Published online in http://ijam.co.in ISSN No: 0976-5921

International Journal of Ayurvedic Medicine, Vol 14 (1), 2023; 244-252

Optimization and Evaluation of Anti-Inflammatory Herbal Emulgel loaded with Vitex Negundo for Enhanced Permeation

Research Article

Keerthana M[1], Akhilesh Dubey[2], Manohar Mahadev[3], Amitha Shetty[3]*

1. PG Scholar, Nitte (Deemed to be University),
NGSM Institute of Pharmaceutical Sciences, Department of Quality Assurance, Mangaluru. Karnataka. India.
2. Assistant Professor Grade III, 3. Assistant Professor Grade I, Nitte (Deemed to be University),
NGSM Institute of Pharmaceutical Sciences, Department of Pharmaceutics, Mangaluru. Karnataka. India.

Abstract

The use of conventional drugs for the treatment of pain and inflammation has largely resulted in various side effects. These challenges have triggered the search for alternative therapy such as herbal medication that may serve as a safe, effective, and alternate treatment approach to managing pain and inflammation. *Vitex negundo*, a potential lipophilic molecule, possesses limited solubility and permeability. Emulgels are either water-in-oil or oil-in-water type emulsions, mixed with a gelling agent to form a gel for the delivery of lipophilic active ingredients. Therefore the current study is undertaken for the effective delivery of vitex negundo locally in the form of oil-in-water emulgel for improving solubility and permeability. The emulgel was formulated from carbopol 934 with *Vitex negundo* in mineral oil emulsion using tween 80 (surfactant), and triethanolamine (co-surfactant). The formulations were optimized using the design of experiment software. The optimized emulgel formulation containing Smix (tween 80 and triethanolamine) ratio of 61.90, mineral oil 50.58%, and carbopol 934 3% showed a pH of 6.5±0.3, a viscosity of 17793 – 939 cps, and spreadability of 7.07 g.cm/s. Data from in-vitro and ex-vivo diffusion studies demonstrated improved permeability properties. The stability of the optimized emulgel formulation was additionally examined at ambient temperature for 28 days. The emulgel remained stable for 28 days. Based on the overall results we can conclude that emulgel can be a promising and suitable formulation for the application of *Vitex negundo*.

Key Words: Verbenaceae; *Vitex negundo*; Emulgel; Topical drug delivery; Optimization; Enhanced Permeation.

Introduction

The skin is the body's main organ. Topical drug delivery is the delivery of a drug through the skin. Drug distribution through the skin is advantageous as it averts the threats linked with intravenous therapy and the difficulties connected with altering gastric pH, emptying time, and hepatic metabolism (1). It has advantages over conventional routes such as avoiding first-pass metabolism, the potential for sustained and controlled release of a drug, and a non-invasive mode of drug delivery thereby increasing the bioavailability of the drug (2). Drug molecules may penetrate the skin along the hair follicles or sweat ducts in the initial transient diffusion stage and then be absorbed by the follicular epithelium through the intact stratum corneum which becomes the primary pathway for transdermal permeation.

Gels are topical drug delivery system that contains a larger quantity of hydroalcoholic liquid in a complex network of solid particles (3). The water component of gel is high thus allowing increased solubility of drugs through a vehicle that is easily penetrated. In terms of patient acceptability, these are superior. Gels, on the other hand, have the drawback of being unable to deliver hydrophobic drugs (4).

Emulgels have been produced as successful hydrophobic drug delivery systems to alleviate this limitation. Oil-in-water or water-in-oil type emulsions are combined with a gelling agent to create emulgels (5). Using an oil-in-water emulsion system, emulgels make it simple to include hydrophobic drugs into the gel, increasing stability and controlled release while also having a higher loading capacity (6, 7). Emulgels are suitable for application to the skin since they are non-greasy, unlike other topical preparations that need excessive rubbing (8, 9).

Inflammation remains a serious problem in the present era. Numerous anti-inflammatory medications have been produced, but research on their safety profiles has shown that none of them are secure. They exhibit a variety of negative impacts. Nutraceuticals have returned as a result of the widespread observation of negative drug side effects caused by synthetic and chemical medications (10).

Vitex negundo, also known as *Nirgundi* is included in the Verbenaceae family. Vitex negundo has enormous medicinal value, and the leaf extract has been

*** Corresponding Author:**
Amitha Shetty
Assistant Professor, Department of Pharmaceutics,
NGSM Institute of Pharmaceutical Sciences, Nitte
(Deemed to be University), Deralakatte,
Mangaluru-575018, India.
Email Id: amithashetty@nitte.edu.in

utilized in Ayurvedic medicine as an anti-inflammatory, analgesic, and anti-itching agent (11). This plant is widely grown in America, Europe, Asia, and West Indies and is widespread in Indo-Malaysia. The topical efficacy of *Vitex negundo* can be increased which depends on its solubility and permeability properties by formulating in the form of Emulgel (12).

This work aimed to formulate an emulgel that enhances the permeation of *Vitex negundo* when applied topically. Emulgel was formulated using tween 80 (surfactant), triethanolamine (co-surfactant), and mineral oil. A central composite design (CCD) was used to examine the potential effects of the components and their interactions, which could aid in selecting the optimal formulation. The pH, viscosity, spreadability, and drug content of optimized emulgel were investigated. Afterward, an in-vitro model was used to study the drug permeability and release behavior. Thus, the current study is undertaken for the effective delivery of *Vitex negundo* locally in the form of emulgel which shall enhance the solubility and thereby the permeability of the drug.

Materials and Methods

Materials

Vitex negundo (purity >98%) was purchased from Sigma Aldrich (India). Mineral oil, Clove oil, Carbopol 934, Tween 80, Span 80, Triethanolamine, Propylene glycol, Methyl paraben, Propyl paraben, and Liquid paraffin were purchased from Loba Chemie, Mumbai. All the other chemicals were procured from HiMedia Lab, Mumbai, India. Each solvent was of High-Performance Liquid Chromatography (HPLC) grade.

Phosphate buffer saline (PBS) of pH 6.8 was prepared by dissolving disodium hydrogen phosphate (28.80 g) and potassium dihydrogen phosphate (11.45 g) in distilled water in 100 mL. Analytical grade components for PBS were bought from Sigma Aldrich (India). Porcine skin membrane was collected freshly from the local slaughterhouse (M/s Badriya Mutton Stall, Near BCC Hall, Derlakatte, Mangaluru- 575017)

Solubility Study

10 mL of oils (Clove oil and Mineral oil), surfactants (Tween 80 and Span 80), and co-surfactants (Triethanolamine and Propylene glycol) were combined with an excess of *Vitex negundo* (3000 µg/mL) in a 100 mL volumetric flask. The combination was then placed in a shaking water bath at 37°C and 50 rpm for 48 hours. After that, the sample was centrifuged for 15 minutes at 3000 rpm. 1 mL of the supernatant liquid was taken from each mixture following centrifugation. After a 10 mL phosphate buffer saline (PBS) pH 6.8 dilution, the absorbance was measured UV-visible spectrophotometrically at a maximum of 273 nm (13).

Construction of pseudo-ternary phase diagram

To identify the emulsion present, pseudo-ternary phase diagrams were built. To develop a phase diagram, oil, surfactant, and co-surfactant were chosen based on studies on the solubility of *Vitex negundo*. For each group, a mixture of surfactant and co-surfactant (Smix) in different weight ratios (1:1, 2:1, and 3:1) was added before being sonicated (Sonics Sonicator, USA) for five minutes at a frequency of 20 kHz and a temperature of 25 °C. For each phase diagram, Smix and oil were fully blended in various weight ratios ranging from 9:1 to 1:9 (i.e., 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, and 1:9). Slow aqueous phase titration was used to create pseudo-ternary phase diagrams (14-16).

Preparation of *Vitex negundo* Loaded Emulgel

Three steps were involved in preparing the emulgel. *Vitex negundo* (0.1 g) was dissolved in a mixture of mineral oil, tween 80, and triethanolamine in order to create the oil phase of the emulsion in the first stage. Then gradually, the water phase was incorporated into the oil phase while continually stirring for 10 minutes at 2000 rpm with a magnetic stirrer. In the next stage, the gel basis was created by combining carbopol 934 with 15 mL of distilled water and agitating with a magnetic stirrer. To effectively dissolve, methylparaben (25 mg) was dissolved in 5 mL of distilled water and heated in a water bath (first solution). Following the cooling of the solution, the initial solution was properly mixed with 2 g of polyethylene glycol (PEG) 4000. Finally, to create the gel, the required amount of each combined ingredient was added to the carbopol 934 while stirring continuously. To create a homogenous emulgel, the third stage involved adding o/w emulsion to the gel base in a ratio of 1:1 (6, 17-21).

Optimization by Design of Experiment

The Design of Experiment (DoE) (version 11.0.3.1) approach was used to examine how different process factors affected the creation of emulgel using central composite design (CCD). A common design in response surface methodology is CCD. Factorial, axial, and centre points are the main components. The presence of such a wide range of points enables a thorough understanding of dependent variables with the least amount of testing. Smix ratio (A), oil ratio (B), and percentage of carbopol (C) were chosen as the independent factors, and drug content studies (%) and in-vitro diffusion studies (%) were chosen as the dependent variables for evaluating the effectiveness of Emulgel. According to the screening study, all the other components and variables had no noticeable impact on the qualities of the created emulgel and stayed constant throughout the investigation. Table 1 lists the factors chosen and the experiment levels. For emulgel, it produced 17 runs (trial batches) (table 2).

Table 1: Independent and dependent factors selected and levels for the experiment

Independent factors	Name	Level (-1)	Level (0)	Level (+1)
A	Smix ratio (% v/w)	30	55	80
B	Oil ratio (% v/w)	20	45	70
C	Carbopol (% w/w)	2	2.5	3
Dependent factors				
X	Drug content			
Y	Diffusion studies			

Table 2. Observed responses for emulgel obtained from DOE

Formulation Code	Factor 1 A: Smix Ratio	Factor 2 B: Oil Ratio	Factor 3 C: Carbopol (%)	Response 1 Drug content (%)	Response 2 Diffusion (%)
F1	-1	-1	-1	88.45	50.46
F2	1	-1	-1	92.85	55.78
F3	-1	1	-1	95.16	47.26
F4	1	1	-1	96.78	56.26
F5	-1	-1	1	89.26	53.94
F6	1	-1	1	94.53	57.89
F7	-1	1	1	91.22	48.05
F8	1	1	1	97.55	57.22
F9	-1	0	0	90.62	54.12
F10	1	0	0	93.68	59.87
F11	0	-1	0	94.53	59.91
F12	0	1	0	98.13	49.34
F13	0	0	-1	93.07	84.58
F14	0	0	1	97.51	85.19
F15	0	0	0	96.05	89.35
F16	0	0	0	96.75	89.55
F17	0	0	0	95.23	88.14

Determination of Drug Content

In order to conduct drug content experiments, 1 g of emulgel was dissolved in a 100 mL volumetric flask, and the volume was then brought to 100 mL with freshly prepared PBS (pH 6.8). Solution (1 mL) was taken, and PBS was used to dilute it up to 10 mL (pH 6.8). Using a UV-Visible Spectrophotometer at 273 nm, the drug content in emulgel was calculated spectrophotometrically (22, 23). To determine the homogeneity of the drug content, experiments are carried out in triplicate (n = 3).

In-vitro Diffusion Studies

According to the supplementary material figure S1 for this experiment, a modified vertical Franz diffusion cell apparatus was used for the in-vitro drug diffusion. A cellophane membrane (donor compartment) was covered with accurately weighed 1 g of emulgel, and the entire apparatus was partially submerged in the dissolution media PBS. Using a magnetic stirrer, the dissolving medium was blended at 50 rpm and 37 ±0.5 °C temperature. 1 mL of the sample was taken out at specified intervals of 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes in order to estimate the absorbance at 273 nm with a UV-Visible Spectrophotometer. By adding the same amount of fresh PBS in place of the withdrawn sample, the sink condition was preserved (24-26).

Optimization and Evaluation of Optimized Emulgel Formulation

Optimization

The best-fitting mathematical model was chosen based on comparisons of a variety of statistical data, as well as the standard deviation (SD) and the determination coefficients (R2 and adjusted R2) (20). An optimal formulation was created utilizing a numerical optimization technique and the desirability function approach. To obtain the desired results of the formulation, the right levels of constraints (target) were set (table 3). The ideal formulation was anticipated to have a strong drug diffusion after 360 minutes (> 90%), as well as a maximal drug content (> 99%).

Table 3. Criteria for numerical optimization and optimized emulgel formulation

Parameters	Goal	Lower limit	Upper limit	Lower weight	Upper weight	Impor-tance
A: Smix ratio	Is in range	-1	1	1	1	1
B: Oil ratio	Is in range	-1	1	1	1	1
C: Carbopol	Is in range	-1	1	1	1	1
Y1: Drug Content	Target= 99.00	88.45	98.13	1	1	1
Y2: Diffusion	Target= 90.00	47.26	89.55	1	1	1
F18 (optimized)	**Concentration of Smix (%)**		**Concentration of oil (%)**		**Carbopol (%)**	
	61.90		50.58		3	

Physical Appearance

Visual evaluations of the color, consistency, and appearance of the developed optimal emulgel formulation were conducted (21, 27).

Determination of pH

The pH of the emulgel was measured using a digital pen pH metre. By emulsifying 2.5 g of the emulgel formulation in 25 mL of distilled water, the pH was measured (28).

Viscosity Measurement

Using a Brookfield viscometer equipped with spindle no. S 63 at 50 rpm for 10 minutes, the viscosity of the emulgel was measured.

Spreadability

The spreadability test was carried out with modified equipment, which consisted of a wooden block. It is made up of a wooden block with a pulley attached to one end. Based on the emulgel's "Slip" and "Drag" qualities, the spreading was calculated. On the wooden block, a ground glass slide was fixed. The spreadability experiment was carried out using modified equipment, as seen in supplemental material figure S2, which consisted of a wooden block. The emulgel (2.5 g) was sandwiched between two parent glass slides. To remove air and create a consistent film of emulgel between the two slides, a mass of 100 g was placed on top of them for five minutes. The upper slide was anticipated to release when a predetermined amount of weight (80 g) was added to the pan that was hooked to the pulley. The time required for the upper slide to

move 7.5 cm and separate from the lower slide in the direction of the weight was observed (29-31). In order to determine spreadability, the following formula was used:

Spreadability (S) = (m. l)/t (Eq. 1)

Where, S = spreadability, m = weight tied to the upper slide, l = length of the glass slide, and t = time taken in seconds for the complete detachment of slides from each other.

Determination of Drug Content

The analysis of drug content was performed using the procedure outlined above.

In-vitro Diffusion Studies

The in vitro diffusion studies were carried out in accordance with the procedure outlined above.

Ex-vivo Permeation Studies

Fresh porcine skin membrane was used in an ex-vivo skin permeation test. The porcine skin membrane was set on the donor compartment, partially submerged in the dissolution media, PBS pH 6.8, and then filled with the receptor compartment. 1 g of emulgel was accurately weighed and applied on the porcine skin membrane which is set on the donor compartment. A magnetic stirrer was used to mix the dissolution medium (PBS pH 6.8) at 50 rpm. A constant 37± 0.5 °C was maintained. At intervals of 15, 30, 60, 90, 120, 180, 240, 300, and 360 minutes, a sample (1 mL) was taken out and evaluated using a UV-Visible Spectrophotometer at 273 nm. In order to keep the sink condition, fresh PBS pH 6.8 (2 mL) was introduced to replace the removed sample (32-37).

Drug Release Kinetic Studies

To investigate the drug release mechanism, the in-vitro release kinetic study was applied. The information regarding drug release can be calculated using the following equations (38):

Zero-order equation: Q = Q0 + k0 t (Eq. 2)

Q0 = initial amount of drug, Q = cumulative amount of drug release at time "t", (released occurs rapidly after drug dissolves), K0 = zero order release constant, t = time in hours.

First order equation: log C = logC0 - kt/2.303 (Eq. 3)

C0 = initial amount of drug, C = cumulative amount of drug release at time "t", K = first order release constant, and t = time in hours.

Higuchi model equation: Q = kH t1/2 (Eq. 4)

Q = cumulative amount of drug release at time "t", kH = Higuchi constant, t = time in hours.

Korsmeyer-Peppas model equation: Mt/M = km tn (Eq. 5)

Mt = Amount of drug released at time 't', M = Total amount of drug in dosage form, km = Kinetic constant, n = Diffusion or release exponent, t = time in hours.

Comparison of Experimental Results with Predicted Responses of Optimized Emulgel Formulation

Following the test, the results obtained and the anticipated values received from the software were compared, and the relative % error was calculated.

Stability Studies

The stability of *Vitex negundo* in emulgel was investigated using stability studies. The optimized emulgel formulation was kept in a collapsible tube at 30 °C / 65% Relative humidity (RH) and 40 °C / 75% RH in the humidity chamber for 28 days. After 15 and 28 days of storage, samples were taken and their physical characteristics, pH levels, spreadability, and drug content were calculated. The similarity index between in-vitro diffusion rates was determined to demonstrate the stability of the emulgel.

Results

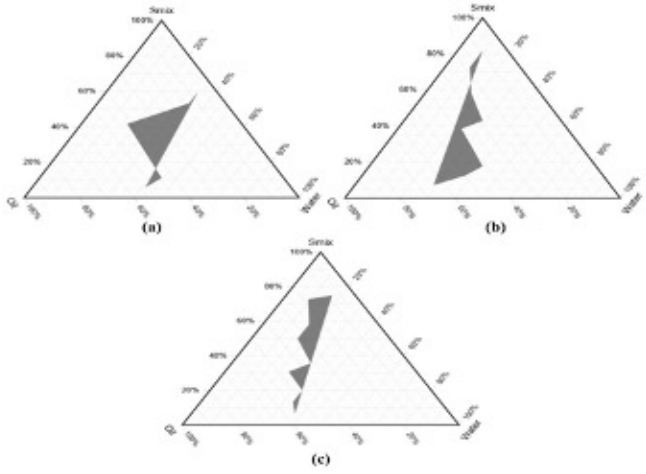
Solubility Study

The maximum solubility of *Vitex negundo* was found to be 0.734±0.021 mg/mL, 0.718±0.015 mg/mL, and 0.763±0.012 mg/mL in tween 80, triethanolamine, and mineral oil, respectively. Tween 80 (a surfactant), triethanolamine (a co-surfactant), and mineral oil were chosen to form the emulsion based on the solubility of nirgundi. Each experimental result is recorded in triplicate (n = 3).

Phase Study

The oil in water emulsion region in the various ratios (i.e., 1:1, 2:1, and 3:1) of Smix to oil was shown in the black area of figure 1a-1c. The ratio of surfactant to co-surfactant that produced the highest emulsion region was 1:1, whereas the ratio of 3:1 produced the smallest emulsion zone. The amount of water needed to achieve turbidity reduces as Smix concentration rises. A larger emulsion region was obtained when tween 80 and triethanolamine were combined in a 1:1 ratio. As a result, this system was chosen for additional research.

Figure 1. Pseudo ternary phase diagram of (a) Smix ratio 1:1, (b) Smix ratio 2:1, (c) Smix ratio 3:1



Optimization by Design of Experiment

The emulgel was optimized using a 2^3-level factorial randomized central composite design (CCD)

with the aid of a design expert. 17 trial batches of emulgel were created, and their in-vitro diffusion and drug content were assessed. For the formulated emulgel, it was expected that drug content should be maximum (to assure uniform drug dispersion and prevent drug loss), and faster diffusion (to give fast performance). Smix ratio (surfactant to co-surfactant ratio) (A), oil ratio (B), and percentage of carbopol (C) were selected as independent variables and drug content studies (%) and in-vitro diffusion studies (%) were chosen as dependent variables. 17 runs were suggested by the CCD for the experiment. Table 1 lists the experimental and coded values for the independent variables. Table 2 provides responses to the 17 experiments. The drug content and in-vitro drug diffusion were observed to be 88.45 to 98.13% and 47.26 to 89.55%, respectively.

Using multiple regression analysis, the CCD model was created to fit the 2nd order polynomial model as shown in the following equation:

$$Y = b0 + b1X1 + b2X2 + b3X21 + b4X21 + b5X1X2\,(1) \quad \textbf{(Eq. 6)}$$

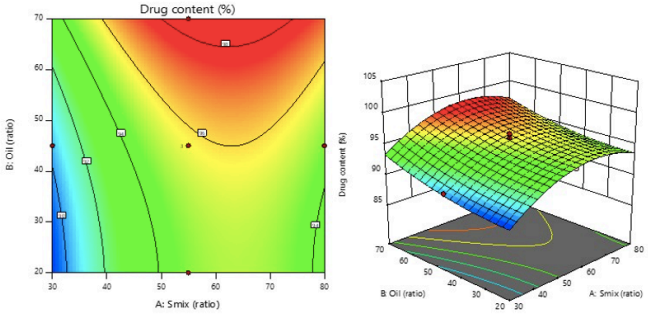
Where Y corresponds to the predicted response, X1 and X2 correspond to the studied factors, b0 is an intercept, and b1–b5 are regression coefficients.

The model created for the drug content has a p-value of 0.0072 and an F-value of 7.54, indicating that it is significant. The Lack of Fit F-value of 4.09 indicates the Lack of Fit is not significant relative to the pure error. Non-significant lack of fit is good. According to the contour plot and response surface plot (figure 2), it was found that the drug content significantly increased as the concentration of Smix and oil ratio rose from -1 to +1 level. The following equation demonstrates how the Smix and oil ratio considerably impacted the drug content in emulgel:

$$\textbf{Drug content} = +95.68 + 2.07A + 1.92B + 0.3760C - 0.2150AB + 0.6975AC - 0.7075BC - 3.28A2 + 0.9017B2 - 0.1383C2 \quad \textbf{(Eq. 7)}$$

Where A, B, and C indicate the coded values for Smix, oil, and carbopol, respectively.

Figure 2. Contour plot and response surface plot for the effect of factors on drug content



The model created for the diffusion has a p-value of 0.0110 and an F-value of 6.50, indicating that it was significant. Since the value of 1.51 indicated a non-significant lack of fit, the model is suitable for computing diffusion.

The following equation illustrates how significantly the amount of Smix and oil ratio impacted the diffusion study of emulgel.

$$\textbf{Diffusion} = +81.15 + 3.32A + 1.99B - 0.7950C + 1.11AB - 0.1500AC - 0.4800BC - 18.26A2 - 20.63B2 + 9.63C2 \quad \textbf{(Eq. 8)}$$

Where A, B, and C indicate the coded values for Smix, oil, and carbopol, respectively.

According to the perturbation plot and response surface plot shown in figure 3, there was a considerable increase and subsequent drop in the in-vitro diffusion as the concentration of Smix and oil ratio increased from (-1 to +1).

Figure 3. Contour plot and response surface plot for the effect of factors on in-vitro diffusion

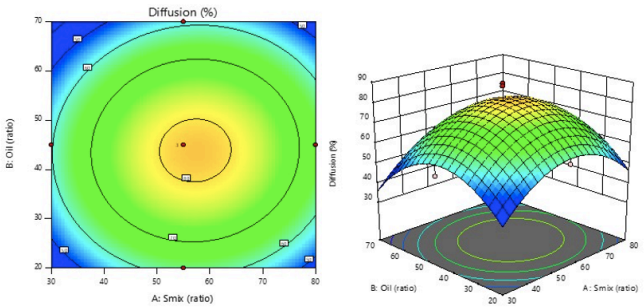


Table 3 illustrates the numerical optimization criteria and the optimum emulgel formulation based on the DOE. Based on the criterion employed, optimized emulgel formulation was suggested by DOE as depicted in table 3 which was formulated and further evaluated.

Evaluation of Optimized Transemulgel Formulation

Physical Appearance

The optimized emulgel formulation was evaluated for color, appearance, and consistency. The physical characteristic of the emulgel was observed to be whitish with a smooth and glossy appearance. There was no evidence of Phase separation.

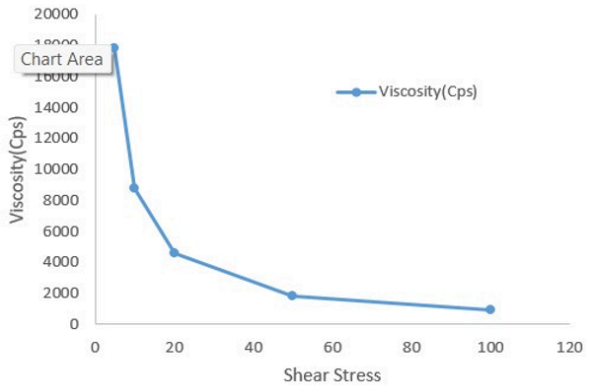
Determination of pH

For topical formulations, pH evaluation is a key parameter because if it deviates from normal skin pH values, it may irritate the skin. The pH of the emulgel was discovered to be 6.5±0.3, closer to the pH of the skin.

Viscosity Measurement

Viscosity of the preparation are a few mechanical and physical characteristics that are important rheological parameters. The optimal emulgel formulation's viscosity was determined to be between 17793 and 939 cps. The outcomes are shown in figure 4. The observed results and plot pattern demonstrated that viscosity reduced when the shear rate was raised and vice versa.

Figure 4. The viscosity of optimized emulgel formulation



Spreadability

Application heavily depends on the spreadability of the gel. The spreadability of the optimized emulgel formulation was observed to be 7.07 g.cm/s, which indicated good spreadability.

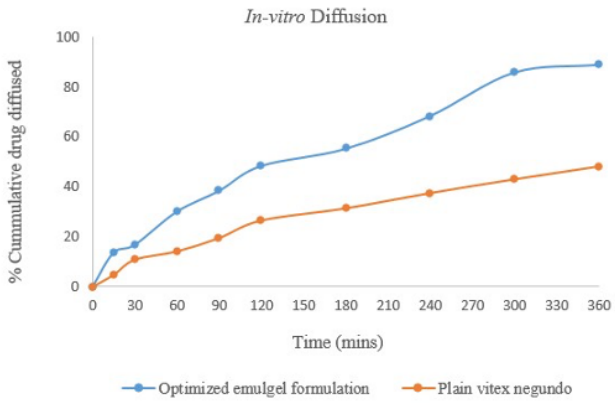
Determination of Drug Content

To achieve the uniform drug dispersion in the entire formulation of a semi-solid preparation, drug content uniformity is required. The drug content of the optimized emulgel formulation was observed to be 96.34 ± 1.74%. The analysis of drug content indicated that the drug was distributed uniformly.

In-vitro Diffusion Studies

In-vitro diffusion tests with a cellophane membrane were performed on the optimal emulgel formulation. The cumulative percentage of drug diffused is shown in figure 5. It was observed that the diffusion of *Vitex negundo* was found to be 89.42 ± 1.02 % after 360 min to plain drug solution, which could diffuse only 47.85 ± 1.12 %. Optimized emulgel formulation of *Vitex negundo* followed a prolonged drug diffusion pattern.

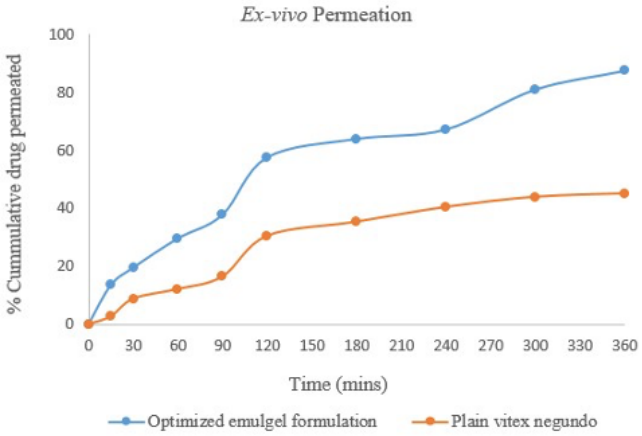
Figure 5. In-vitro diffusion studies of optimized emulgel formulation and plain vitex negundo



Ex-vivo Permeation Studies

Permeability using skin membranes is a key feature of any formulation, which determines the permeation and absorption of the drug from it. Hence, ex vivo permeability studies were carried to determine the permeability of *Vitex negundo* in skin membranes from optimized emulgel formulation and plain drug solution. The cumulative percentage of drug permeated from fresh porcine skin membrane is shown in figure 6. It was found that the permeation of *Vitex negundo* was enhanced from the optimized emulgel formulation, in comparison to plain drug solution, as 87.70±1.28 % of the drug from optimized emulgel formulation permeated through the skin membrane after 360 min to plain drug solution, which could permeate only 45.68 ± 1.08 %.

Figure 6. Ex-vivo permeation studies of optimized emulgel formulation and plain *Vitex negundo*



Comparison of Experimental Results with Predicted Responses of Optimized Emulgel Formulation

All response variables are listed in Table 4 along with their anticipated and experimental values and percentage error. The mean percentage error was determined to be 0.26 when the observed results were compared to the estimated responses.

Table 4: Selected Formulation and % Error between the Predicted and the Observed Values

Factors (%)		Responses		
A	**B**	**C**	**Drug Content (%)**	**Diffusion (%)**
Predicted				
61.90	50.58	3	96.73	89.55
Observed				
			96.34	89.42
Relative % error			0.39	0.13

Stability Studies

Stability tests were also performed on optimized emulgel formulation. The difference and similarity factor between dissolution profiles of optimized emulgel formulation before and after storage was found to be 3 and 83. There exists a similarity between the diffusion profile before and after storage, as the difference factor was observed to be less than 15, and the similarity factor was observed to be greater than 50. The results shown in Supporting Information (Tables 5 and 6) demonstrated sufficient stability.

Table 5. Stability studies of optimized emulgel formulation

Time Period	Physical Appearance		pH		Spreadability (g.cm/		Drug Content (%)	
	30°C/ 65% RH	40°C/ 75% RH	30°C/ 65% RH	40°C/ 75% RH	30°C/ 65% RH	40°C/ 75% RH	30°C/ 65% RH	40°C/ 75% RH
Before storage	No phase separation	-	6.7±0.3	-	7.07	-	96.34±1.74	---
After 15 days	No phase separation	No phase separation	6.7±0.1	6.7±0.3	7.07	6.9	96.35±1.72	96.04±1.63
After 28 days	No phase separation	No phase separation	6.7±0.3	6.7±0.2	7.06	6.9	96.54±1.60	95.00±1.70

Table 6. Difference and Similarity Factors of Diffusion Profile of Optimized Formulation

Time (min)	Before storage	After 28 days		Difference factor	Similarity factor
		30°C/ 65% RH	40°C/ 75% RH		
360	89.42±1.02%	89.23±1.22%	88.58±1.00%	3	83

Discussion

Although gels are considered to be superior topical drug delivery systems, gels have the drawback of being unable to deliver hydrophobic drugs. On the other hand, chemically synthesized drugs have several adverse effects. To overcome these problems, improving the permeability, reducing the dose, and increasing the absorption are the main strategies.

The initial goal of this work was to study how the parameters affected the formulation and develop an optimized emulgel containing *Vitex negundo*. The second goal of our work was to enhance and verify the permeability and stability of the optimized emulgel formulation.

Oil, surfactant, and co-surfactant are the primary ingredients used in the formulation of emulsion. Solubility studies of the drug were carried out in various oil (Clove oil and Mineral oil), surfactants (Tween 80 and Span 80), and co-surfactants (Triethanolamine and Propylene glycol). Based on the maximum solubility, mineral oil which has moisturizing, good spreadability, and stability properties was utilized as oil in the development of an o/w type microemulsion. The mineral oil was accompanied by tween-80 as a surfactant and triethanolamine as a co-surfactant, which helps in adjusting the HLB value of formulation. The range of component concentrations for existing emulsions can be ascertained by creating a phase diagram. The region of the transparent microemulsion was visually examined. Only ratios that were black in colour were in the microemulsion area. Turbid and conventional emulsion are represented by the remaining area on the phase diagram. A larger emulsion region was obtained when tween 80 and triethanolamine were combined in a 1:1 ratio and it was observed that as the concentration of Smix escalates, the volume of water necessary for obtaining turbidity escalates. Therefore, 1:1 was chosen for further study.

Vitex negundo was prepared as o/w emulsion and introduced into carbopol gel to form emulgel. To study the effect of formulation parameters affecting the product, optimization was carried out using the Design of Experiment approach using central a composite design. Smix ratio, oil ratio, and percentage of carbopol were chosen as independent variables. To make sure the uniformity of the dispersed drug, prevent drug loss and get faster diffusion, drug content and in-vitro diffusion studies were selected as dependent variables. The 17 formulations proposed were formulated and evaluated to obtain the optimized formulation. It was found that as the concentration of Smix and oil ratio raised, drug content also increased significantly which may be attributed to the solubility effect of tween-80 further triethanolamine reduces the interfacial tension which helps in the solubilization of *Vitex negundo* in the oily phase. The drug content of emulgel was significantly affected by Smix and oil ratio. Additionally, it was noted that there was a considerable increase in in-vitro diffusion when the concentration of Smix and oil ratio increased, followed by a reduction. According to the criterion employed, an optimized emulgel formulation containing 61.90 % of Smix, 50.58 %, and 3 % carbopol was suggested by DOE which was formulated and further evaluated.

The emulgel was clear and transparent in appearance. The pH of the formulation was found to be 6.5±0.3, which is in the acceptable pH range for the skin. Thus, showing that the formulation may not irritate the skin when it is applied topically. The viscosity was found to be in the range of 17793 – 939 cps which was expected to aid in the easy removal of the product from the container, and easy topical application. The spreadability was 7.07 g.cm/s indicating good spreadability, as poor spreadability gradually shortens the amount of time a drug resides on the skin, which leads to poor bioavailability. The prepared emulgel exhibited uniform drug content and was in the acceptable range, which implies that the drug dispersion in the emulgel is uniform. The loss of drugs during the formulation processes was insignificant.

An extended duration of drug diffusion was anticipated from the formulation, therefore experimental investigation of drug release was performed. The cumulative drug release at 360 min delivers details on the release over a longer time. The drug release properties and plain drug solution were compared. A significantly higher percent drug diffusion value compared with the plain drug solution showed more favorable drug diffusion.

Medication administered topically ought to penetrate the skin and reach the site of action. The formulation and the composition can influence the penetration as well. In our work, optimized emulgel formulation enhanced drug penetration compared to plain drug solution. Optimized emulgel formulation also increases permeability due to increased drug diffusion. This was because of the maximum concentration of tween-80 as a surfactant, which is a permeation enhancer, and can also be caused by emulsion droplet size, which helps in drug diffusion and permeation by offering a significant interfacial surface area. The outcomes of the experiment show that emulgel promotes penetration since it has greater permeability values than plain drug solution.

The low magnitudes of error between the anticipated and observed results of all the response variables showed an excellent fit for the model. All the formulations obeyed Korsmeyer–Peppas model suggesting super case-II drug transport and the release kinetics was zero order. The stability studies support that the formulation is unstable over a prolonged period.

Conclusion

Thus, the *Vitex negundo* emulsion was effectively developed and then added into the carbopol gel. *Vitex negundo* is weakly water-soluble, which is a major disadvantage when formulating it for topical drug delivery. Thus, the solubility was enhanced by adding *Vitex negundo* into an oil-in-water emulsion and then adding it to the gel. According to the results, the emulgel has shown better permeation, maintaining the sustained release profile. Overall results indicated that emulgel can be a potential formulation for the application of *Vitex negundo*.

Funding

The authors are grateful to Nitte (Deemed to be University) for the grant Project “Formulation and evaluation of anti-inflammatory emulgel containing vitex negundo” 2020. Ref: N(DU)/RD/Grant NUSR2/ NGSMIPS /2020-21/07.

References

1. Rehman K, Zulfakar MH. Recent advances in gel technologies for topical and transdermal drug delivery. Drug Development and Industrial Pharmacy. 2014; 40(4): 433-40.
2. Krishna Veni N, Yashwanthi P, Hemalatha B, Padmalatha K. A review on emulgels as a novel approach for topical drug delivery. Asian Journal of Research in Pharmaceutical Sciences. 2022; 12(2): 163-168. doi: https://doi.org/10.52711/2231-5659.2022.00028
3. Yadav S, Wairkar S, Invally M, Ranade S. Topical emulgel of tolnaftate with penetration enhancer: development, characterization and antifungal activity. Indian Journal of Medical Research and Pharmaceutical Sciences. 2017; 4(10): 28-35.
4. Singla V, Saini S, Joshi B, Rana AC. Emulgel: a platform for topical drug delivery. International Journal of Pharma and Bio Sciences. 2012; 3(1): 485-98.
5. Thanushree H, Kumar G, Acharya A. Formulation development of diclofenac sodium emulgel using aloe vera gel for transdermal drug delivery system. International Journal of Pharmaceutical Sciences and Nanotechnology. 2017; 10(5): 3858-3865. doi: https://doi.org/10.37285/ijpsn.2017.10.5.7
6. Venkataharsha P, Maheshwara E, Raju Y, Reddy V, Rayadu B, Karisetty B. Liposomal aloe vera trans-emulgel drug delivery of naproxen and nimesulide: A study. International Journal of Pharmaceutical Investigation. 2015; 5: 28-34. doi: https://doi.org/10.4103%2F2230-973X.147230
7. Malavi S, Kumbhar P, Manjappa A, Disouza J, Dwivedi J. Emulgel for improved topical delivery of Tretinoin: Formulation design and characterization. Annales Pharmaceutiques Françaises. 2022; 80(2): 157–68. doi: https://doi.org/10.1016/j.pharma.2021.05.004
8. Begum S, Chetty M, Voleti V, Pavithra B, Akhila B, Gayathri C et. al. A review on emulgels: a novel approach for topical drug delivery. Asian Journal of Pharmaceutical Research and Development. 2019; 7(2): 70-77.
9. Teku RL, Mylangam CK, Kolapalli VRM. Formulation of capsaicin loaded emulgels using natural gums and oils for topical delivery. World Journal of Pharmacy and Pharmaceutical Sciences. 2015; 5(1): 1017-34.
10. Mosaddek A, Rashid M. A comparative study of the anti-inflammatory effect of aqueous extract of neem leaf and dexamethasone. Bangladesh Journal of Pharmacology. 2008; 3(1): 44-47. doi: https://doi.org/10.3329/bjp.v3i1.836
11. Khan A, Naz S, Farooq U, Shahid M, Ullah I, Ali I, Rauf A, Mabkhot Y. Bioactive chromone constituents from Vitex negundo alleviate pain and inflammation. Journal of Pain Research. 2018 Dec; 11: 95-102. doi: https://doi.org/10.2147%2FJPR.S145551
12. Sahare KN, Anandharaman V, Meshram VG, Meshram SU, Gajalakshmi D, Goswami K et. al. *In vitro* effect of four herbal plants on the motility of *Brugia malayi* microfilariae. Indian J Med Res. 2008: 467-71.[A15]
13. Costanzo MT, Yost RA, Davenport PW. Standardized method for solubility and storage of capsaicin-based solutions for cough induction. Cough. 2014, 10, 6. doi: https://doi.org/10.1186/1745-9974-10-6
14. Jhawat V, Gulia M, Sharma AK. Chapter 15—Pseudoternary phase diagrams used in emulsion preparation. In Chemoinformatics and Bioinformatics in the Pharmaceutical Sciences; Sharma N, Ojha H, Raghav PK, Goyal RK, Eds.; Academic Press: Cambridge, MA, USA, 2021; pp. 455–481.
15. Sudhakar K, Mishra V, Hemani V, Verma A, Jain A, Jain S, Charyulu R. Reverse pharmacology of phytoconstituents of food and plant in the management of diabetes: Current status and perspectives. Trends in food science and technology. 2021; 110: 594-610. doi: https://doi.org/10.1016/j.tifs.2020.10.024

16. Ma Y, Yuan X, Huang H, Xiao Z, Zeng G. The pseudo-ternary phase diagrams and properties of anionic–non-ionic mixed surfactant reverse micellar systems. Journal of Molecular Liquids. 2015; 203: 181-186.
17. Shafiq S, Shakeel F, Talegaonkar S, Ahmad F, Khar R, Ali M. Development and bioavailability assessment of Ramipril nanoemulsion formulation. European Journal of Pharmaceutics and Biopharmaceutics. 2007; 66: 227-243. doi: https://doi.org/10.1016/j.ejpb.2006.10.014
18. Gaikwad D, Jadhav N. Formulation design and evaluation of an emulgel containing Terminalia arjuna bark extract for transdermal delivery. Pharmacognosy Magazine. 2018; 14: 249-255.
19. Morsy M, Abdel-Latif R, Nair A, Venugopala K, Ahmed A, Elsewedy H, Shehata T. Preparation and evaluation of atorvastatin-loaded nanoemulgel on wound-healing efficacy. Pharmaceutics. 2019; 11: 609. doi: https://doi.org/10.3390/pharmaceutics11110609
20. Naga V, Maheshwari P, Navya M, Reddy S, Shivakumar H, Gowda D. Calcipotriol delivery into the skin as emulgel for effective permeation. Saudi Pharm J 2014; 22: 591-599. doi: https://doi.org/10.1016/j.jsps.2014.02.007
21. Khullar R, Kumar D, Seth N, Saini S. Formulation and evaluation of mefenamic acid emulgel for topical delivery. Saudi Pharmaceutical Journal. 2012; 20(1): 63-67. doi: https://doi.org/10.1016/j.jsps.2011.08.001
22. Thapa B, Skalko-Basnet N, Takano A, Masuda K, Basnet P. High-performance liquid chromatography analysis of capsaicin content in 16 Capsicum fruits from Nepal. Journal of Medicinal Food. 2009; 12(4): 908-913. doi: https://doi.org/10.1089/jmf.2008.0187
23. Khan BA, Rashid F, Khan MK, Alqahtani SS, Sultan MH, Almoshari Y. Fabrication of Capsaicin Loaded Nanocrystals: Physical Characterizations and In Vivo Evaluation. Pharmaceutics. 2021; 13(6): 841. doi: https://doi.org/10.3390/pharmaceutics13060841
24. Al-Othman ZA, Ahmed YB, Habila MA, Ghafar AA. Determination of capsaicin and dihydrocapsaicin in Capsicum fruit samples using high performance liquid chromatography. Molecules. 2011; 16(10): 8919-8929. doi: https://doi.org/10.3390/molecules16108919
25. Vandana KR, Yalavarthi PR, Sundaresan CR, Sriramaneni RN, Vadlamudi HC. In-vitro assessment and pharmacodynamics of nimesulide incorporated Aloe vera transemulgel. Current Drug Discovery Technologies. 2014; 11(2): 162-167. doi: https://doi.org/10.2174/1570163810666131202233721
26. Nikumbh KV, Sevankar SG, Patil MP. Formulation development, in vitro and in vivo evaluation of microemulsion-based gel loaded with ketoprofen. Drug Delivery. 2015; 22(4): 509-515. doi: https://doi.org/10.3109/10717544.2013.859186
27. Mohamed M. Optimization of chlorphenesin emulgel formulation. AAPS Journal. 2004; 6: e26. doi: https://doi.org/10.1208%2Faapsj060326
28. Ajazuddin, Alexander A, Khichariya A, Gupta S, Patel RJ, Giri TK, Tripathi DK. Recent expansions in an emergent novel drug delivery technology: Emulgel. Journal of Controlled Release. 2013; 171(2): 122-132. doi: https://doi.org/10.1016/j.jconrel.2013.06.030
29. Shen Y, Ling X, Jiang W, Du S, Lu Y, Tu J. Formulation and evaluation of Cyclosporin A emulgel for ocular delivery. Drug Delivery. 2015; 22(7): 911-917. doi: https://doi.org/10.3109/10717544.2013.861883
30. Sengupta P, Chatterjee B. Potential and future scope of nanoemulgel formulation for topical delivery of lipophilic drugs. International Journal of Pharmaceutics. 2017; 526(1-2): 353-365. doi: https://doi.org/10.1016/j.ijpharm.2017.04.068
31. Jelvehgari M, Montazam H. Evaluation of mechanical and rheological properties of metronidazole gel as local delivery system. Archives of Pharmacal Research. 2011; 34(6): 931-940. doi: https://doi.org/10.1007/s12272-011-0610-5.
32. Ravikumar R, Ganesh M, Ubaidulla U, Young Choi E, Tae Jang H. Preparation, characterization, and in vitro diffusion study of nonwoven electrospun nanofiber of curcumin-loaded cellulose acetate phthalate polymer. Saudi Pharmaceutical Journal. 2017; 25(6): 921-926. doi: https://doi.org/10.1016/j.jsps.2017.02.004
33. Nayak D, Thathapudi NC, Ashe S, Nayak B. Bioengineered ethosomes encapsulating AgNPs and Tasar silk sericin proteins for non-melanoma skin carcinoma (NMSC) as an alternative therapeutics. International Journal of Pharmaceutics. 2021; 596: 120265. doi: https://doi.org/10.1016/j.ijpharm.2021.120265
34. Valenta C, Almasi-Szabo I. In Vitro Diffusion Studies of Ketoprofen Transdermal Therapeutic Systems. Drug Development and Industrial Pharmacy. 1995; 21: 1799–1805. doi: https://doi.org/10.3109/03639049509069267
35. Egbaria K, Ramachandran C, Weiner N. Topical application of liposomally entrapped cyclosporin evaluated by in vitro diffusion studies with human skin. Skin Pharmacology and Physiology. 1991; 4(1): 21-28. doi: https://doi.org/10.1159/000210920
36. Stahl J, Blume B, Bienas S, Kietzmann M. The comparability of in vitro and ex vivo studies on the percutaneous permeation of topical formulations containing Ibuprofen. Alternatives to Laboratory Animals. 2012; 40(2): 91-98. doi: https://doi.org/10.1177/026119291204000208
37. Luo L, Patel A, Sinko B, Bell M, Wibawa J, Hadgraft J, Lane ME. A comparative study of the in vitro permeation of ibuprofen in mammalian skin, the PAMPA model and silicone membrane. International Journal of Pharmaceutics. 2016; 505(1-2): 14-19. doi: https://doi.org/10.1016/j.ijpharm.2016.03.043
38. Geeva Prasanth A, Sathish Kumar A, Sai Shruthi B, Subramanian S. Kinetic study and in vitro drug release studies of nitrendipine loaded arylamide grafted chitosan blend microspheres. Materials Research Express. 2020; 6: 125427.

*****