FORMULATION OF *VITEX NEGUNDO* ETHOSOMAL LOADED TOPICAL HERBAL GEL AND ITS EVALUATION FOR ARTHRITIS TREATMENT

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ABSTRACT

The current research aims to develop and test a system of vesicular drug carriers for topical drug administration of *Vitex negundo* Linn. to provide sustained drug delivery. The ethosomes of *V. negundo* Linn. were produced using thin-film hydration, and their *in vitro* drug release profiles, size, drug content, and other characteristics were examined. To attain the desired results, for drug release and entrapment effectiveness, the composition of lecithin and ethanol was changed to form ethosomes. The ethosomal size of the vesicle of the optimized formulation batch was measured to be the units 13.47 nm with -3.97 mV as zeta potential. The percent drug release of ethosomal gel was 83.24 %, and the percent entrapment efficiency was 89.40 %. The formation of spherically shaped vesicles was confirmed by optical and scanning electron microscopy observations. It was observed that as the ethanol concentration increased, the formulation's *in vitro* profile for drug release increase, and lipid concentration decreases.

Keywords: *Vitex negundo*, ethosomes, sustained drug delivery, thin film hydration

INTRODUCTION

Within the category of auto immune disorders, arthritis affects 0.51 % of the world's population. NSAIDs, disease-modifying anti-rheumatics, along with immunosuppressive medications, are among the treatments for rheumatoid arthritis that are often utilized that have been linked with gastrointestinal problems, immunodeficiency and humoral disturbances. As an alternative approach to arthritic treatment, siddha along with Ayurvedic treatment are gaining popularity. Two major plants employed in the system of traditional medicine to treat arthritis are *Vitex negundo* Linn. and *Cardiospermum halicacabum*. A huge fragrant shrub of the Verbenaceae family is also known as the *'five-leaf chaste tree'* or '*Nirgundi*', '*V. negundo'* or *'Sarvaroganivarani'* traditional Indian medicine and its anti-inflammatory, expectorant, tranquilizing, antispasmodic, rejuvenating, anti-arthritic, anthelmintic, antifungal and antipyretic properties result in reduced gastrointestinal side effects, improved patient compliance, bioavailability, and solubility. In this report, an herbal gel loaded with ethosomes of *Nirgundi* was formulated and characterized[1].

On wastelands, this shrub grows. It is cultivated as a hedge plant. The flavonoids in this plant include catechin, isoorientin, luteolin, lutecin-7-O-glucoside and terpenoids. *V. negundo* Linn. has been shown to have anti-arthritic, anti- inflammatory, anti- pyretic, anticonvulsant, hepatoprotective, bronchial relaxant tonics, vermifuges and lactagogues properties[1].

ETHOSOMES

Ethosomes are known to contain ethanolic phospholipids which act as a carrier like vesicles that are flexible, soft, and carry medications to the systemic circulation or deep skin layers. The transdermal pathway is widely used by ethosomes to deliver drugs. Several physicochemical characteristics, such as amphophilicity, lipophilicity and hydrophilicity, can be used to entrap drugs in ethosomes. Phospholipids, a lot of water and alcohol make up the ethosomal system. The presence of ethanol distinguishes ethosomes. A vesicle's capacity to enter the stratum corneum is improved when ethanol is integrated into the membrane[2].

Classic mechanical dispersion of the powder was carried out with soy lecithin 200-800 mg and ethanol was used to make the *Nirgudi* ethosomal formulations 10 to 40 mL. The drug concentration was calculated as 50 mg. Accurately weighed phospholipid was dissolved along with methanol and propylene glycol in the ratio of 3:1 to generate a thin lipid film in the rotary

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Table I: Formulation for ethosomal gel

Formulation	Soya lecithin (%w/V)	Ethanol (%V/V)	Propylene glycol (%V/V)	Drug (%w/w)	Cholesterol (%w/w)
F1	1	25	12	1.3	1.8
F2	2	35	14	1.3	2.5
F3	3	45	18	1.3	3.8

evaporator at a speed of 60 rpm and lipid transition temperature 55 ^{0}C. Following the formation of the thin film, traces of the organic solvent combination were removed by maintaining the temperature at a reduced pressure for another 30 minutes. The film on lipids was then hydrated in the rotary evaporator at 60 rpm for 1h at room temperature with a particular concentration of hydro ethanol mixture containing *Nirgudi* extract. The preparation was vortexed in the bowl followed by cold sonication at a temperature of 4 ^{0}C. The sonication was carried out at 40 Watt for 3-5 min. After sonication, the ethosomal formulation was kept in the refrigerator at 4-5 ^{0}C for further investigation process[3].

MATERIALS AND METHODS

Materials

V. negundo L. powder was purchased from Bagwan Ayurvedic Medical, Karad, Satara, Maharashtra. Ethanol (AR grade), methanol (AR grade), triethanolamine (AR grade), soya lecithin (AR grade) and cholesterol (AR grade) were used and provided by Loba Chemicals Pvt. Ltd., Mumbai, India.

Methods

Preformulation research

To determine, characterize, and validate the percent purity of the medication as well as to investigate the adaptibility of drug and excipients these analyses were performed. The drug's organoleptic characteristics were assessed. The capillary method was used to establish the drug's melting point and its maximum rate of absorption using methanol as the solvent. FTIR was done to observe changes in physical and chemical composition of ethosomal gel and DSC test was performed to examine the endothermic and exothermic responses of the drug. With the FTIR-4600 (Jasco Corporation, Japan) spectrophotometer, FTIR scans were produced, spanning the wave number ranging from 4000 - 400 cm^{-1}. Drug and physical mixtures (drug+cholesterol+lipid) were studied using DSC for both the pure drug and in a 0.8:1.2 ratio. The samples were provided with heating at a constant rate of 12 ^{0}C min^{-1} to produce thermograms. For all runs, a dry nitrogen gas purge was used[4,5].

Microwave assisted extraction (MAE)

Microwave assisted extraction was carried out at 340 Watt for 12 minutes by taking ethanol and *V. negundo* powder in the ratio of 9:1. Microwave assisted extraction was carried out at several extraction times such as 1, 5, 10, 20, and 30 minutes. Following a filtering process, the mixture was evaporated to leave a residue, which was then measured[6].

Formulation of *V. negundo* Linn loaded ethosomes by using thin film hydration

The technique used to create the ethosomal compositions was thin film hydration. Cholesterol, soya lecithin and *V. negundo* Linn extract was dissolved in the mixture of propylene glycol and ethanol in the ratio of 3:1. For the lipid transition, a rotary evaporator was used to evaporate the organic solvent at appropriate pressure and temperature, as shown in Fig.1. On the flask's inner wall, a uniform thin layer was produced. After that, the drug-lipid film was rehydrated using purified water and the proper amount of ethanol. The formulation was kept in cold for storage. By adjusting the dosage and soya lecithin concentration, three formulations were created, as shown in Table I[7].

Fig. 1: Rotary evaporator

Ethosomal gel preparation

As a vehicle, for *V. negundo* Linn ethosomes preparation, 1 % w/w Carbopol 940 was used. To create a homogenous ethosomal gel, the hydrated solution of

Carbopol progressively integrated and the ethosomal formulation was stirred continuously. Triethanolamine was used to bring the gel's pH back to neutral, and it was carefully stirred and ethosomal gel was obtained as shown in Fig. 2.

Fig. 2: *V. negundo* ethosomal gel

EVALUATION OF ETHOSOMES

Optical microscopy

An electron microscope was used to perform optical microscopy of the developed ethosomes. On the glass slide, a very small volume of the ethosomal sample solution was dispersed. This slide was centered, and the images were taken under different magnification lenses. The optical image is shown in Fig. 3.

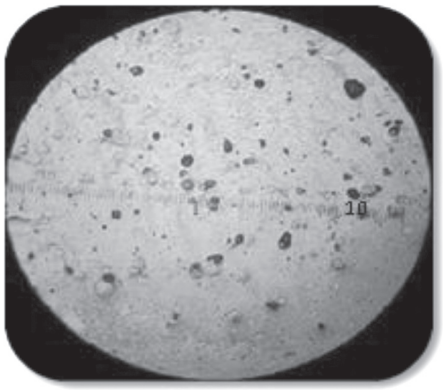

Fig. 3: Optical microscopy

Size of vesicle and zeta (ζ) potential

DLS i.e. Dynamic light scattering can be utilized to estimate vesicle's particle size. A computerized inspection system can also be used to assess the ethosomal vesicle's zeta potential. Vesicular features like stability and skin-vesicle interactions can also be affected by the ethosomal vesicle's charge. Using zeta sizer, manufactured by Malvern Instruments Ltd., the three produced formulations were evaluated for particle size. Double distilled water was used as the medium of dispersion during the analysis, which was conducted at temperature of 25 $^0C^8$. The graphical representation of size distribution and zeta potential is shown in Fig. 4 and Fig. 5, respectively.

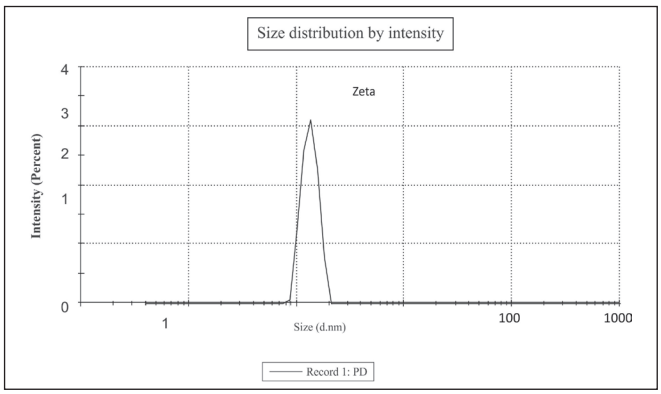

Fig. 4: Particle size

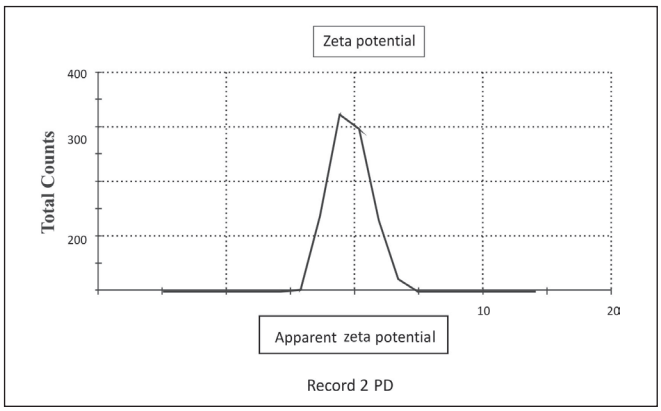

Fig. 5: Zeta potential

Drug content

The three developed formulations' drug content was evaluated. Using a UV spectrophotometer, the content of drug present in the ethosomal formulations was identified. The formula given below was used to find out the content of drug:

$$\text{Drug content} = \frac{[\text{Drug content (practical)}}{\text{drug content (theoretical)}]} \times 100$$

Entrapment efficiency

Entrapment effectiveness was assessed using the microcentrifugation method. For 15 minutes, the ethosomal sample was kept for centrifugation at 1400 rpm. The supernatant was separated and collected after

centrifugation, and the concentration of medication in each was assessed with the help of UV spectrophotometer instrument. It was calculated with the help of given equation and the data for entrapment efficiency is given in Table II.

$$\text{Entrapment efficiency of a drug} = \frac{(T-C)}{T} \times 100$$

where;

T stands for the total final amount of medication found in both the resident and supernatant layers, and

C is the maximum drug concentration that can be found in the supernatant layer.

Table II: Entrapment efficiency of the drug

Formulation	Entrapment efficiency (%)	Vesicle size (nm)
F1	79.35	434.64
F2	89.40	365.56
F3	84.14	414.23

Differential scanning colorimetry (DSC)

The vesicular lipid system's transition temperature is determined using differential scanning calorimetry (DSC). Several phase transitions that occur in lipid bilayers are being researched for their potential applications in triggering medication release. Lipid bilayers can exist in two different phases: a solid ordered phase at low temperatures and a fluid disordered phase at a particular temperature. By choosing the right lipids, it is possible to control the temperature of this phase transition. By the application of DSC, the melting endotherm observed is 102.10 ^{0}C as indicated in Fig. 6 [9].

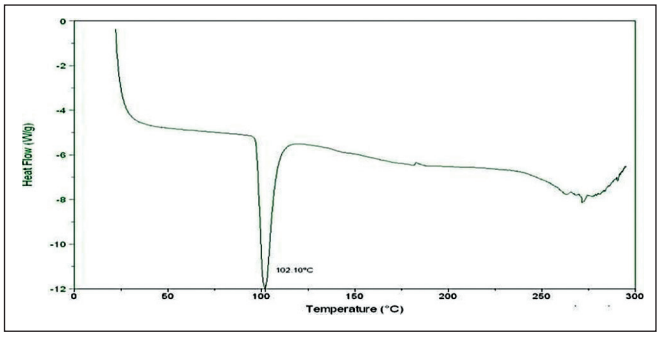

Fig. 6: DSC for ethosomes

In vitro drug penetration analysis by using rat skin

The Franz diffusion cell was employed for all *in vitro* diffusion experiments. The diffusion cell apparatus was created locally as a 3.7994 cm^2 open-ended cylindrical tube with a height of 100 mm, a 3.8 cm^2 diffusion area, and a height of 3.7994 cm^2. Phthalate buffer served as the receptor media (pH 7.4). Rat abdomen skin served as the dialysis membrane's material.

The donor cell (diffusion cell) was connected to the skin in such a way that the stratum corneum side is exposed with the release surface from donor compartment. The phosphate buffer solution measuring 100 mL (isotonic with a pH of 7.4) was added before attaching the donor compartment to the diffusion cell. 1 g of formulation was weighed and put to the rat's skin before being barely submerged in 100 mL of constantly stirring receptor media. A constant temperature of 371 ^{0}C was maintained throughout the entire system. A 5 mL sample was obtained at predetermined intervals for upto 8 h, and the wavelength was spectrophotometrically determined to be 275 nm. The diffusion medium was replaced after each withdrawal with a fresh batch of the same volume. The data of % cumulative drug release at 8h is indicated in Table III and its graph obtained is shown in Fig. 7.

Table III: % Cumulative drug release at 8h

Formulation	% Cumulative drug release at 8h
F1	66.12
F2	83.24
F3	62.14

For each time interval, the cumulative percentage release was determined (in hours)[10]. The Rajarambapu College of Pharmacy, Kasegaon based Institutional Animal Ethics Committee, Maharashtra, registered with CPCSEA, India (Reg. No. 179/GO/Re/S/2000/CPCSEA), approved the study on February 22, 2023.

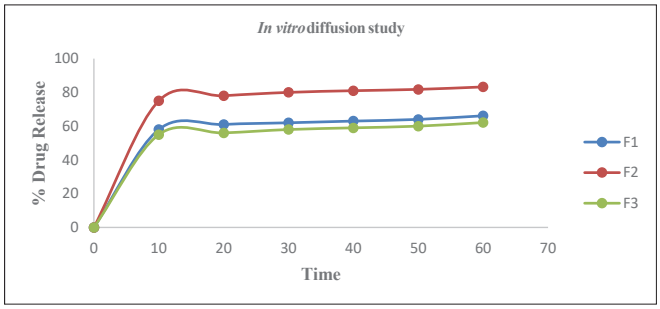

Fig. 7: *In vitro* diffusion study

Scanning electron microscopy (SEM)

Scanning electron microscopy was used to examine ethosomal morphology. On all F1 to F3 batches, the ethosomes SEM image was calculated, individually.

The ethosomes in *V. negundo* appeared to be in spherical and discrete in shape, with sharp boundaries and a large internal aqueous space in SEM images as shown in Fig. 8.

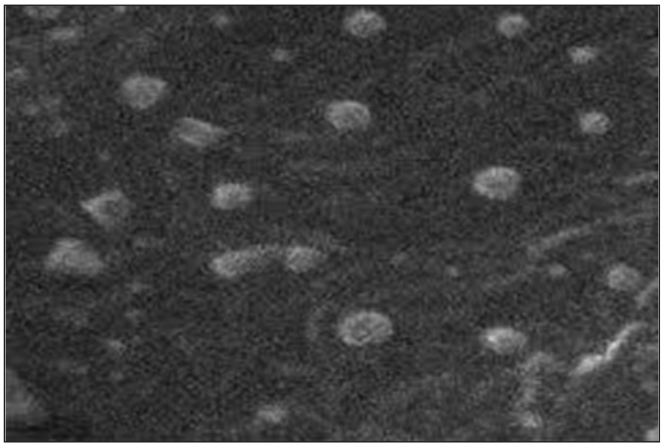

Fig. 8: SEM for ethosomes

Fourier transform infrared spectrometry (FTIR)

An infrared spectrum of pure ethosome, with each polymer and physical mixture was recorded using a Jasco FTIR 4100, Japan. The scanning range was 650-4000 cm^{-1}, and FTIR was done to observe changes in physical and chemical composition of ethosomal gel. Any changes in the drug spectrum pattern caused by the presence of polymers were investigated for chemical interactions.

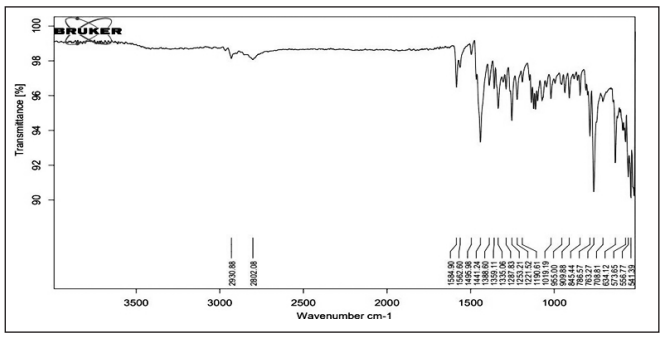

Fig. 9: FTIR for ethosomes

The FTIR spectrum of pure ethosome showed -OH (alcohol), -NH (amide), -CH, - C=O (Carbonyl), -C=O (Aldehyde), -C=C (Aromatic), -C-O (Acid) and -C-O (Ether) groups obtained in 3363, 3390, 2841, 1734, 1732, 1448, 1238 and 1041 cm^{-1}, respectively, as shown in Fig. 9.

EVALUATION OF ETHOSOMAL LOADED GEL

Physical characteristics

The developed ethosomal loaded gels were smooth, homogeneous, and light green white in colour, and were assessed visually.

pH, drug release (*in vitro*) and percent drug content

The pH, content of drug, and cumulative drug release profile of three formulations were determined. The data for pH, drug release is given in Table IV.

Table IV: Drug release (*in vitro*) and percent drug content

Formulation	pH	Content of drug (%)	Release of drug (%)at 8 h
F1	6.1	83.53	66.12
F2	6.4	90.13	83.24
F3	6.7	87.23	62.14

Extrudability

A clamp was used to stop any rollback after the crimped end of a collapsible tube that was closed with 15 g of gel, was vigorously pushed. Gel was extruded, after the cap was taken off. Volume of extruded gel was collected and measured. It was computed how much gel was extruded and is reported in Table V below.

Table V: Extrudability of ethosomal gel

Formulation	Weight of formulation (g)	Amount of gel extruded (g)	Extrudability amount
F1	15.51	13.20	86.06 %
F2	17.12	15.23	88.96 %
F3	17.48	14.98	83.89 %

Viscosity

A Brookfield viscometer was used to determine the gel's viscosity at a temperature of 25 ^0C and a spindle speed of 12 rpm.

Spreadability

Two standard-sized glass slide sets were used. One of the slides was treated with the gel formulation. The other slide was positioned on top of the gel, leaving 7.5 cm between them, as if the gel were being sandwiched between the two slides. Before distributing the liquid uniformly, 100 g of gel was incorporated on the upper slides and compressed between the two slides like a sandwich to form a thin layer. The gel that had stuck to the slides was scraped off after the weight was taken off[11, 12].

$$S = m \times l / t$$

where,

m = Weight affixed to the upper slide

l = The glass slides' length

t = Duration of slide separation

Spreadability of gel were calculated and the results are reported in the Table VI.

Table VI: Spreadability of gel

Formulation	Spreadability (g.cm/sec)
F1	21.88
F2	25.87
F3	23.64

Appearance and homogeneity

Visual perception was used to assess the physical attributes and uniformity of the gels that were developed.

RESULTS AND DISCUSSION

Normal calibration curve was obtained for *V. negundo* ethosomes by mapping absorbance vs. concentration with UV spectroscopy. The calibration curve for ethosomes in phosphate buffer (pH 7.4) showed the straight-line figure passing from the source. Beer-Lambert Law was considered to be obeyed in the 0-20 µg mL^{-1} range. The data from the *V. negundo* ethosome's calibration curve is shown in Fig. 10.

The stretch for -NH, -CH and -OH were observed at 3390 cm^{-1}, 2841 cm^{-1} and 3363 cm^{-1}, N- H bend at 1563 cm^{-1}, as characteristic peaks were identified to be consistent with the chemical structure of the *V. negundo* ethosomes in its FTIR spectrum. The usual O-H stretch bands were observed at 3307 cm^{-1} and 2927 cm^{-1} after

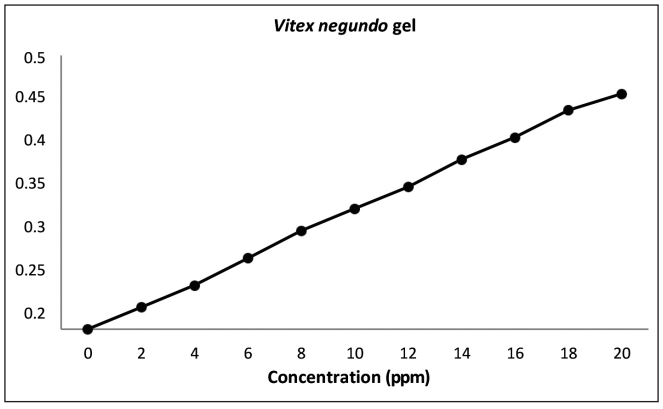

Fig. 10: Calibration curve of *V. negundo*

the medication was physically combined with cholesterol and soya lecithin. The primary peak was intact, and the spectra showed that the tiny shift in the band indicate, that the drug and the excipients were compatible. The elevated temperature peak at 148.8 ^{0}C on the pure ethosome thermogram confirmed the sample's purity, whereas the physical mixture DSC thermogram revealed a peak shift to 107 ^{0}C. The presence of lipids and cholesterol could explain the temperature shift.

The preliminary batches of *V. negundo* ethosomal gel were evaluated. Entrapment efficiency was found to be in the range between 79.53% and 89.40 % in three preliminary batches. The preliminary ethosomal batches showed a range of 66.12 % to 83.24 % drug release. F2 was chosen for the anti-arthritic investigation, out of the formulations F1 to F3, because its drug release performance (*in vitro*) of the created gel F2 was fairly good. Its quality management evaluation results were also good.

CONCLUSION

The goal of *V. negundo* ethosomal loaded gel formulation was to enhance treatment response and lessen the likelihood of unfavorable effects. Here, rotary evaporators and thin hydration films were used to prepare *V. negundo* Linn. ethosomes. The % entrapment efficiency was optimized, after study the impact of different ethanol content levels in the formulation. The highest efficiency of trapping was found to be 89.40 %. It was discovered that the formulated ethosomes had good morphological characteristics and size distribution. According to DSC thermograms of ethosomes, the drug and lipid components of the vesicles interacted significantly, increasing the efficacy of entrapment. The percentage of drug release from the *V. negundo* Linn. ethosomes after 8 h of *in vitro* testing was reported to be 83.24 %. These findings conclusively demonstrate that the ethosomes provides better disease relief and shortens the duration of therapy.

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