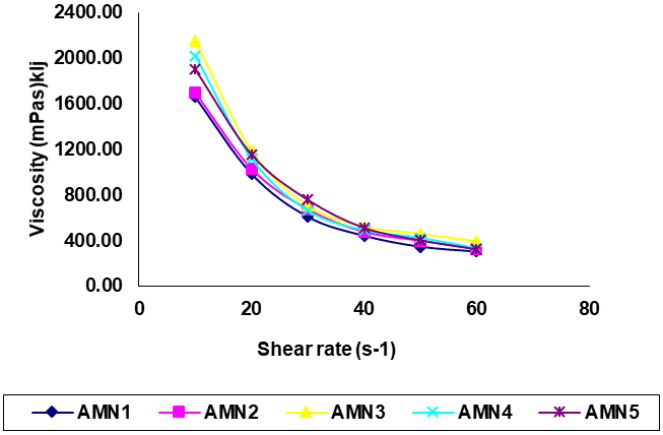

Fig. 4: Rheological behavior of agomelatine ethosomal formulations, data is given as mean of 6 replicates

The ethosome formulations' viscosity, which is reduced with an increase in shear rate, and rheological behavior determine the percutaneous application. The findings show that the viscosity of the formulations is influenced by the amount of propylene glycol, which is consistent with previous publications. We could see a linear relationship between the formulation's viscosity increase with the amount of propylene glycol. Furthermore, as the accompanying table 1 illustrates, the thixotropic degree (n value), which was determined using the rheological data, appeared to be influenced by the amount of propylene glycol present. It was shown to decrease as the concentration of propylene glycol increased.

In vitro drug release

It was investigated to comprehend AMN's *in vitro* release behaviour from ethosomes. The percentage of drug released from various formulations is shown in fig. 5. Within 24 h, the release of AMN from the control group was almost 75%, indicating that the drug's sink condition was prevalent and the membrane was permeable. When using ethosome formulations, a standard biphasic release pattern was seen. Fig. 5 illustrates that the release occurred in two stages: first, in a rapid burst and then continuously for a full day. One possible explanation for the observed rapid drug release and faster release of the unentrapped drug from the ethosomal formulations is the presence of a significant concentration gradient at the early time points. Nevertheless, further increases in propylene glycol content led to a rise in. According to the data, drug release from ethosomal formulations is regulated by diffusion, which is in line with table 3's zero-order kinetics results. The *in vitro* drug release of the optimized formulation was 60.89% which was shorter than that of the ethosomes made by N. Nerella *et al.*, which had an *in vitro* drug release of 89.26 [24].

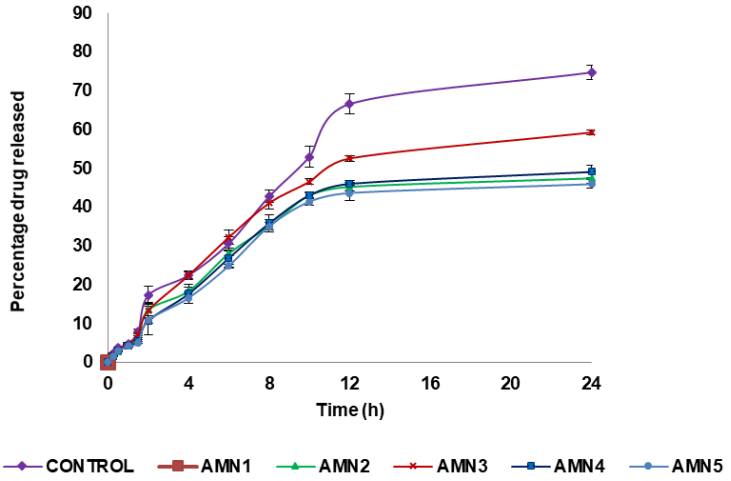

Fig. 5: Agomelatine release profile *in vitro* across the cellophane membrane from different ethosomal formulations. Data is given as mean±SD of 6 replicates

Table 3: I*n vitro* release kinetics of agomelatine from ethosomes across cellophane membrane

Formulation code	Zero-order		First order		Higuchi
	K_0 (h^{-1})	R^2	K_0 (h^{-1})	R^2	R^2
Control	0.431	0.846	0.452	0.589	0.794
AMN-1	0.229	0.894	0.691	0.735	0.810
AMN-2	0.201	0.923	0.564	0.627	0.904
AMN-3	0.251	0.967	0.698	0.644	0.918

AMN-4	0.226	0.931	0.552	0.611	0.873
AMN-5	0.184	0.914	0.529	0.612	0.824

K_0 and K represent Zero and first-order release rate constants of respective formulations.

***Ex vivo* permeation study**

Rats' excised abdomen skin was used for *ex vivo* permeation assays to assess the viability of transdermal administration of AMN ethosomes. The total amount of medication that has seeped is depicted in fig. 6.

The entire amount of medication that entered the rat's abdomen skin during the ex-vivo permeation study is shown in fig. 6. AMN does not considerably penetrate until the drug is liberated from the ethosomes that form once the ethosomes are moistened with the skin fluids. There was no lag time seen in any of the formulations, which amply demonstrates the ethosomes formation process. Furthermore, the drug was found within 0.25 h, which states how quickly the drug was released from the skin membrane and how water diffused from the receptor fluid. The formulation without propylene glycol had much less penetration, which could be the result of poor drug entrapment, less stability, and improper vesicle formation. When propylene glycol was added to the formulation, there was a noticeable increase in permeation. However, following an increase in propylene glycol content, there was a decrease in penetration. The vesicle's high hydrophobicity may have contributed to this by reducing the stimulation of AMN partitioning into the skin bilayers. The maximum flux for the ethosome formulation (AMN-3) was 34.29 µg. h/cm^2, which was shorter than that of the ethosomes made by Ashok *et al.*, which had a maximum flux of 19.00±2.22 µg. h. cm^2 [25],

Whereas the control formulation's maximum flux was 15.05 µg. h/cm2. The regulated delivery of AMN from formulations is indicated by the flow, which was larger for the ethosomal formulations at all-time points compared to the control and no propylene glycol (fig. 6). A table was created and showed the permeation enhancement as determined by the permeation parameters (flux, permeability coefficient, and enhancement ratio). The steady-state flux and permeability coefficient significantly increased for the ethosomes gel formulation (AMN-2). Improved permeation is indicated by an enhancement ratio that is well above 1, and our results show that all ethosomal formulations have an ER higher than 1 when compared to the control. A statistically significant improvement has been made (table 4). The formulations are based on the permeation parameters Ethosomes: AMN-3>AMN-2>AMN-4>AMN-1>AMN-5>Control. It's interesting to note that the quantity of medication released through the cellophane membrane was much less than that which permeated through the skin, indicating the skin's barrier qualities. This implies that direct vesicle-skin contact and interaction are significant contributing factors to enhanced AMN transdermal delivery. The reports and our results have a strong correlation (Fang *et al.*, 2001). Overall, the data clearly show that AMN permeation has significantly improved over ethosome formulations. Numerous mechanisms have been proposed in the literature to account for the factors influencing the enhanced drug permeation from ethosomes. It is commonly known that propylene glycol and phospholipid surfactants interact to change the intercellular lipids in the stratum corneum.

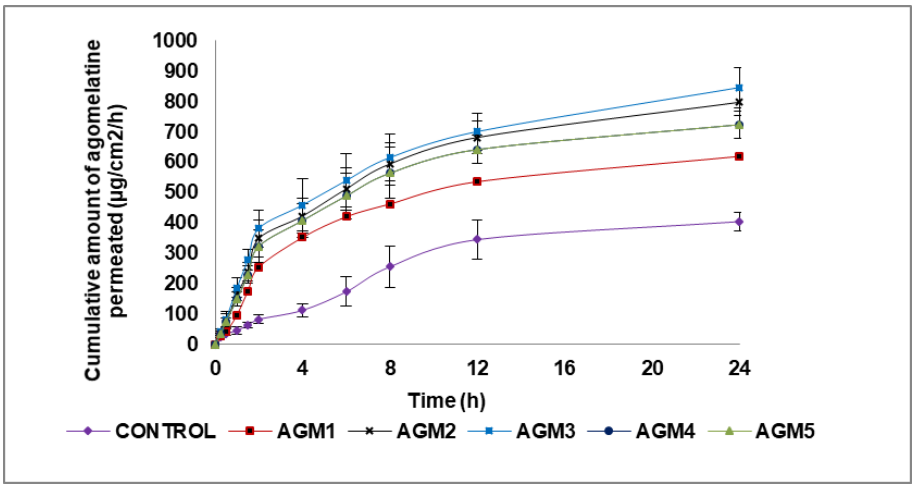

Fig. 6: Total Agomelatine that penetrated the rat skin from different ethosome formulations Cumulative amount permeated per square. Centimeter per hour, data is given as mean±SD of 3 replicates

Table 4: Permeation parameters of AMN from different ethosomal formulations across rat skin

Formulation code	Q_{24} (µg)	Jss (µg/cm^2/h)	Kp (cm/h) x10^3	ER	DCS
Control	1636.86±24.10	15.05±0.96	2.74	-	528.94±25.35
AMN-1	2500.14±46.35^1	22.90±2.50^1	2.96	1.81	1287.90±9.69
AMN-2	3276.88±57.15^{1,2,3,5}	29.32±1.01^{1,2,3}	3.21	1.96	1057.20±6.33
AMN-3	3859.31±87.05^{1,2,3,4,5}	34.29±1.37^{1,2,3,4,5}	3.86	2.13	841.35±25.64
AMN-4	2918.19±24.23^{1,2}	25.72±0.59^1	3.01	1.92	1220.65±10.51
AMN-5	2330.45±42.66^1	29.61±2.33^{1,2,3}	3.20	1.96	926.83±24.10

Data is given as mean±SD of 6 replicates. ^{1,2,3,4,5}Represents control, mAMN-1, AMN-2, AMN3, AMN4, and AMN5 respectively.

Q24, Jss, Kp, ER, DCS, and steady-state flux, respectively ($p<0.05$), represent the number permeated in 24 h, permeability coefficient, enhancement ratio, and drug content retained in the skin layers. The control, AMN-1, AMN-2, AMN-3, AMN-4, and AMN-5 are represented by DCS-Drug Content Retained in the Skin 1, 2, 3, and 5, respectively.

Drug content retained in the skin layers and skin irritation study

Fig. 7 shows the drug content in the skin layers after rat skin was treated with all of the ethosomes formulations and drug solutions. Given that all

of the ethosomes formulations increased penetration, it follows that the drug's concentration in the epidermal layers rose as well. This makes sense because the higher drug deposit in the skin values could have resulted from the skin layers being saturated at the experiment's termination point, or 24 h in this case. The increased AMN deposition in skin layers with ethosomes as compared to control suggests that these formulations may be able to better distribute the drug into the skin's viable regions while circumventing the stratum corneum's barrier function. The drug content of the optimized formulation was 1057.20 µg which was higher than that of the ethosomes made by Jukanti *et al.*, which had a drug content of 357 µg [26]. After the application of optimized Ethosomes formulations (AMN-3) in three rats, could not notice any marked erythema during seven days*($p<0.05$), (n=3, mean±SD).

Pharmacokinetic studies

The plasma concentration Vs time profile following oral administration and transdermal application of ethosomal formulation is shown in fig. 8. A 10.71fold increment was observed in the bioavailability of optimized formulation than control (oral suspension). Which was higher than that of the formulation made by Ashok Mateti *et al.*, which had a 3.07-fold increase in bioavailability [27]. The *ex vivo* and *in vivo* correlation was done between the cumulative amount permeated (µg) and AUC (µg h ml^{-1}). The higher regression coefficient with an R^2value of 0.9904 indicates point to point-to-point correlation following level a correlation.

Stability studies

For 12 mo, the ethosomal formulations' physical characteristics, vesicle size, and drug leakage were observed. The microscopic investigations reveal that the vesicles derived from the ethosome were multilamellar, and there was no significant alteration in the morphological behavior that we could witness. The size and percentage of AMN retained in the vesicles of the ethosomes that developed following hydration were assessed. When the medication for the AMN-2 formulation was kept in a refrigerator, we were unable to observe any discernible changes in the drug's size or percent retention (fig. 9 and 10). However, there has been a discernible increase in size and medication leakage ($p<0.05$) after 120 d. Conversely, as shown in fig. 11 and 12, the size grew and the percentage of drug retention dropped when the preparations were stored at room temperature. The observed data clearly stated that there was an influence of temperature on finished ethsosomal formulations. Comparatively, when kept at ambient temperature and in the refrigerator, the formulations without propylene glycol (AMN-1) were more stable in terms of vesicle size ($p>0.05$). On the other hand, a decreased retention % demonstrated the importance of vesicle composition. Formulations stored at 4 °C in a refrigerator generally showed more stability than formulations kept at ambient temperature.

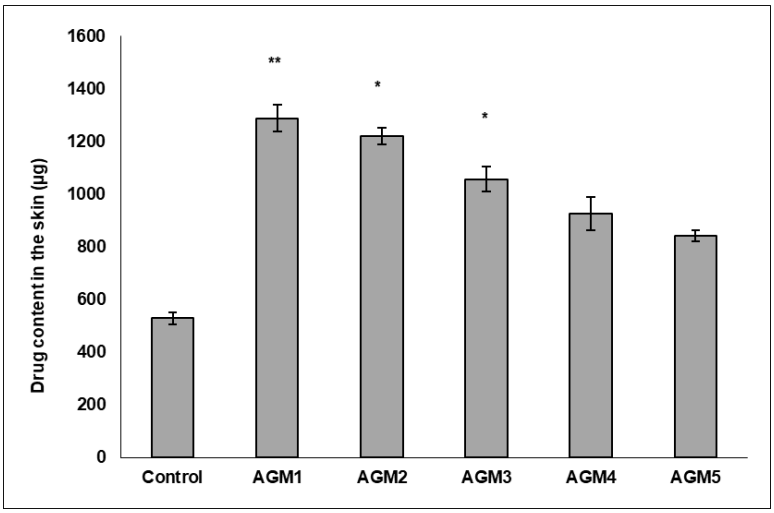

Fig. 7: Medication applied to the skin layers 24 h after treatment with various ethosome formulations, data is given as mean±SD of 3 replicates

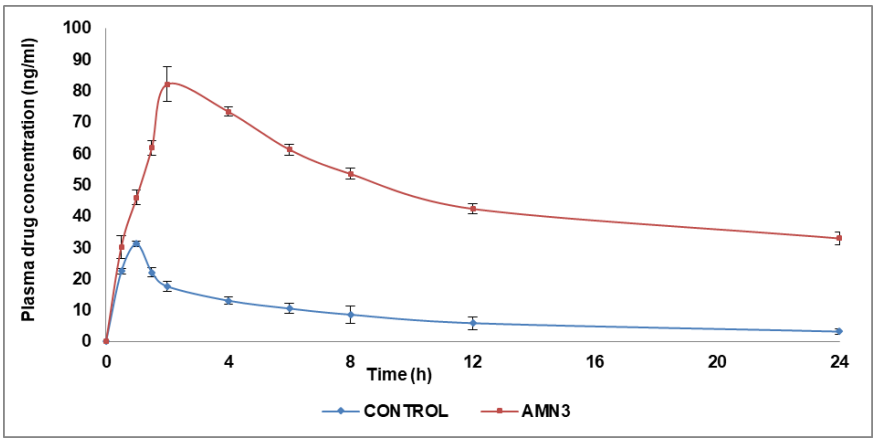

Fig. 8: AMN3's optimized ethosomal formulation was used in the treatment, and the control group was administered after the AMN's mean plasma concentration versus time profile, data is given as mean±SD of 3 replicates

Table 5: Pharmacokinetic parameters of agomelatine in rats following administration of optimized ethosome (AMN 3) control (oral suspension)

F. code	C_{max} (µg/ml)	T_{max}(h)	$AUC_{(0-t)}$ µg/h/ml	$AUC_{(t-i)}$ µg/h/ml	$AUC_{(0-i)}$ µg/h/ml	K (h)	F
Control	31.19±0.89	1.00±0.00	199.92±35.52	100.45±26.35	300.37±59.96	0.0324	
AMN-3	73.38±1.37	4.00±0.00	1138.41±22.22	2044.89±302.22	3183.31±307.69	0.016	10.71

Data is given as mean±SD of 6 replicates.

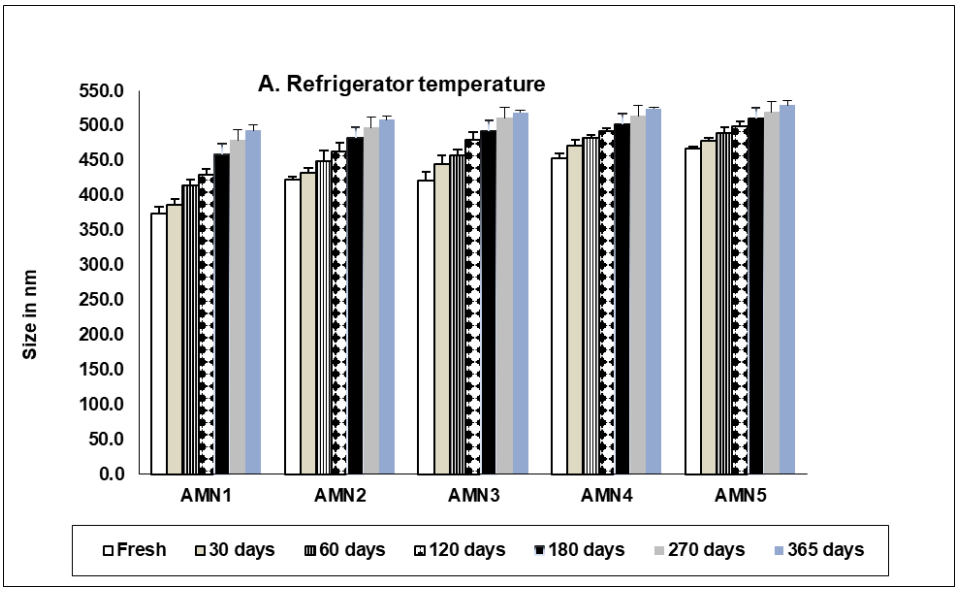

Fig. 9: Particle size changes in formulations containing ethosomes when stored at refrigerator temperature, data is given as mean±SD of 6 replicates

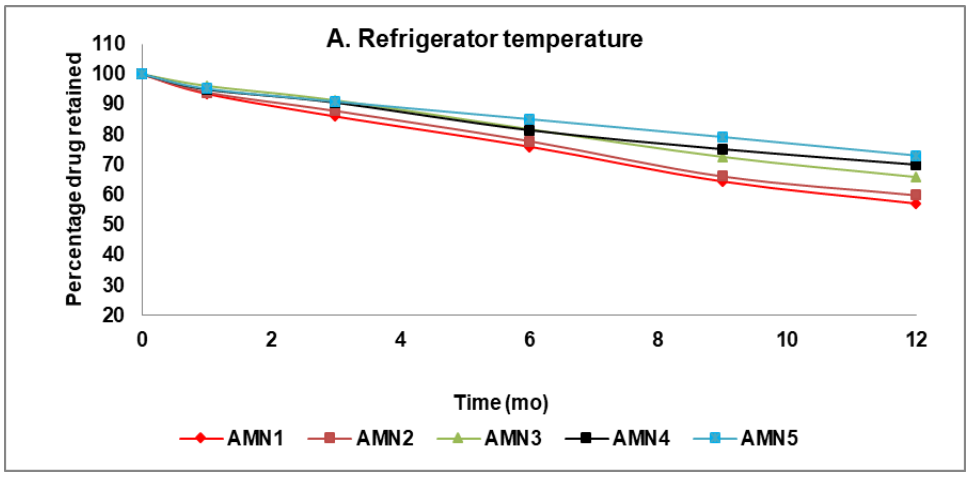

Fig. 10: Percentage of AMN retained in ethosomal formulations after refrigerator storage, data is given as mean of 6 replicates

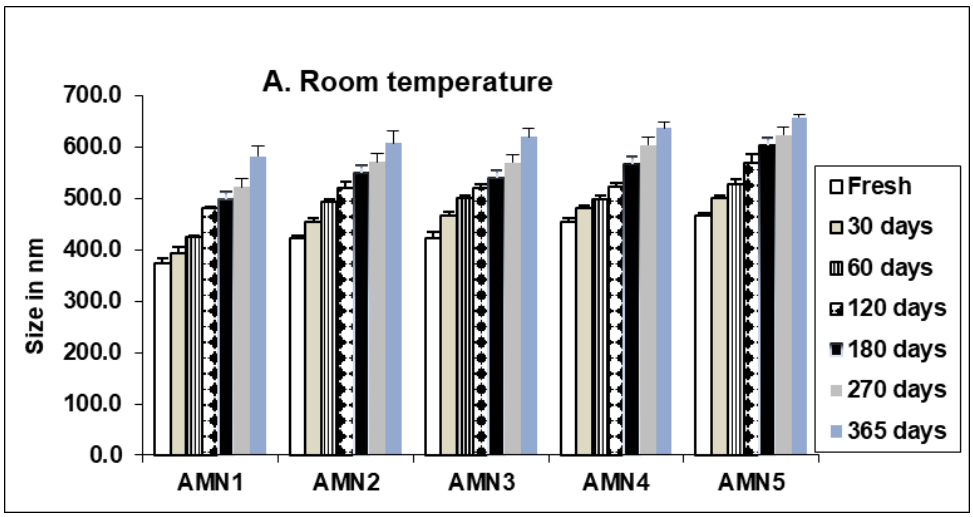

Fig. 11: Modification of particle size formulations containing ethosomes in AMN are stored at room temperature, data is given as mean±SD of 6 replicates

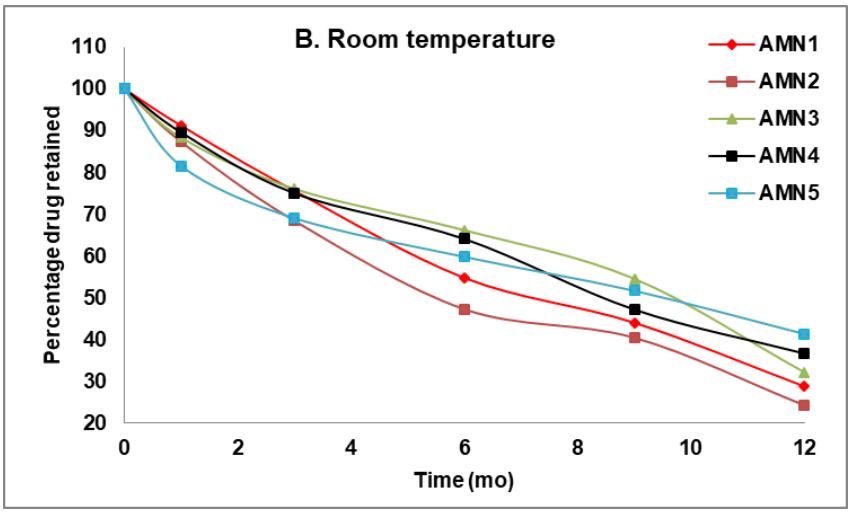

Fig. 12: Percentage retention of AMN in Ethosome formulations upon storage at room temperature, data is given as mean of 6 replicates

CONCLUSION

AMN-loaded ethosomes were developed successfully. The rheological study findings indicate a higher thixotropic degree towards ethosome formulations with good flow patterns. The findings of the permeation study revealed the benchmarking potency of ethosomes in transporting AMN across the skin in a controlled pattern by following zero-order kinetics. The results indicate that AMN-3 (40% ethanol, 1.0% Propylene glycol, and 2.0% Phosphatidylcholine) reasoning in the stability of vesicle size (optimum), entrapment efficiency, thixotropic degree, and enhanced permeation kinetics. Ethosome (AMN-3) was selected as an optimized formulation based on observations. However, further *In vivo* studies are our future prospective to know how potential for transdermal delivery of AMN ethosome.

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Nil

AUTHORS CONTRIBUTIONS

The authors report that this publication is based on the Ph. D. thesis (Rajitha Nallagandla), who conducted the preliminary research, collected the data, carried out the work, and produced the entire manuscript. (Kothapally Daniel) was the supervisor, and he revised the text and validated the data for this study.

CONFLICTS OF INTERESTS

All authors have none to declare

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