A Use of Medicinal Plant with Transfersome as Avesicular Carrier that Increase the Permeation

Miss. Mayuri Balu Jagtap¹, Mr. Vaibhavkumar Bapu Bhagwat²

¹Final Year Student, Vidya Niketan College of Pharmacy, Lakhewadi, Tal- Indapur, Dist- Pune (MH), 413103, India
²Assistant Professor, Department of Pharmaceutics, Vidya Niketan College of Pharmacy, Lakhewadi, Tal- Indapur, Dist- Pune (MH), 413103, India

Abstract
Transfersomes are a novel type of vesicular drug carrier system. Phospholipid, surfactant, and water combine to form transfersomes, which improve the transdermal medication delivery mechanism. Elastic or malleable vesicles are the shape of transfersomes. Increase the transdermal distribution of bioactives. In the present study Vitex negundo loaded Transfersomes are prepared by using thin hydration technique. Vitex negundo have anti-inflammatory properties. The topical Transfersome gel is prepared. The phytochemical components of the plant extract from Vitex negundo were examined in this study using TLC and UV research. In a preliminary phytochemical analysis, flavonoids, saponins, cardiac glycoside, terpenoids, quinones, alkaloids, and tannins were discovered. Chloroform, methanol, ethyl acetate, cyclohexane, and acetic acid were combined in a ratio of 10:2:5:1:1 for TLC, and the Rf value was ascertained. A spot was found using TLC analysis, and its Rf value was 0.70. By using UV-Vis spectroscopy, the existence of an absorption peak at 640 nm was confirmed.

Keywords: Vitex negundo, Transfersomes, Thin hydration technique, gel

Introduction
Transdermal Drug Delivery Systems (TDDS), also known as transdermal therapeutic systems (TTS), are topical dose forms that contain medications with systemic effect. Transdermal medication delivery is the process of delivering a medicinal component through "intact" skin to the systemic circulation in a sufficient amount that is beneficial following the administration of a therapeutic dose. Transdermal delivery devices are generally well-suited for the management of chronic illnesses. Therefore, therapeutically used anti-inflammatory drugs were tested transdermally.

Transfersomes:
The transdermal route offers several potential benefits over the conventional routes, including prolonged duration and consistent activity, avoiding first-pass metabolism, minimizing side effects, improving pharmacological and physiological response, preventing fluctuations in drug levels between and within patients, and—above all—facilitating patient convenience. Pharmaceutical researchers are highly interested in the transdermal route of medication administration. Various approaches are being employed nowadays to increase the transdermal distribution of bioactives. They primarily consist of the vesicular system, which includes niosomes, liposomes, elastic liposomes like transfersomes and ethosomes,
microneedles, chemical permeation enhancers, and sonophoresis. Among these tactics, transfersomes seem to have promise. Transfersomes are a novel type of vesicular drug carrier system. Phospholipid, surfactant, and water combine to form transfersomes, which improve the transdermal medication delivery mechanism. Elastic or malleable vesicles are the shape of transfersomes [1,2,3]. Gregor Cevc coined the phrase "Transfersome concept" in 1991. A transfersome is a complex, highly adaptive, and stress-responsive aggregation. It is characterized as a very malleable vesicle with an aqueous core encircled by a lipid bilayer complex. The lipid bilayer's structure and shape enable the vesicle to self-regulate and self-optimize. This Transfersome functions as a drug carrier for the non-invasive targeted administration of therapeutic medicines and the efficient crossing of various transport obstacles.

Figure 3- Structure of Transfersome [4]

Advantages of Transfersomes:
1. Transfersomes with a high entrapment efficiency—roughly 90% in a lipophilic drug—have been seen.
2. Its great deformability may allow intact vesicles to penetrate more easily.
3. They serve as carriers for both high and low molecular weight medications, such as insulin, sex hormone, analgesics, corticosteroids, anesthetics, and gap junction proteins.
4. The hydrophobic and hydrophilic moieties combined in the structure of transfersomes allow them to accommodate medicinal molecules with a broad range of solubility.[5]

Limitations of Transfersomes:
1. Because of their propensity for oxidative destruction, chemical transfersomes are unstable.
2. Natural phospholipids are pure.
3. Formulating transfersomes is costly [6].

Composition of Transfersomes:
The two main parts of the transfersome are as follows:
- The amphipathic ingredient (phosphatidylcholine, for example) self-assembles into a lipid bilayer in an aqueous solvent to produce a basic vesicle.
- The lipid bilayer membrane exhibits enhanced flexibility and permeability due to the presence of a bilayer component, such as an amphiphilic medication or biocompatible surfactant. A transfersome vesicle can quickly and readily restore its shape by changing the concentration of a bilayer
component, such as an amphiphilic medication or surfactant. Therefore, physical characteristics like
globule size, softer, more flexible, and better modifiable artificial membrane distinguish the
transfersome from ordinary vesicles.

Vesicle formation (from small to large vesicle):
The solute to be entrapped was present in the vesicular solution, which was then heated to 25°C. The
surfactant concentration was then quickly added to the solution and stirred, resulting in a final
combination with a 1:2 ratio of surfactant to phospholipids. Large vesicles begin to form right away, and
as the solution's light scattering increases, the small vesicles' nearly clear appearance changes to a
transparent opalescence.

At 40°C, a vesicle forms in 15–30 minutes. Next, the extra detergent was wiped off. The surfactant
residue can be detergent that has become stuck inside the vesicle.

General procedures for vesicular preparations are:
- **Direct hydration**: The process of organic solvent hydration is utilized to produce multilamellar
  vesicles (MLVs). Large unilamellar vesicles are produced by MLVs extrusion through 0.2 μm and
  0.45 μm polycarbonate filters, reverse phase evaporation, and detergent removal. The process of
  sonication creates tiny unilamellar vesicles. The direct hydration technique has the benefit of being a
  quick process, but it also has drawbacks, such as uneven solute distribution and low trapped volume
  and efficiency.

- **Hydration from organic solvent**: One benefit of hydration via organic solvent approach is its high
  drug-trapping effectiveness; nevertheless, one drawback is its restricted lipid solubility in the organic
  phase.

- **Detergent removal**: The method of detergent removal offers benefits such as increased trapped
  volume and protein reconstitution; however, it has drawbacks such as low drug efficacy and
difficulty in entirely removing detergents [7,8].

METHODS AND MATERIAL

**Materials:**
Vitex Negundo plant, Potato starch, Span 80 and Tween 80, Chloroform, phosphate buffer.

**Method:**
Thin film hydration technique is employed for the preparation of transfersomes which comprised
of three steps:
- Vitex negundo extract and surfactant, which are the elements that create vesicles, are dissolved in a
  volatile organic solvent (chloroform-methanol) to form a thin film. Using a rotary evaporator, the
  organic solvent is then evaporated above the lipid transition temperature of 45°C for pure PC
  vesicles. To create a dry lipid layer, the last remnants of solvent were extracted under vacuum for a
  whole night.
- A thin film that has been created is hydrated using phosphate buffer saline (pH 7.4) by rotating at 60
  rpm for a duration of one hour at the appropriate temperature. At room temperature, the resultant
  vesicles swelled for two hours.
- The tiny vesicles were prepared by sonicating them for 30 minutes at either room temperature or
  50°C using a bath sonicator or a probe sonicated for 30 minutes at 4°C. Ten hand extrusions across a
sandwich of 200 and 100 nm polycarbonate membranes were used to homogenize the sonicated vesicles.[9]

PREPARATION OF VITEX NEGUNDO-LOADED TRANSFERSOMES:
Using a traditional rotary evaporation process, transfersome formulations were created utilizing a medication, vitex negundo extract and surfactant (Span 80 and Tween 80). Every formulation has the same amount of medication. Various formulations were made with varying surfactant ratios. Phospholipid, surfactant, and medication were weighed precisely and placed in a dry, clean round-bottom flask. A little amount of a mixture of chloroform and methanol was then added to dissolve the lipid mixture.

The final traces of solvent were eliminated under vacuum by rotating evaporation at 45°C with decreasing pressure. Multi-lamellar vesicles are produced when the deposited lipid film is hydrated with the phosphate buffer (pH 7.4) by rotating at 60 rpm for one hour at room temperature. By forming tiny unilamellar vesicles with a bath sonicator, they were further decreased in size [10].

Preparation of Preliminary Formulation of Vitex Negundo Transfersomes:
In preliminary batches, different concentration of Vitex Negundo and other excipients used to make a final Trasfersomal suspension.

Table 6- Prepared preliminary Formulations

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Formulation code</th>
<th>Drug (ml)</th>
<th>Potato Starch (gm)</th>
<th>Tween 80 (ml)</th>
<th>Dichloromethane (ml)</th>
<th>Methanol (ml)</th>
<th>Ethanol (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F-1</td>
<td>0.3</td>
<td>2</td>
<td>3</td>
<td>10</td>
<td>5</td>
<td>-</td>
</tr>
</tbody>
</table>
Preparation of topical transfersome gel:
Carbopol gels were created as a medium for the integration of Transfersomes for therapeutic administration. The aqueous dispersion of transfersomes was used to make the topical gel. Transfersomal gel was prepared using a gel polymer such as carbopol-934. To prevent the production of dispersible lumps, a 0.5 gram powder of carbopol-934 was mixed violently with distilled water using a magnetic stirrer Remi5MLH. The mixture was left to hydrate for a whole day. Subsequently, a tiny quantity of triethanolamine was gradually added to the neutralized pH 7.4 and mixed until a transparent gel was visible. Next, a gel was created by adjusting the base's quantity, and the pH was determined using a pH meter (Equip-Tronics Eq-610) [11].

<p>| | | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>F-2</td>
<td>0.2</td>
<td>5.2</td>
<td>5</td>
<td>15</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>F-3</td>
<td>0.4</td>
<td>4.7</td>
<td>2</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Figure 9- Preliminary Formulations of Transfersomal Suspension

Figure 10 - Prepared Vitex Negundo transfersomal gel
CHARACTERIZATION AND EVALUATION OF VITEX NEGUNDO TRANSFERSOMES

CHARACTERIZATION OF VITEX NEGUNDO DRUG:

Boiling Point:
Approximate amount of Vitex Negundo drug fill in fusson tube. One end of capillary is seal. Fussion tube and capillary tube is attach to the thermometer and that thermometer is place in to the thiele tube. With the help of burner heat supply to the tube meanwhile the temperature of oil increased the drug present in fussion tube start bubble from capillary tube is start to and note down the Boiling point of drug [12].

Spectrofluorometric and UV System:
Spectral was runs on a Shimadzu Ultra Violet (UV)-Visible spectrophotometer and the spectral bandwidth of 0.5 nm and wavelength accuracy of ± 0.3 nm with wavelength 247 nm with 10 mm quartz cells[13].

Chemical Test

1. Test for carbohydrates
Add 1 ml molisch’s reagent a few drop of concentrated sulfuric acid and 2 ml of vitex negundo extract carbohydrates are indicated by presence of purple or reddish color.

2. Test for tannins and phenol
Vitex negundo extract combined with 2ml 5% FeCl3 solution. Phenol and tannin indicated by blue-green or black color.

3. Test for flavonoids
A small amount of plant extract mixed with 1 or 5 drops of concentrated hydrochloric acid. Presence of flavonoid indicated by rapid development of red color.

4. Test for saponins
Vitex negundo extract combined with 5ml of distilled water and shaken briskly. Formation of foam indicates presence of saponins.

5. Killer-kilani test
2ml of glacial acetic acid containing 1 or 2 drops of 2% FeCl3 solution were combined with vitex negundo extract. After that, mixture was transferred to second test tube that holds 2 ml of concentrated H2SO4. Presence of cardiac glycoside was indicated by brown ring at interphase.

6. Test for terpenoids
Vitex negundo extract combined with 2ml chloroform. It was dried out. After adding 2ml of concentrated H2SO4, heated for approximately 20 min. Grayish color indicates the presence of terpenoids.

7. Test for quinines
Concentrated HCL was added in small amount of vitex negundo extract, formation of yellow colored indicates presence of quinines.

8. Test for alkaloids
A test tube is filled with 2ml of vitex negundo extract, 0.2 ml of diluted HCL and 1 ml of mayer’s reagent. The presence of alkaloid is indicated by a yellowish color.
Characterization of Transfersomal Formulation:

**Figure 12- Light Microscope**

**Vesicle morphology:**
Photon correlation spectroscopy or DLS method generally used for vesicle diameter determination. Prepared sample in distilled water was filtered through 0.2 mm membrane filter and diluted with filtered saline and then size measurement done using photon correlation spectroscopy or DLS measurements. Transmission electron microscopy (TEM) and phase contrast microscopy can be commonly used for visualization of transfersomes vesicles. The stability of vesicle can be determined by assessing the size and structure of vesicles with respect to time. DLS and TEM used for mean size and structural changes, respectively\(^{[14,15,17,18]}\).
Degree of deformability:
The deformability study is done by taking pure water as standard. Transfersomes preparation is passed through a number of pores of known size (through a sandwich of different microporous filters, with pore diameter between 0.2µ, 0.45µ and 1µ depending on the starting transfersomes suspension)[19].

**In vitro drug Release Study:**
Modified Franz diffusion cell with a receiver compartment volume of 22 ml and effective diffusion area of 2 cm² was used for this study. In vitro drug release study was performed by using 0.45µ cellophane membrane in phosphate buffer solution (pH 7.4). To perform in vitro drug release study, cellophane membrane was mounted horizontally on the receptor compartment of franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2 cm² and capacity of receptor compartment was 22 ml. The receptor compartment was filled with 22 ml of phosphate buffer (pH 7.4) maintained at 37±0.5°C and stirred by a magnetic bar at 100 rpm. Transfersome gel formulation equivalent to 5 mg drug was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 3 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 7.4) to maintain sink conditions. The samples were analysed spectrophotometrically at 247 nm [20].

**Temperature**: 37±0.5°C  
**Diffusion Medium**: 7.4 pH Phosphate Buffer  
**Volume of Diffusion medium**: 22 ml  
**Aliquot withdrawn**: 3ml  
**Aliquot Replaced**: 3ml

Physical Appearance and determination of pH:
Outward look The Vitex Negundo transfersomal gel formulations that were made were visually evaluated for pH, homogeneity, color, and consistency. Using a digital pH meter (Equip-Tronics Eq-610), the pH values of the produced gel's 1% aqueous solutions were determined [21].

Spreadability:
After 60 seconds, the spreadability of the Vitex Negundo gel formulation was measured by measuring the diameter of 1 gm of gel between horizontal plates with an area of 20 by 20 cm². The device, which consists of a wooden block with a pulley at one end, was used to measure spreadability. A gram or so of gel was applied to the ground slide. Next, the gel was attached with a hook and placed between this slide and a second glass slide that was secured to the ground. For five minutes, a one-kilogram weight was positioned over the two slides to eliminate any air and create a consistent layer of gel between them. The borders had excess gel removed from them. After that, a 20 gram weight was used to pull on the top plate. With the string fastened to the hook, record the number of seconds needed for the top slide to travel 6 cm. a reduced interval, signifying strong spreadability. Spreadability was determined by applying the subsequent formula:

\[ S = M \times L / T \]

Where,  
\( S \) = Spread ability,  
\( M \) = Weight in the pan (tied to the upper slide),  
\( L \) = Length moved by the glass slide (6 cm),
T= Time (in sec) taken to separate the slide completely each other\textsuperscript{[22,23,24]}.

**Drug Content:**
Using a UV spectrophotometer, the drug content was estimated. 150 mg of gel was extracted, dissolved in methanol, filtered, and then made up to a volume of 100 ml using phosphate buffer with a pH of 7.4. A 10 ml aliquot was created by adding solvent to 1 ml of it. The absorbance in the UV spectrophotometer was measured at 247 nm. At 247 nm, the resultant solutions were measured \textsuperscript{[31]}.

**Result and Discussion**

**Boiling point**
The boiling point of methanolic extract of vitex negundo was found to be 105°C.

**UV spectroscopic determination**
In the present study UV –vis spectral profile showed peak 640 nm.

![UV Spectroscopic Determination](image)

**TLC**
TLC of methanolic extract of vitex negundo revealed the presence of spots having RF value of 0.70 when solvent phase of chloroform, ethanol, ethyl acetate, acetic acid, hexane in ratio of (10:2:5:1:1) a solvent system is used.

![TLC Image](image)
Phytochemical test
Flavonoids, saponins, cardiac, glycoside, terpenoids, Quinones, Alkaloids, Carbohydrates present in methanolic extract of vitex negundo.

Degree of Deformability

F-1 Before

F-1 After
### In Vitro Drug Release Study

<table>
<thead>
<tr>
<th>Time (hrs)</th>
<th>% DR of F-1 SD</th>
<th>% DR of F-2 SD</th>
<th>% DR of F-3 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>9.71</td>
<td>12.453</td>
<td>12.962</td>
</tr>
<tr>
<td>2</td>
<td>12.547</td>
<td>17.702</td>
<td>18.233</td>
</tr>
<tr>
<td>3</td>
<td>14.811</td>
<td>23.192</td>
<td>23.797</td>
</tr>
<tr>
<td>4</td>
<td>21.245</td>
<td>26.16</td>
<td>27.04</td>
</tr>
<tr>
<td>5</td>
<td>25.381</td>
<td>29.428</td>
<td>30.031</td>
</tr>
<tr>
<td>6</td>
<td>44.917</td>
<td>39.377</td>
<td>44.83</td>
</tr>
<tr>
<td>7</td>
<td>71.319</td>
<td>59.703</td>
<td>62.604</td>
</tr>
<tr>
<td>8</td>
<td>94.912</td>
<td>83.678</td>
<td>91.267</td>
</tr>
</tbody>
</table>
Reference