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Review Article

REVIEW ON EMERGING ROLE OF INVASOMES IN TOPICAL AND TRANSDERMAL DRUG DELIVERY

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ABSTRACT
Transdermal drug delivery systems (TDDS) have emerged as an effective alternative to conventional drug administration by overcoming first-pass metabolism, improving patient compliance, and providing controlled drug release. However, the barrier properties of the stratum corneum significantly restrict the permeation of many therapeutic agents. To address this limitation, novel vesicular carriers such as invasomes have gained considerable attention due to their superior skin penetration and enhanced drug delivery capabilities. Invasomes are elastic phospholipid vesicles composed of phospholipids, ethanol, and terpenes, which act synergistically to disrupt the stratum corneum and facilitate transdermal transport of both hydrophilic and lipophilic drugs. This review summarizes the composition, structure, mechanism of skin permeation, methods of preparation, physicochemical characterization, and evaluation parameters of invasomes. It also discusses the influence of formulation components, including ethanol and terpenes, on vesicle properties and drug permeation. Furthermore, the review highlights the pharmaceutical applications of invasomes in the delivery of antifungal, anti-inflammatory, antihypertensive, anticancer, immunosuppressive, and anti-acne agents. Recent advances demonstrate that invasomes improve drug bioavailability, enhance skin permeation, prolong drug release, and reduce systemic adverse effects. Owing to their versatility, biocompatibility, and efficient transdermal delivery, invasomes represent a promising nanocarrier platform for both topical and systemic drug administration. Future research should focus on large-scale manufacturing, long-term stability, regulatory considerations, and clinical translation to facilitate the commercialization of invasome-based drug delivery systems.

Keywords: Transdermal Drug Delivery System, Invasomes, Vesicular Drug Delivery, Terpenes, Ethanol, Skin Permeation, Penetration Enhancers, Nanocarriers, Controlled Drug Delivery, Topical Drug Delivery.

INTRODUCTION
Currently, oral delivery of drugs is the most prevalent mode of drug delivery. Even though it has the benefit of ease of delivery, it still comes with its demerits such as poor bioavailability as a result of first-pass effect and formation of peaks of blood concentration (high and low), hence requiring a large amount of the dose which is both costly and cumbersome. There is, therefore, need for the creation of a new drug delivery system that will enhance the safety and efficiency of drugs through site-specific, spatial, and temporal delivery of the drug within the body

hence resulting in smaller and fewer doses. New drug delivery system is important for the delivery of new pharmaceutical products (peptides and proteins) through gene engineering without causing any immunological problems and deactivation. Transdermal drug delivery can be defined as self-contained, distinct dosage systems that, upon application on the skin surface, release the drug to the systemic circulation through the skin at controlled rates. Transdermal drug delivery system (TDDS) has proven to be one of the components of the novel drug delivery systems (Jain, 2002).

Drugs administered in the conventional dosage forms usually produce large range in fluctuations in plasma drug concentrations leading to undesirable toxicity or poor effectiveness. These factors as well as other factors such as repetitive dosing and unpredictable absorption, led to the concept of the controlled drug delivery system or therapeutic system. A dosage form that releases one or more drugs continuously in a predetermined pattern for a fixed period of time, either systemically or to a specified target organ is a controlled drug delivery system. The primary objectives of controlled drug delivery are to ensure safety and to improve efficacy of drugs as well as patient compliance. This is achieved by better control of plasma drug levels and less frequent dosing. Transdermal therapeutic systems are defined as self-contained discrete dosage forms which, when applied to the intact skin, deliver the drug(s), through the skin, at controlled rate to the systemic circulation. The first Transdermal drug delivery (TDD) system, Transderm-Scop developed in 1980, contained the drug Scopolamine for treatment of motion sickness (Chowdary and Naidu, 1995).

Basic Components of TDDS

Polymer matrix

i) Polymer is a crucial and indispensable part of the transdermal drug delivery system. Rate-controlled medication delivery has been accomplished using a variety of polymeric material types.

ii) The physicochemical characteristics of the drug and polymer used in the device's construction determine the drug release mechanism.

iii) For a polymer to be employed in a transdermal system, it must meet the following requirements.

- The polymer’s chemical functionality, glass transition temperature, and molecular weight must permit the diffusion and release of the particular medication.
- The polymer should make it possible to include a significant quantity of drug.
- Neither physically nor chemically should the polymer and the medication interact.
- The polymer should be pricey and simple to build into the required product.
- In the presence of the medicine and any other excipients used in the formulation, at high humidity levels, or at body temperature, the polymer must be stable and must not disintegrate (Hadgraft and Guy, 2004).

Drug substance

Choosing the right drug substance is crucial to the creation of a successful transdermal product. Important pharmacological

characteristics that influence how well it diffuses through the apparatus and through the skin include:

Physical and chemical attributes

- The drug's molecular weight should be below 600 Dalton.
- The log P should fall between 1 and 7
- The melting point must be below 200° C.
- The minimum number of hydrogen bonding groups is two.
- It must have an advantageous oil:water partition coefficient.
- Transdermal administration is not appropriate for medications that are strongly acidic or alkaline in solution.
- Solubility should be more than 1 mg/ml in both mineral oil and water (Heather, 2005).

Penetration Enhancers:

- They are regarded as an essential component of the majority of transdermal formulations and increase skin penetration.
- They can alter the skin's resistance to penetration by reacting with the skin's surface or the applied substance.

The following qualities should be present in an ideal penetration enhancer:

- Pharmacological inertness, affordability, and Cosmetically Acceptable.
- Nontoxic.
- Nonirritating.
- Nonallergenic.
- Quick onset; predictable and appropriate duration of action for the medicine used, Chemical penetration enhancers' reversible impact on the stratum corneum's barrier properties.
- Compatible with the delivery system both chemically and physically.
- Easily fitted into the delivery system (Azhar *et al*., 2011).

Two types of principles have been employed to increase drug permeation through skin;

- Physical Enhancers
- Chemical Enhancers

Types of Permeation Enhancers:

Sulfoxides Dimethylsulfoxide (DMSO)

- It is an efficient penetration enhancer that enhances permeation by lowering skin resistance to drug molecules or promoting drug partitioning from the dose form.
- It has been proposed that DMSO either denatures the intercellular structural proteins of the SC or enhances lipid fluidity by disrupting the ordered structure of the lipid chains.
- DMSO may also change the physical structure of the skin via elution of lipid, lipoprotein, and nucleo protein components from the SC.

Alcohols

- Alcohols can alter transdermal penetration through a variety of methods. The alkanols alkyl chain length is a key characteristic in the promotion of permeability enhancement.

Polyols

- Propylene glycol action is hypothesised to come from keratin solvation inside the SC; the occupancy of proteinaceous hydrogen bonding sites lowering drug tissue binding and hence enhancing penetration.

d) Alkanes

- Long chain alkanes (C7-C16) have been demonstrated to improve skin permeability via non-destructively altering the SC barriers.

Adhesives

All transdermal devices must be adhered to the skin with a pressure sensitive adhesive that can be applied to the face or the rear of the device. Both adhesive layers must meet the following requirements:

- When in touch with the skin, it should not produce irritation, sensitization, or an imbalance in the natural skin flora.
- Should actively adhere to the skin (Ahmed *et al*., 2011)

Marketed products

The market for transdermal products has been in a significant upward trend that is likely to continue for the future. More than 35 TDD products have been approved for sale in the US, and approximately 16 active ingredients are approved for use in TDD products globally. The table gives detail information of the different drugs which are administered by this route and the common names by which they are marketed, it also gives the conditions for which the individual system is used (Koteshwar *et al*., 2020).

Table 1: TDDS Marketed Products

Product name	Drug	Indication
Alora	Estradiol	Post menstrual syndrome
Androderma	Testosterone	Hypogonadism in males
Captapres-TTS	Clonidine	Hypertension
Climaderm	Estradiol	Post menstrual syndrome
Climara	Estradiol	Post menstrual syndrome
Combipatch	Estradiol/Nore thindrone	Hormone replacement therapy
Deponit	Nitroglycerin	Angina pectoris
Duragesic	Fentanyl	Moderate/severe pain

Applications of TDDS

- Nicotine transdermal patch marketed as Nico dermis to help in smoking cessation. It is the highest selling patch in United State.
- Two opioid medications Fentanyl (marketed as Duragesic) and Buprenorphine (marketed as BuTrans) used to provide round-the-clock relief for severe pain available in patch form
- Estradiol patches available as Estraderm for treat menopausal symptoms as well as postmenopausal osteoporosis. It is also available in combination with levonorgestrel as Climara Pro for menopausal symptoms.
- Nitroglycerin transdermal patches for the treatment of angina pectoris, prescribed in place of sublingual pills.
- Transdermal patch of clonidine available for treatment of hypertension.
- Transdermal patch of the selegiline (MAO inhibitor) became the first transdermal delivery agent for major depressive disorder.

- Transdermal delivery agent Methylphenidate for the Attention Deficit Hyperactivity Disorder (ADHD) (Sankar *et al*., 2003).

Invasomes

Liposomal vesicles known as invasomes contain small amounts of ethanol and terpenes, or terpene combinations, and they have the ability to penetrate the skin more deeply than other carriers. When it comes to skin penetration, invasomes are more common than liposomes and ethosomes. Improved drug efficacy, patient compliance, and comfort are just a few of the benefits that come with invasomes (Moradi *et al*., 2019). Invasomes are a unique category of vesicles that are generally responsible for improving the transdermal penetration of active medicinal substances. These vesicles' structure includes phospholipids, ethanol, and unique terpenes or terpene combinations. As These components exhibited remarkable transdermal penetration abilities.

Controlled drug delivery system has been designed to obtain an optimal drug action and targeting the drug to the particular sites in order to reduce the side effect and improve therapeutic efficacy by preventing undesired drug localization in healthy tissue site and decreasing rapid degradation or elimination of drugs. Transdermal drug delivery system is used as an alternative delivery of drug into the systemic circulation. Although advantageous in avoiding problems of poorly absorbable drugs and enzymatic degradation. In order to increase the number of drugs administered via transdermal route, novel drug delivery systems has to be designed. These systems include physical means, such as iontophoresis, sonophoresis, micro needles, etc. and chemical means like penetration enhancers and biochemical means using liposomes, niosomes, invasomes, transferosomes and ethosomes also have been reported to enhance permeability of drug through the stratum corneum (Syeda and Krishna, 2017). Invasomes are novel vesicles with enhanced percutaneous penetration compared to the conventional liposomes. Invasomes are novel elastic phospholipid vesicles composed of phosphatidylcholine, ethanol and one or mixture of terpenes. Many researchers have already confirmed the capability of terpenes in enhancing percutaneous penetration. Their penetration-enhancing activity is through the disruption of the stratum corneum lipids, interaction with intracellular proteins, and improvement of partitioning of the drug into the stratum corneum. Ethanol improves the vesicular ability to penetrate the stratum corneum. In addition, ethanol provides net negative surface charge and prevents vesicle aggregation due to electrostatic repulsion. A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed (Mohamed *et al*., 2018).

Terpenes, the naturally occurring volatile oils are included in the list of generally recognised as safe substances with low irritancy at lower concentrations (1-5%), with reversible effect on the lipids of stratum corneum are considered as clinically acceptable penetration enhancers. Invasomes are characterised for size, surface morphology, zeta potential, stability.

The transdermal delivery of drugs is rapidly increasing in formulation development in enhancing the bioavailability of many drugs. When drugs are administered via the transdermal route skin barrier is harmfully affected. In recent years, vesicular systems have been intensively studied as drug carrier systems for the dermal and transdermal administration of drugs (Dragicevic *et al*., 2016). Traditional liposomal formulations, compared to conventional dosage forms, have shown in vitro an enhanced cutaneous drug accumulation allowing a reduction of the dose applied onto the skin. In the last two decades, new classes of lipid vesicles were introduced by different researchers. More recently, researchers investigated the novel vesicular systems called as invasomes. Briefly, invasomes contain not only phospholipids but also ethanol and terpenes, which make the vesicles deformable, and also serve as penetration enhancers [1=3]. This system has been shown to improve skin penetration of hydrophilic and lipophilic drugs. A synergistic effect between terpenes and ethanol on the percutaneous absorption has been significantly observed. In this study, we investigated the ability of invasomes to increase the topical transport of ketoconazole in order to develop a topical formulation with enhanced ketoconazole skin delivery to achieve an effective fungal treatment. Ethanol is a good penetration enhancer while terpenes have also shown potential to increase the penetration of many drugs by disrupting the tight lipid packing of the stratum corneum (Kamran *et al*., 2016).

Structural features and composition of invasomes

Invasomes are the soft liposomal vesicles with minute amounts of ethanol and terpene or terpene mixtures that act as potential transporters with enhanced skin penetration. These special lipid vesicles are composed of water, terpenes or a combination of terpenes (such as citral, cineole, limonene, and eugenol; 1–5% v/v), low concentrations of ethanol (3% to 3.3% v/v), and phospholipids (phosphatidylcholine, phosphatidylserine, soya phospholipid, egg lecithin, phosphatidylinositol, phosphatidic acid and phosphatidylglycerol) (Lakshmi *et al*., 2013). The percutaneous absorption of hydrophilic and hydrophobic medicines is accelerated by terpenes, which have a general formula of (C5H8)n.

Terpenes are natural-source components of essential oils that are widely employed as penetration enhancers. Terpenes, however, also have the benefit of minimal skin irritancy at low dosages. Terpenes are additionally categorised by FDA as usually safe (Jain *et al*., 2021).

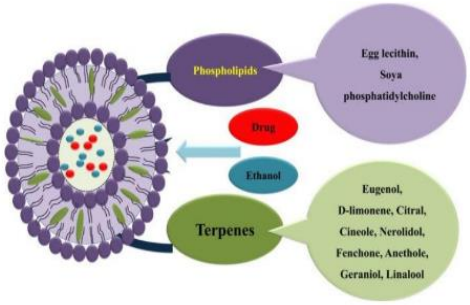

Figure 1: Structure and Constituents of Invasomes

Advantages of invasomes

- Non-invasive technique of drug delivery.
- Enhanced permeation of drug through the skin for transdermal drug delivery.

- Delivery of hydrophilic and lipophilic drug is possible.
- Contains non-toxic raw material in formulation.
- Simple method of drug delivery in comparision to iontophoresis and phonophoresis and other complicated methods (Saudagar and Bornare, 2016).

Invasome preparation technique

Invasome made using the film hydration technique filled with ketoconazole. In ethanol, ketoconazole and phosphatidylcholine were dissolved. The solution was then treated in a rotary flask evaporator after being moved to a round bottom flask (RBF). To produce a dry RBF film, the mixture was subsequently thoroughly dried under vacuum at 40 °C, 100 rpm, and 16 mm Hg. After being at ambient temperature for two hours under vacuum (1 mbar), the film is flushed with nitrogen (Karimi *et al*., 2015). Next, the deposited film is hydrated using a phosphate buffer (pH: 6.8; PBS) mixture that contains ethanol and Dlimonene for 30 minutes at lipid phase transition. To obtain varied invasome pore diameters, the resulting vesicles are vortexed and ultrasonically sonicated at 45% amplitude for 10 minutes after cooling to room temperature.

Characterization of invasomes

- Entrapment Efficiency
- Surface Morphology
- Stability Studies
- Drug Content
- Vesicular size
- Ex-Vivo Permeation Studies

Entrapment Efficiency: Entrapment efficiency was studied by ultracentrifugation method. 1ml of invasomal formulation was transferred to Ephendroff tubes, centrifuged at 15000 rpm, 4℃ for 15 min in two cycles to separate the unentrapped drug. The clear fraction was used for determination of free drug. Percentage entrapped is calculated indirectly from the amount of free drug from the formula (Lakshmi *et al*., 2014).

Entrapment Efficiency (%) = $total\ drug$ / $free\ drug\ total\ drug$ × 100

Surface Morphology: Surface morphology was studied by placing a drop of preparation on clear glass slide, air dried, coated with gold using sputter coater (Polaron E5100, Watford, UK) and visualized under scanning electron microscopy (Lakshmi *et al*., 2014).

Stability Studies: Optimised invasomal formulation was sealed in 10ml glass vial and stored at refrigeration temperature (4 - 8°C) and room temperature for one month. Entrapment efficiency, physical appearance was determined at regular intervals (Lakshmi *et al*., 2014).

Drug Content: Drug content of the invasomes can be determined by using ultraviolet spectrophotometer. This can be quantified by a modified high-performance liquid chromatographic method (Saudagar and Bornare, 2016).

Vesicular Size and Shape: Invasomes can be visualised by using Transmission Electron Microscopy (TEM) and by Scanning Electron Microscopy (SEM). Vesicle size and zeta potential particle size of the invasomes can be determined by Dynamic Light Scattering (DLS) and photon correlation spectroscopy (Saudagar and Bornare, 2016).

Ex Vivo Permeation Studies: The permeation of invasome formulations was determined by usingthe Franz diffusion cell. The effective surface area of cell was 2.0 cm^2

and has a receptor volume of 20ml. The skin was mounted on the receptor compartment with the stratum corneum side facing upwards into the donor compartment. The top of the diffusion cell was covered with lid.

The donor compartment was applied with invasomal preparation and 20 ml of pH 7.4 phosphate buffer saline maintained at 37ºC was used as receptor medium. Aliquot amounts were withdrawn and replaced by fresh media to maintain a sink condition. Samples were analyzed using UV spectrophotometer (Kalpana and Lakshmi, 2013).

Pharmaceutical applications of invasomes

Improves bioavailability

In this study the solubility of curcumin was increased by complexing with Cyclodextrin (CD) and Hydroxy Propyl β Cyclodextrin (HPβCD). Improved solubility and stability was observed with HPβCD prepared by co-precipitation method than physical mixture method. Optimized complex i.e., 1:2 proportion complex is incorporated into invasomes with limonene, fenchone, nerolidol as terpenes which acted as phospholipid membrane flexibilizers, penetration enhancers. 1% soya phosphatidylcholine, 0.5% limonene was optimized which showed Q24OF 70.32 µg/cm2 , SSTF of 3.34 µg/cm2 /hr-1 , permeability coefficient of 5.35 cm/hr, and decreased lag time of 1hr. formulation CHL1 was found to be better in all characteristics and was incorporated into 2% HPMC K4M gel formulation (Lakshmi *et al*., 2014).

Effective permeability of drug into cells

(Gauhar *et al*., 2016) has worked to prepare Invasome of israpidine for enhanced transdermal delivery against hypertension formulation, characterization and in vivo pharmacodynamic study. It was observed that prepared isradipine-loaded invasomes delivers ameliorated flux, reasonable entrapment efficiency, and more effectiveness for transdermal delivery. The optimized formulation presented the particle size of 194±18nm, entrapment efficiency (88.46%), and attained mean transdermal flux of 22.80±2.10µg/cm^2 /h through rat skin. Confocal laser scanning microscopy revealed an enhanced permeation of rhodamine-red-loaded isradipine invasome to the deeper layers of the rat skin. During hypertensive study, the treatment group showed a substantial and constant decrease in blood pressure, for upto 22hr. the isradipine invasomes formulation was found to be effective, with a 20% reduction in blood pressure by virtue of better permeation through wistar rat skin.

- Prolong the existence of the drug in systemic circulation
- Overcome the problems of the drug insolubility, instability and rapid degradation
- Both hydrophilic and lipophilic drugs can incorporated.

Immunosuppressive drug delivery

Immunosuppression is the primary approach to treating autoimmune diseases. Also, it is useful for the clinical application of existing immunosuppressive agents that have been suffered from various drug side effects. The nanotechnology centered approaches can correct the major limitations by enhancing immunosuppressant delivery to target cells of the immune system. Also, reducing the recommended dose for therapeutic function and reducing drug distribution to non-target

tissues can be a key alternative to immunosuppressive drug delivery. From the sub-structure of lipidic vesicles in drug delivery, it receives the primary consideration of the investigators to develop advanced nanosized vesicles. A literature survey mentioned that cyclosporine A (CsA, CyA) is a lipophilic drug, and it exhibits poor penetration efficiency into the skin layers (partition coefficient: 4000). The topical applications of CsA can be a suitable alternative for the management of psoriasis and other dermatological diseases (Dragicevic-Curic *et al*., 2011).

Anticancer drug delivery

From its inception, cancer treatment is still a challenging field in the era of biomedical science. Due to the ineffectiveness of currently engaged therapeutic strategies, a large number of deaths have been occurring each year. Therefore, there is an urge to develop an advanced substitute to resolve cancer ineffective treatment issues. Interestingly, the temoporfin is a potent (second-generation) photosensitizer. It shows high tumor selectivity and residual photosensitivity of only 2 weeks. Thus, this could be an effective anticancer agent in photodynamic therapy of early or recurrent carcinomas. Briefly, temoporfin-loaded invasomes have been synthesized using the mechanical dispersion method. In that, the temoporfin and 1%w/v terpene (limonene/citral/cineole) were dissolved in ethanolic phospholipid solution (phosphatidylcholine:ethanol: 75:25 w/w) and subjected to vertexing followed by sonication for 5 min.

Finally, PBS was added into the above-mentioned clear solution with constant vortexing for 5 min. The drugloaded invasome (1%, w/v cineol) showed about 105.4 nm particle sizes and about 0.066 PDI. After plastic surgery, the human female abdominal skin was obtained, and penetration was carried out using the nominal surface of the Franz cells (3.14 cm2). The use of cineole (1% w/v) showed the highest penetration ability followed by a mixture (1% w/v) of cineole: citral: D-limonene (45:45: 10 v/v).

Experimental outcomes revealed that the single terpene could make an efficient delivery of temoporfin, and a combination of terpenes could lead to the synergistic effect of active penetration to the subcutaneous and deeper skin layers.

In the future, invasome can be an efficient carrier for the delivery of hydrophobic active molecules with effective concentration to the systemic/local site. However, there is no thumb rule for the use of terpene and its mixture towards penetration (Chen *et al*., 2011). As abovementioned, temoporfin is a second-generation synthetic photosensitizer used for the treatment of oral carcinomas, refractory oral carcinomas, non-melanomatous tumors of the skin of the neck, and head. For photodynamic therapy, there is a need for sufficient skin penetration of photosensitizer. The invasome can promote the efficient delivery of temoporfin drug. In this context, they have prepared the temoprofin-loaded invasomes via mechanical dispersion method. In brief, temoporfin and 1% w/v of terpenes mixture [cineole, citral and D-limonene] were dissolved in ethanolic phospholipid solution (phosphatidylcholine: ethanol;75:25 w/w). Further, the mixture was subjected to vortexing and sonication to obtain a clear solution.

After that PBS was added followed by constant stirring (5 min) to obtain multilamellar vesicles and then extruded using an Avestin hand-extruder via polycarbonate membranes (400 nm, 200 nm, 100 nm, 50 nm).

Delivery of anti-acne agent

Acne is a widespread skin disorder worldwide in recent times. Dapsone is an exceptionally active pharmaceutical ingredient for leprosy treatment. It has significant potential for acne therapy due to its anti-inflammatory action. As essential for the delivery of topical drugs, an effective carrier must be established for the delivery of dapsone to the specific site. Briefly, dapsone (20 mg) and terpenes with different concentrations were mixed with a clear solution of phosphatidylcholine (200 mg) in methanol/chloroform, 2:1 v/ v.

After that, this mixture was subjected to rotary evaporation to remove the organic solvent at 120 rpm (60°C) for 15 min. Finally, thin-film was hydrated using 3% v/v ethanolic: water mixture at 120 rpm (60°C) for 1 h and further filtered (pore size 25 μm) to separate the drug crystals from invasomes. The high concentration of terpene showed the superior entrapment efficiency of dapsone. Furthermore, the limonene containing invasomes showed the highest entrapment efficiency followed by cineole, fenchone, and citral. Possibly, it may because of the lipophilicity of the used terpenes.

The optimized drug-loaded unilamellar invasome exhibited uniform spherical discreet shape, and − 37.5 mV zeta potential that confirms the stability of invasome vesicles. The differential scanning thermogram revealed that the invasomecontaining drug was converted into the amorphous region with uniform distribution. Interestingly, the in vivo permeation study on Wistar rats resulted in the enhancement of penetration rate of invasome by 2.5-fold as compared with the solution form. Besides, they found the in vivo rat skin deposition amount of dapsone to be 4.11 μg/cm^2 for invasomes, which was high than the drug-alcoholic solution (1.71 μg/cm^2).

Particularly, the Wister albino rats have been shown invasome increases the 2-fold AUC0–10 than the solution of dapsone. Overall, invasomes significantly increased the skin deposition of dapsone. Therefore, invasome can be potential vehicles for the delivery of an anti-acne agent. A literature survey reported that capsaicin is a major pungent photo component and widely studied in the pharmaceutical arena. Particularly, it is used for the management of oral and topical pain. The optimized capsaicin-loaded invasomes showed narrow size distribution (0.01–0.30), smaller than 100 nm in size. Furthermore, negative (−20 mV) zeta potential confirmed the stability of drug-loaded invasomes. Furthermore, it gives excellent skin permeability over the conventional liposome formulation and commercial product (0.15% capsaicin in ethanolic solution). Hence, the optimized capsaicin invasomes can be used as a substitute for transdermal drug delivery.

Evaluation parameter

A] Evaluation of Invasomes

Entrapment efficiency: Entrapment efficiency of Clotrimazole Invasomes formulation was determined using centrifugation method (Dragicevic-Curic *et al*., 2008). The entrapment efficiency of acyclovir in invasomes vesicle was determined by ultracentrifugation, 10mL of

invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 260 nm using UV spectrophotometer.

Vesicle Size: Microscopic analysis was performed to determine the average size of prepared invasomes (Dragicevic-Curic *et al*., 2011). Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer.

B] Evaluation of Invasomes Containing Gel

Measurement of Viscosity: Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH Measurements: pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Ayman *et al*., 2001).

Drug Content: Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol (Aqil *et al*., 2007). This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λmax 260 nm.

Extrudability Study: Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load (Kalpana and Lakshmi, 2013). More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility: Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by (Multimer *et al*., 1956). An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan. To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80 g of weight was noted. Good spreadibility show lesser time to spread.

***In-Vitro* Drug Diffusion Study:** The in-vitro diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion (Aqil *et al*., 2007). The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14 sq. cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one

side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell.

Stability Studies: Stability study was carried out for drug loaded invasomal gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25- 28 ± 2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay.

Effect of composition on the physicochemical characteristics of invasomes

Effect of ethanol

The addition of ethanol in the formulation of lipid nanovesicles is an effective strategy to increase the fluidity of the lipid bilayer of the skin. The interaction of ethanol with the lipid elements in the polar group area of the SC leads to alterations in the structure of the keratinized or lipophilic domains, decreased transition temperature of lipids, and consequently fluidization and disruption of the tightly packed SC lipids. Ethanol-based nanocarriers can fluidize and disturb the SC lipids. The presence of ethanol increases the flexibility of the intercellular lipid matrix due to the rotating freedom of the lipid acyl chains. Thus, ethanol increases the fluidity of lipids in the vesicle structure, resulting in a structure that has softer and less rigid properties than conventional liposomes. In addition to enhanced penetration ability, ethanol creates a net negative surface charge and limited vesicle aggregation due to electrostatic repulsion, leading to increased stability of invasomes under storage conditions (Dragicevic-Curic *et al*., 2010).

Effect of terpenes

Effect of terpene on penetration

X-ray diffraction and differential scanning calorimetry (DSC) results showed that terpenes lead to increased drug penetration by disrupting the tight bilayers and lipid packing in the SC. Furthermore, breaking the hydrogen bonds and extracting SC lipids, enhancing the partition into the SC by improving lipid fluidity and increasing diffusion via the intercellular lipids are another mechanisms that have been reported to increase drug permeability by terpenes. (Dragicevic-Curic *et al*., 2010) revealed that various types of terpenes have a synergistic effect on the permeation of temoporfin.

Invasomes containing a 1% mixture of three types of terpenes (citral, cineol, and limonene) demonstrated higher temoporfin permeability than invasomes containing 1% citral alone. In another study, (Dragicevic-Curic *et al*., 2010) demonstrated the relationship between the permeated amount of temoporfin and the amounts of terpenes in the invasomes. They indicated that vesicles containing 1% terpenes have a 1.7-fold higher temoporfin penetration effect than vesicles containing 0.5% terpenes. Therefore, incorporation of temoporfin in vesicles containing 1% terpenes could lead to deeper penetration.

Effect of terpene on the size of the invasomes

Examination of particle size demonstrated that the size of the invasomes is directly correlated to the number of terpenes; the size of the invasomes increases as the amount of terpene increases. The size of vesicles containing 1% terpenes was 124 nm, whereas the size of vesicles with 0.5% terpenes was 93.0 nm. The size of invasomes containing nerolidol (molecular size 222 g/mol) was around 11 to 13 μm. The vesicle sizes of nimesulide-loaded liposomes containing citral, limonene, and cineole were 194 nm, 216 nm, and 244 nm, respectively.

Effect of terpene on the shape of the invasomes

The results of cryo-transmission electron microscopy (cryo- TEM) were in agreement with the DSC and ESR results, indicating the influence of terpenes on the shape of the invasomes, i.e., in addition to spherical vesicles, malformed vesicles of varied shapes also existed in invasomal dispersions. To observe the lamellarity and shape of invasomes with various percentages of terpenes cryo-electron microscopy used. Their results revealed that invasomes with Nanomaterials 2020, 10, 341 5 of 13 0.5% terpenes were mostly unilamellar and bilamellar or oval and spherical in shape; however, in the invasomal formulation with 1% terpenes, the invasomes appeared to be unilamellar and bilamellar. Therefore, the combination of 1% terpenes with the invasomes increased the membrane elasticity of invasomes, the percentage of terpenes, and the number of deformed vesicles.

Synergistic effects

A synergistic effect between phospholipids, ethanol, and terpenes on dermal absorption has been visibly observed. (Dragicevic-Curic *et al*., 2010) suggest that one part of the invasome disintegrates during permeation in the upper layers of skin and releases the phospholipids and terpenes, which act as permeation enhancers that fluidize the intercellular lipids. Furthermore, the ethanol in the invasome fluidizes the intercellular lipids and enhances the penetration of flexible vesicles. Invasomes increased the transdermal permeation of cyclosporine A compared to an ethanolic solution. The improved efficiency of invasomes compared to an ethanolic solution suggests a synergistic effect of phospholipid, terpenes, and ethanol.

The improved permeation of temoporfin (mTHPC) with 1% terpenes was due to the concentration of terpenes and the synergistic effects of terpenes and ethanol. Thus, the results from the aforementioned studies point toward the synergistic effect of phospholipid, terpenes, and ethanol in the reformed activity of invasomes in comparison with liposomes.

Conclusion

Invasomes have emerged as a promising vesicular carrier system for transdermal drug delivery due to their superior skin penetration, high drug-loading capacity, and ability to deliver both hydrophilic and lipophilic drugs. The synergistic action of phospholipids, ethanol, and terpenes enhances drug permeation through the stratum corneum, resulting in improved bioavailability, controlled drug release, and enhanced therapeutic efficacy. Numerous studies have demonstrated the potential of invasomes in the delivery of antifungal, anti-inflammatory,

antihypertensive, anticancer, and other therapeutic agents. Despite these advantages, further investigations on long-term stability, large-scale manufacturing, and clinical evaluation are required to facilitate their successful translation into commercial pharmaceutical products. Invasomes represent a versatile and effective nanocarrier with significant potential for the future of topical and transdermal drug delivery.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

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