INVASOMES NOVEL VESICULAR CARRIERS FOR TRANSDERMAL DRUG DELIVERY
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ABSTRACT
Invasomes are the liposomal vesicles embodying small amounts of ethanol and terpenes or terpene mixtures, which act as potential carriers with increased skin penetration. Invasomes have higher penetration rate through the skin as compared to liposomes and ethosomes. Invasomes provide a number of advantages including improving the drug’s efficacy, enhancing patient compliance and comfort. This article reviews various aspects of invasomes including their preparation, characterization, potential advantages in drug delivery. Enhanced delivery of drugs through the skin and cellular membranes by means of an invasomal carrier opens numerous challenges and opportunities for research and future development of novel improved therapies. This review is mainly focused on effectiveness and permeation enhancing controversy of lipid vesicles as dermal and transdermal drug delivery with special emphasis on recent advances in this field, including the development of deformable vesicles, ethosomes and invasomes. Only specially designed lipid vesicles have been shown to be capable of achieving enhanced delivery. The incorporation of additives, such as anionic surfactants and ethanol, fluidize the phospholipid bilayers, thus can penetrate the intercellular pathways of the skin.
INTRODUCTION:
Transdermal delivery provides a leading edge over invasive methods and conventional oral routes increasing patient compliance and avoiding first pass metabolism respectively.\textsuperscript{[1]} It brings forth many attractive advantages over other routes of administration, like sustained and controlled delivery over a prolonged period of time, reduction in side effects associated with systemic toxicity, direct access to target or diseased site, convenient and painless administration and so on.\textsuperscript{[2]}
The major limitation for topical drug delivery is the low diffusion rate of drugs across the stratum corneum (SC), which acts as the barrier.\textsuperscript{[3,4]} SC is the outermost layer of skin and its structure is often compared to a brick wall, with the keratin rich corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae.\textsuperscript{[5]} This layer consists of cells enriched with keratin embedded in lipid lamellae. This layer consists of cells enriched with keratin embedded in lipid lamellae. The highly organized crystalline lipid lamellae play an essential role in the barrier properties of the SC.\textsuperscript{[6]} Many approaches have been aimed to disrupt or weaken the highly organized intercellular lipids of SC such as chemical enhancers, iontophoresis, microneedles, vesicles, nanoparticles, etc.\textsuperscript{[7,8]} Since, past two decades colloidal lipid aggregates (liposomes) were developed as vesicular drug carrier systems. Vesicles are used in dermal and transdermal drug delivery as they might:\textsuperscript{[9]}

\begin{itemize}
  \item Act as drug carriers to deliver entrapped drug molecules into or across the skin
  \item Act as penetration enhancers for the penetration of the individual lipid components into the SC and subsequently altering the intercellular lipid lamellae within this skin layer
  \item Serve as a depot for sustained release of dermally active compounds
  \item Serve as a rate limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled transdermal delivery system.
\end{itemize}

There are several new vesicle types, depending on the additives used for vesicle preparation. Such as niosomes, transferosomes, ethosomes, flexosomes, invasomes, vesosomes, ufasomes, and polymerosomes.\textsuperscript{[10]}

Only specially designed liposomes have been shown to be capable of achieving enhanced delivery. The incorporation of additives, such as anionic surfactants and ethanol, can fluidize the phospholipid bilayers, thus increasing the depths to which liposomes can penetrate the intercellular pathways of the skin. Also, liposomes that have been conjugated with PEG or antibodies can increase the residence time of anticancer drugs in the circulation and enhance drug accumulation in tumors. A new class of lipid vesicles is the highly deformable (elastic or ultra flexible) liposomes, which have been termed Transfersomes. Recent studies have reported that deformable liposomes
were able to improve in vitro skin delivery of various drugs. Ethosome is another novel lipid carrier, recently developed by Touitou et al., showing enhanced skin delivery.\textsuperscript{[7,10]} Invasomes are novel vesicles incorporating terpenes with enhanced penetration compared to the conventional liposomes. These are soft liposomal vesicles with very high membrane fluidity, containing terpenes, which are playing the role of penetration enhancement.\textsuperscript{[29]} The presence of terpenes and ethanol makes invasomes unique. These vesicles have shown to possess the combined advantages of liposomes, which are potential carriers and penetration enhancement of the terpenes, which are having the ability to modify the order of SC packing thus promoting skin delivery.\textsuperscript{[30,31]} Terpenes, the naturally occurring volatile oils are included in the list of generally recognized as safe substances with low irritancy\textsuperscript{[31]} at lower concentrations (1-5%), with reversible effect on the lipids of SC are considered as clinically acceptable penetration enhancers.

**ADVANTAGES**

- Non-invasive technique of drug delivery.\textsuperscript{[20]}
- Enhanced permeation of drug through the skin for transdermal drug delivery.\textsuperscript{[21]}
- Delivery of hydrophilic\textsuperscript{[16]} and lipophilic\textsuperscript{[16]} drugs is possible.
- Contains non-toxic raw material in formulation.\textsuperscript{[21]}
- Patient compliance as the drug can be administered as semisolid form (gel or cream).\textsuperscript{[21]}

Simple method for drug delivery in comparison to iontophoresis and phonophoresis and other complicated methods.\textsuperscript{[21]}

**DISADVANTAGES**

The various disadvantages associated with Invasomes are:

- Its high production cost
- Leakage and fusion of encapsulated drug / molecules.
- The phospholipids present may undergo oxidation or hydrolysis, thus affecting stability of Invasomes.\textsuperscript{[10]}

**PENETRATION ENHANCEMENT MECHANISM**

A combination of processes contributes to the enhancing effect of the invasomes. The SC lipid layers at physiological temperature are densely packed and highly conformationally ordered. Ethanol is known for its disturbance of skin lipid bilayers organization; therefore, when integrated into a vesicle membrane, it gives that vesicles the ability to penetrate the SC.\textsuperscript{[18]} Furthermore because of the presence of ethanol, the lipid membrane is packed less tightly than conventional vesicles, but has equivalent stability, allowing a more malleable structure, giving it more freedom and ability to squeeze through small places such as the openings created in disturbing the SC.
Ethanol interacts with lipid molecules in the polar head group region, resulting in reducing the rigidity of the SC lipids, and increasing their fluidity. In addition to the effect of ethanol on SC structure, the vesicle itself may interact with the SC barrier. The inter digitated, malleable vesicle can forge paths in the disordered SC.

**Invasomes as carriers for skin delivery systems and their mechanism:**

Several lipophilic and hydrophilic penetration enhancers (i.e. labrasol, transcutol and cineole) were tested and penetration enhancer – containing vesicles have been introduced where there is enhancement in penetration due to the penetration enhancers. Such penetration enhancer containing vesicles with terpenes as penetration enhancers were termed as invasomes. Invasomes composed of phosphatidylcholine, ethanol and a mixture of terpenes as penetration enhancers have been introduced by Verma and Fahr’s group. In addition to the mechanism of penetration enhancement of elastic vesicles terpenes which are considered as potent penetration enhancers increase drug permeation by disrupting lipid packaging of stratum corneum and/or disturbing the stacking of the bilayers. Penetration enhancer-containing vesicles have been used as carriers for Minoxidil, Diclofenac and Temoporfin.

![Cross section of the human skin](image.png)
### Table 1: Different vesicles as carriers in transdermal delivery

<table>
<thead>
<tr>
<th>Vesicle</th>
<th>Composition</th>
<th>Permeant</th>
<th>Description</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liposome</td>
<td>Phospholipids and cholesterol</td>
<td>Triamcinolone acetonide</td>
<td>Four to five fold increase in drug concentration in epidermis and dermis when compared with conventional formulations</td>
<td>[11]</td>
</tr>
<tr>
<td>Niosome</td>
<td>Composed of non-ionic amphiphiles[surfactants]</td>
<td>Ammonium glycyrrhizinate</td>
<td>Improved percutaneous permeation by bolasurfactant</td>
<td>[12]</td>
</tr>
<tr>
<td>Transferosome</td>
<td>Phospholipids, cholesterol and an edge activator</td>
<td>Valsartan</td>
<td>When compared to rigid liposomes, amount of drug permeated was enhanced by 33.97 fold</td>
<td>[13]</td>
</tr>
<tr>
<td>Ethosomes</td>
<td>Phospholipid, ethanol and water</td>
<td>Econazolenitrate</td>
<td>Percent drug diffused was two fold higher than liposomal and hydroethanolicgels</td>
<td>[14]</td>
</tr>
<tr>
<td>Flexosome</td>
<td>Contained phospholipid, an edge activator and positively or negatively charged lipids</td>
<td>Low molecular weight heparin</td>
<td>2.6 fold higher permeability co-efficient than Ethosomes</td>
<td>[15]</td>
</tr>
<tr>
<td>Invasome</td>
<td>Composed of phosphatidylcholine, ethanol and terpene</td>
<td>Temoporfin</td>
<td>Enhanced deposition of drug (3.87 fold) in stratum corneum when compared to liposomes</td>
<td>[16]</td>
</tr>
<tr>
<td>Vesosomes</td>
<td>Large lipid bilayer enclosing many smaller Liposomes</td>
<td>Tetanus toxoid</td>
<td>Effective for topical delivery of vaccines</td>
<td>[17]</td>
</tr>
<tr>
<td>Ufasomes</td>
<td>Fatty acid vesicles</td>
<td>Methotrexate</td>
<td>Three to four fold increase in permeation when compared to plain drug solution</td>
<td>[18]</td>
</tr>
<tr>
<td>Polymersomes</td>
<td>Self assembled vesicles of diblock/triblock copolymers</td>
<td>Insulin</td>
<td>Enhanced insulin activity</td>
<td>[19]</td>
</tr>
</tbody>
</table>

**METHODS OF PREPARATION**

- **Mechanical Dispersion Technique**

Drug and terpene or mixtures of terpenes are dissolved in ethanolic phospholipid solution. The mixture is vortexed for 5 min and then sonicated for 5 min in order to obtain a clear solution. Phosphate buffer saline (PBS) (pH: 7.4) is added to the solution by a syringe under constant vortexing. The vortexing is continued for an additional 5 min. The last step is the extrusion of
multilamellar vesicles through polycarbonate membranes of different pore sizes. The invasome dispersions are extruded through each polycarbonate membrane for several times. \[29,27,28\]

**Figure: 2 Mechanical dispersion Technique of Invasomes**

- Film Hydration Technique

Invasomes can also be prepared by the conventional film method. Phospholipids in ethanol are dissolved in methanol: Chloroform(2:1, v/v). This mixture is dried to a thin film by slowly reducing the pressure from 500 to 1 mbar at 50°C using the rotary flash evaporator. The film is kept under vacuum (1 mbar) for 2 h at room temperature and subsequently flushed with nitrogen. Then, the film deposited is either hydrated for 30 min at lipid phase transition with a mixture of phosphate buffer (pH: 7.4; PBS) containing ethanol and terpenes or it is hydrated using PBS (pH: 7.4) and after cooling to room temperature, ethanol and a single terpene or a terpene mixture are added in order to obtain invasomes. The obtained vesicles are vortexed, ultrasonicated and subsequently
sized by extrusion for several times through polycarbonate membranes of different pore sizes.\textsuperscript{[41,38,39]}

**CHARACTERIZATION:**

- **Vesicle shape**

Invasomes can be easily visualized by using transmission electron microscopy (TEM) and by scanning electron microscopy. Temoporfin vesicles were reported to be spherical and oval shape, unilamellar, bilamellar and also oligolamellar.\textsuperscript{[17]} Finasteride invasomes were reported to be unilamellar and spherical shape.\textsuperscript{[22]} By cryo-TEM carboxyfluoresce in invasomes and temoporfin invasomes were reported to be almost unilamellar and bilamellar.\textsuperscript{[23]} Hence, invasomes are observed as spherical or deformed vesicles with uni, bi, or oligolamellarity. Vesicle size and zeta potential Particle size of the invasomes can be determined by dynamic light scattering (DLS) and photon correlation spectroscopy.

With temoporfin invasomes, the sizes of vesicles were reported to increase with increasing the amount of terpenes in the vesicles. Wherein invasomes with carvone (molecular size 150.22 g/mol) resulted in vesicles of $4.54 \pm 0.30 \mu m$ to $4.80 \pm 0.01 \mu m$ and with nerolidol (molecular size 222.37 g/mol) vesicles of $11.23 \pm 0.26 \mu m$ to $13.00 \pm 0.20 \mu m$.\textsuperscript{[22]} Hence, vesicle size is influenced by the
molecular size of the terpene incorporated\[^{22}\] and the concentration of terpene mixture added\[^{17}\]. Zeta potential of the formulation can be measured by Zetasizer.

- **Drug entrapment**
  The entrapment efficiency of invasomes can be measured by the ultra-centrifugation technique or the other techniques used for determining entrapped drug in vesicles.\[^{41}\] With finasteride invasomes maximum entrapment efficiency was reported with hydrophobic terpene limonene and minimum entrapment efficiency with nerolidol.\[^{45}\] With limonene, maximum entrapment was reported with 0.5% concentration (84.56 ± 0.25%) when compared with 1.5% concentration (71.56 ± 0.20%).\[^{22}\] Entrapment efficiency was found to be influenced by hydrophilicity of drug and terpene added and concentration of terpene added.\[^{28,22}\]

- **Drug content**
  Drug content of the invasomes can be determined using ultraviolet spectrophotometer. This can also be quantified by a modified high performance liquid chromatographic methods.

- **Stability studies**
  The stability of vesicles can be determined by assessing the size and structure of the vesicles over time. Mean size is measured by DLS and structure changes are observed by TEM. Both temoporfin and finasteride invasomal suspensions were reported to be stable at 4°C.\[^{16,22}\]

- **In vitro skin permeation studies**
  For the penetration studies human abdominal skin after the removal of the subcutaneous fatty tissue, obtained after plastic surgery, can be used. The diffusion studies can be carried out by using Franz diffusion cells, with PBS (pH: 7.4) in the receptor compartment. The ability of the invasomal preparation to penetrate into the skin layers can be determined by using confocal laser scanning microscopy. Curic et al.\[^{26}\] reported invasomes with 1% terpenes delivered temoporfin 2.7-fold higher than liposomes containing 3.3% ethanol. Moreover 1% terpenes, invasomes showed 2-fold higher deposition of temoporfin in the SC when compared to the ethanolic solution and 3.5-fold higher deposition when compared to conventional liposomes.\[^{22}\] Chen et al. reported the influence of dose applied with carboxyfluorescien and temoporfin invasomes. With finite dose, carboxyfluorescien delivered into the deep skin layer was 52.9% and with infinite dose, it decreased to 37.8%. With temoporfin, with finite dose 8.8% was delivered into deep skin layer and with infinite dose 6.1%.\[^{23}\]

**CONCLUSION**
In order to overcome the barrier properties of SC several techniques were developed, including iontophoresis, electroporation, ultrasound, chemical penetration enhancement using different
penetration enhancers and the use of vesicular systems, i.e., liposomes, ethosomes. One such technique is the formulation of invasomes, which could be a promising tool for delivering drugs through the skin and can provide better skin permeation than liposomes. Invasomes have been tested to encapsulate hydrophilic drugs and hydrophobic drugs. Hence, they can open up new challenges and opportunities for the development of novel improved therapies.

REFERENCES:


