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A comprehensive review on invasomal carriers incorporating natural terpenes for augmented transdermal delivery

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Abstract

Background: Transdermal drug delivery is one of the most widely used drug administration routes, which offer several advantages over other routes of drug delivery. The apical layer of the skin called the *stratum corneum* is the most dominant obstacle in the transdermal drug delivery, which restricts the passage of drugs across the skin. Considerable strategies have been applied to enhance the rate of permeation across the epithelial cells; however, the most widely used strategy is the use of sorption boosters, also known as permeation enhancers.

Main body: Terpenes were considered as efficient skin permeation enhancers and are generally recognized as safe as per Food and Drug Administration. Terpenes improve the permeability of drugs either by destructing the *stratum corneum*'s tightly packed lipid framework, excessive diffusivity of drug in cell membrane or by rampant drug partitioning into epithelial cells. Various vesicular systems have been developed and utilized for the transdermal delivery of many drugs. Invasomes are one such novel vesicular system developed which are composed of phospholipids, ethanol and terpenes. The combined presence of ethanol and terpenes provides exceptional flexibility to the vesicles and improves the permeation across the barrier offered due to the *stratum corneum* as both ethanol and terpenes act as permeation enhancers. Therefore, utilization of invasomes as carriers to facilitate higher rate of drug permeation through the skin can be a very useful approach to improve transdermal drug delivery of a drug.

Conclusion: The paper focuses on a broad updated view of terpenes as effective permeation enhancers and invasomes along with their applications in the pharmaceutical formulations.

Keywords: Terpenes, Invasomes, Permeation enhancer, Transdermal drug delivery, Skin, *Stratum corneum*

Background

Drug delivery via the percutaneous pathway has various benefits over drug delivery through oral and intravenous routes [1, 2]. Skin acts as a natural obstacle for the movement or deeper penetration of drug molecules applied topically. Epidermis is the uppermost layer of the skin. Structural composition of epidermis and membranes of epidermal cells are the key obstacles that restrict the permeation of drugs across the skin when applied topically, which decreases the bioavailability of drug by significantly reducing the transdermal flux of drug across the skin [3]. Strategies like utilization of chemical penetration enhancers, ultrasound, iontophoresis, microneedles, nanoparticles and vesicular drug delivery carrier systems have been employed for modification of barrier function of *stratum corneum* (SC) [4–7]. One of the most widely used strategies is the utilization of sorption boosters, also known as permeation/penetration enhancers [1, 8].

Terpenes have been used as efficient penetration boosters for enhancing the rate of drug delivery across the skin for the past 60 years [9]. Terpenes, as per Food and Drug Administration (FDA), are considered as generally recognized as safe (GRAS) and a powerful

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category of permeation enhancers derived from biological sources [10]. In certain instances, they trigger just slight discomfort but still no toxic effects to the skin were reported [11, 12]. Essential oils contain terpenes, which are the secondary metabolites of the plant. They are made up of numerous isoprene (C_5H_8) units, connected via tail to head [13, 14]. Terpenes are categorized on the basis of number of isoprene units present in their chemical composition as shown in Table 1. Oxygen-containing terpenes are termed as terpenoids, e.g. carvone, thujone, menthol, etc. Terpenes are used in pharmaceutical formulations as carminatives, antispasmodics, flavouring agents and in perfumeries because of their aroma. Menthol produces mild antipruritic effect when incorporated in an emollient, which is a highly beneficial inhalational pharmaceutical product.

Vesicular drug delivery carrier systems like invasomes have been designed by using terpenes as one of their formulation components. Vesicular systems were investigated extensively in recent years for the development of transdermal drug delivery systems owing to their ability to deliver the entrapped drug molecule across the skin. They were reported to increase penetration of lipoidal materials into the SC and also permit sustained drug release by serving as a depot system [15]. Vesicles can also provide controlled release of drugs as they can act like a rate-limiting membrane barrier which can modulate the systemic absorption of drug [15]. Physicochemical properties of vesicular carriers like size range, surface charge and membrane properties like deformability or elasticity also confer suitability to them to be used as efficient drug carriers [16, 17]. In this article, different categories of terpenes that are used as penetration enhancers in pharmaceutical products, their mechanism to improve the penetration, and factors affecting penetration power of terpenes were discussed along with the pharmaceutical application of invasomes in the transdermal drug delivery.

Table 1 Terpenes classification

Classification on the basis of composition of chemicals		Classification on the basis of units of isoprene	
Terpenes	**Class**	**Terpenes**	**Isoprene number**
Ascaridole	Peroxides	Tetraterpenes	C_{40}
Cineole, anethole	Ethers	Triterpenes	C_{30}
Thymol, eugenol	Phenols	Sesterterpenes	C_{25}
Thujone, carvone	Ketones	Diterpenes	C_{20}
Aldehydes of cinnamic	Aldehydes	Sesquiterpenes	C_{15}
Menthol, acetate of linalyl and linalool	Alcohols and esters	Monoterpenes	C_{10}
Geraniol, linalool	Alcohol	–	–

Main text

Factors determining the penetration effect of terpenes

There are several factors, which influence the skin penetration capability of terpenes. Some of these factors are discussed below.

Lipophilicity

Terpenes with high lipophilic character act as strong boosters for sorption of lipophilic drug [18]. Terpenes with non-polar hydrogen (e.g. limonene) are much more efficient boosters than oxygen-rich polarized terpenes [19–21].

Size and chirality

Size plays a crucial role in defining the permeation capability of terpenes. Terpenes that are smaller in molecular size appear to be more effective in penetrating the skin than the large-sized terpenes [2]. Stereoisomerism of terpenes also influences their permeation capabilities. Research has revealed that the (−) enantiomer of a terpenoid is much more competent in permeation when compared with its correlating (+) isomer or (±) racemic mixture [22].

Vaporization energy and boiling point

It has been observed that the higher the boiling point of a terpenoid, the lower is the skin permeation enhancement ability. Cineole, with a boiling temperature of 173 °C, is the most powerful permeation booster of zidovudine relative to many other terpenoids with higher boiling points (224 °C for pulegone and 230 °C for carvone) [18]. Vaporization energy is inversely related to the permeation boosting property of terpenes [18].

Degree of unsaturation

Tiny alcoholic terpenoids with a greater degree of unsaturation are strong contenders for enhancing the permeation of water-soluble drugs [18]. It has also been stated that terpenoids with limited unsaturation, such as menthol and cineole, are strong boosters of adsorbents for polarized and water-soluble substances [22].

Different types of terpenes as penetration enhancers

Various types of terpenes have been utilized for the enhancement of drug permeation across the skin (Table 2). The main mechanism of action of terpenes for permeability enhancement is their ability to disturb or break the hydrogen bonds between the components of cellular bilayer membrane. Fourier transform infrared (FTIR) and differential scanning calorimetry (DSC)

Table 2 Terpenes utilized as permeation enhancer in transdermal systems

Terpenes	Structure	Type	Characteristics	Ref
Ascaridole (C10H16O2)		Monoterpene peroxide	Source is *Chenopodium ambrosioides* It is bicyclic in nature and contains peroxides	[1]
Terpinolene (C10H16)		Monoterpene	Utilized in industries of textile and for providing fragrance	[24]
Carvone (C10H14O)		Ketone	Majorly present in seeds of caraway Utilized in aroma therapy	[25]
Geraniol (C10H18O) [		Monoterpene alcohol	Found in citronella, lemon and geranium Utilized to provide aroma	[26]
Farnesol (C15H26O)		Sesquiterpene alcohol	Found in oils of tolu, balsam, musk, rose, lemon grass, neroli Utilized in perfume industry	[27]
Nerolidol (C15H26O)		Sesquiterpene alcohol	Present in flowers and plants like tea tree, lavender, jasmine, ginger, etc., in the form of oil Utilized in perfume industry and as flavouring agent	[28]
Cineole (C10H18O)		Ether	Source is *Eucalyptus globulus* It is cyclic in nature and also known as cajeputol	[29]
Linalool (C10H18O)		Monoterpene alcohol	Source is *Coriandrum sativum* (fruits) Occurs naturally, present in seeds of coriander and has pleasant aroma	[10]
Limonene (C10H16)		Monoterpene	Source is *Citrus lemon* (peel) and has lipophilic and chiral properties	[30]
Menthol (C10H20O)		Monoterpene alcohol	Source is *Mentha piperita* (flowering tops) Used as decongestant and antipruritic	[31]

methodologies are widely used to evaluate how terpenes modulate the skin permeability. DSC analysis has provided evidence regarding the changes in the thermotropic activity of lipids and protein components of cell membranes upon interacting with terpenes. FTIR showed evidence of structural and conformational modifications in bilayer membrane upon interacting with terpenes [23].

Terpenes usually improve the product permeability across the skin by utilizing any of the three mechanisms. One mechanism involves the destruction of SC's tightly packed lipid framework. Excessive diffusivity of drug in

cell membrane or rampant drug partition into epithelial cells was also suggested [32, 33]. Another proposed mechanism of action was that the terpenes enhance the electrical properties of the body tissue by opening of polar routes inside the dermal *stratum* [34]. The mechanism by which terpenes act as penetration enhancers is depicted in Fig. 1.

Zhao et al. studied the mechanism by which terpenes (menthone and limonene) in combination with ethanol enhanced the in vitro permeation of propranolol hydrochloride. The porcine epidermis skin model and Franz diffusion cells were used for performing in vitro permeation study. Solution of propranolol hydrochloride containing ethanol was used as control for this experiment. Solutions of propranolol hydrochloride containing menthone (5%) or limonene (5%) in combination with ethanol remarkably enhanced the permeation of propranolol hydrochloride across porcine epidermis. In the case of both terpenes (menthone and limonene), FTIR study demonstrated a decline in peak height and peak area both for symmetrical and for asymmetrical stretching of C–H as compared to the untreated epidermis [35]. This decrease in peaks and area reflects the extraction of lipids of SC [35].

Zhao et al. also performed a similar research work with tamoxifen in which they investigated the permeability-enhancing effect of three different permeation enhancers such as eugenol, D-limonene and menthone. All the three enhancers were used in combination with 50% ethanol. Porcine skin epidermis was used for the in vitro permeation experiment. The effect of enhancers on the structural and biophysical integrity of SC was analysed by FTIR. The FTIR analysis of porcine epidermis treated with permeation enhancers showed that in the case of all the enhancers a reduction in height and areas of peaks related to the symmetric and asymmetric C–H stretching absorbance was observed when compared with the FTIR of untreated SC. This change in FTIR patterns is an indication of lipid extraction. They also investigated the partitioning pattern of tamoxifen under experimental set-up. Powdered SC was used as one phase and control solution or solution of drugs with various penetration enhancers was used as the second phase. In the case of solutions of tamoxifen with eugenol and D-limonene, the results showed that the increment in drug permeation is due to extraction of lipids from SC and enhanced partitioning of drug into the SC. In the case of tamoxifen solution containing menthone, enhanced permeation was reported to be due to the extraction of SC's lipids only [36].

Menthol

Menthol is a powerful sorption promoter and generally recognized as safe (GRAS) for use in pharmaceutical products. It was utilized either alone or in combination with other terpenes like limonene for boosting the permeation of several active pharmaceutical ingredients across the skin like propranolol, triamcinolone, caffeine and imipramine after administration via topical route [37, 38]. Synergistic use of terpenes along with iontophoresis technique has also been reported in the scientific

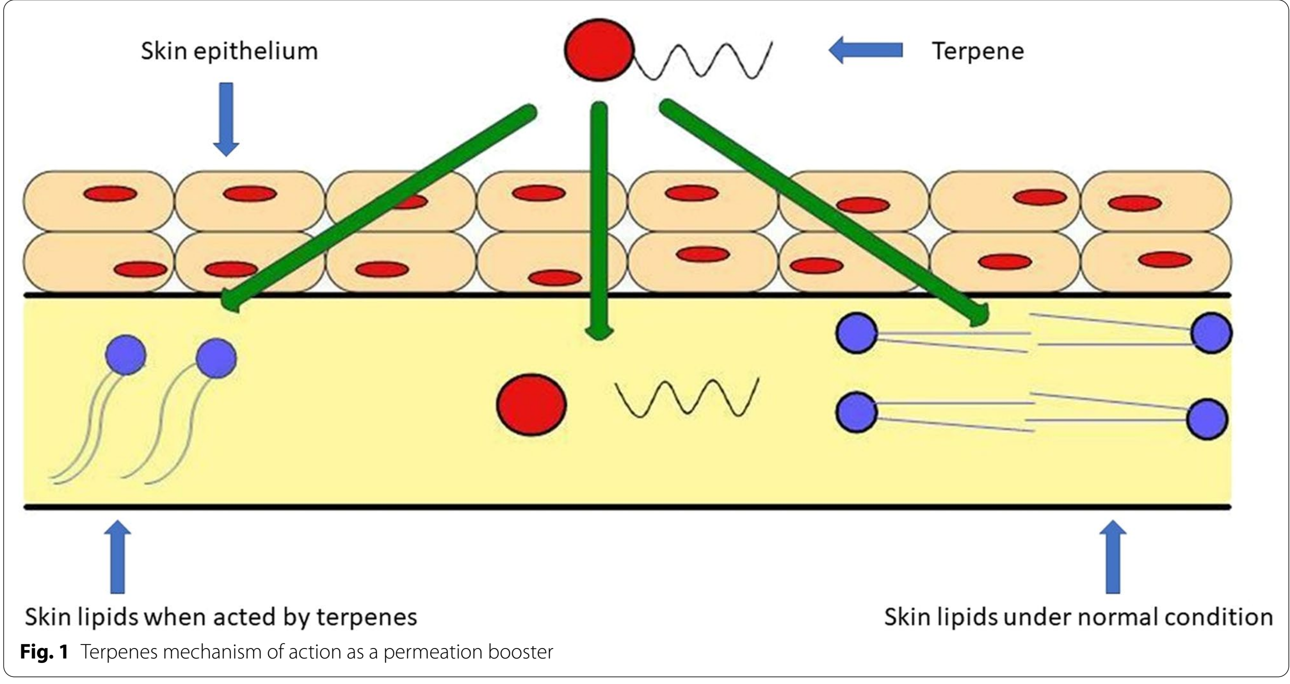

Fig. 1 Terpenes mechanism of action as a permeation booster

literature studies. Al-Khalili et al. formulated ethanol/water (50:50 v/v)-based gel formulations of buspirone hydrochloride (1% and 2%, respectively). Two types of gel formulations were prepared using two different polymers (carboxymethylcellulose and hydroxypropylmethylcellulose). They evaluated the effect of API concentration, density and vehicle pH on the iontophoresis-assisted transdermal delivery of buspirone hydrochloride. The study results showed that increasing the buspirone hydrochloride concentration produced no impact on iontophoretic flux of the drug. Iontophoretic flux of drug was doubled with increment in density of formulation from 0.05 to 0.1 MA/cm^2. The study also revealed that ethanol/water (50:50 vol/vol) system does not produce any impact on the iontophoretic flux of drug. However, upon adding menthol, cineole and terpineol to the buspirone hydrochloride gel formulation, the transdermal flux of the increases significantly. The results showed that upon application of iontophoresis technique alone the flux of drug across the skin increases up to 15 times. However, terpenes increased the flux of drug by 200 times. Highest enhancement of flux was observed for the gel formulations containing methanol. Menthol when used alone increased the flux of drug up to 300 times. Higher increment in the flux of buspirone hydrochloride was observed when terpenes were used in combination with iontophoresis technique. Menthol containing carboxymethylcellulose gel of drug when applied in combination with iontophoresis technique synergistically increased the flux of drug up to 546.84 ± 40.54 μg/h.cm^2 in comparison with the flux of drug obtained by utilization of iontophoretic technique (27.19 ± 4.15 μg/h.cm^2) or menthol (347.10 ± 14.96 μg/h.cm^2) alone, respectively. Similar results were obtained in the case of hydroxypropylmethylcellulose gel of buspirone hydrochloride. The flux of drug increased from 55.59 ± 11.18 μg/h.cm^2 (iontophoresis) and 523.10 ± 80.34 μg/h.cm^2 (menthol) to 637.81 ± 92.88 μg/h.cm^2 when iontophoresis and menthol used together. Therefore, the results obtained not only clearly showed the permeation enhancing capability of the menthol, cineole and terpineol, but also that these permeation enhancers could increase the flux of buspirone hydrochloride synergistically across the skin after topical application when utilized together with iontophoresis [39].

Menthol always seemed a stronger transdermal enhancer for propranolol hydrochloride delivery. Kunta et al. studied the impact of four different types of terpenes and their concentration on the diffusion of propranolol hydrochloride across the excised hairless mouse skin by utilizing side-by-side diffusion cells. They used L-menthol, limonene, linalool and carvacrol for the study, and all the terpenes were used in three different concentrations (1, 5 and 10% w/v), respectively. Propranolol permeation was not impacted very much when terpenes used in by a 5 to 10%w/w concentration, except for the linalool which demonstrated exaggerated response. Among all the aforementioned terpenes, 1% menthol was considered to be a great penetrating enhancer because it had comparatively high skin permeation and a relatively short time lag than other substances [40]. In another study, drugs indomethacin and antipyrine were utilized along with skin of micropig to compare the effect of L-menthol metabolite (p-methane-3,8-diol) and L-menthol on permeation enhancement. They reported that both p-methane-3,8-diol and L-menthol do have a comparable impact on indomethacin's permeability, while p-methane-3,8-diol has less impact on antipyrine transport [41].

Limonene

Limonene was reported to improve the permeability of hydrophobic (butyl paraben) and amphiphilic drugs (6-mercaptopurine) but found to be not very effective for increasing the permeability water-soluble substances, like mannitol. Limonene was found to have more stronger skin penetrating capability in comparison with oleic acid, span 20, ethanol, 1,8-cineole, bisabolol, etc. [42]. Krishnaiah et al. formulated a transdermal drug delivery system for administration of nicardipine hydrochloride. Development of transdermal system starts with formulation of 2% hydroxypropyl cellulose gel used as reservoir for drug. Reservoir also contains a permeation enhancer, limonene (4% w/w). Ethylene vinyl acetate (EVA) copolymer composed of 28%w/w vinyl acetate was utilized as the rate-controlling membrane. Finally, pressure-sensitive adhesives, viz. MA-31, MA-38 or TACKWHITE A 4MED, were attached to the membrane to develop the whole delivery system. Rat abdominal epidermis was used for evaluating the performance of the developed transdermal system. The results revealed that the transdermal system of nicardipine hydrochloride having TACKWHITE A 4MED showed higher permeation in comparison with the systems developed with MA-31 and MA-38. Study conducted in the human volunteers showed that a steady state in the drug plasma concentration was achieved for up to 20 h after application of the transdermal system along with the increment in bioavailability when compared with conventional capsule dosage form of nicardipine hydrochloride [43].

Lim et al. utilized organogels formulated with dibutyl lauroyl glutamide (GP1) 2–10%, propylene glycol (PG) and limonene 5% for transdermal administration of haloperidol. Gels with other permeation enhancers like cineole and linalool were also prepared. Rheological study showed that gel consistency and density vary accordingly

with the variation in the concentration of gelling agents used, i.e. GP1 and PG. The permeation study reported that the organogels prepared with limonene boosted the permeation of haloperidol up to 26.5 times, while the gels containing linalool and cineole enhanced the permeability up to eightfold and sevenfold, respectively. Inverse relation between gelling agents concentrations and flux of drug across the skin is observed [44]. Ota et al. studied the impact of different terpenes (geraniol, citronellol, L-menthol and D-limonene) on the percutaneous permeation of midazolam. They reported that using a combination of 5% w/v of terpenes with ethanol (30%) and propylene glycol (20%) enhanced the permeation of midazolam to a significant extent. The formulation prepared with the combination of 5% D-limonene, ethanol and propylene glycol showed maximum improvement in the permeation of the midazolam across the rat skin when compared with control [45]. Clarys et al. investigated the relation permeation of radiolabelled dihydrotestosterone across the bald rat skin and temperature of the rat skin. They prepared formulations with two different terpenes or permeation enhancers: limonene and oleic acid. They studied the permeation of radiolabelled drug across the skin via scintillation counting. The results showed the highest drug penetration was achieved at a temperature of 38.2 °C with the formulation containing limonene as a penetration enhancer [46].

Linalool

This is a naturally produced terpene alcohol with several industrial uses but mostly used because of its nice fragrance. Vaddi et al. compared linalool's penetration-enhancing ability and compared it with other penetration enhancers, terpineol and carvacrol. They formulated 5% w/v solution of each penetration enhancer in propylene glycol and used these solutions as vehicle for haloperidol to facilitate its transdermal administration. They reported an increase in haloperidol flux and solubility both. The results demonstrated that the highest increment in the drug permeability was achieved when linalool is used. The mechanism behind the increased flux was reported to be the extraction of skin lipids by the permeation enhancers and the propylene glycol itself. One more mechanism was suggested for the penetration-enhancing activity of linalool. It was reported that the way the linalool molecules orient themselves within the skin lipoidal bilayer also has a role in increasing the penetration of drug across the skin. They utilized analysis techniques, DSC and FTIR for detecting the thermal behaviour and the molecular and conformational changes that lipids present in the skin layers have undergone after transdermal application of prepared formulation [10].

Cineole

Cineole was found to be the most powerful enhancer of the penetration of propranolol through skin of rat relative to propylene glycol and menthol [29]. Amnuaikit et al. reported the formulation of various transdermal films for administration of propranolol hydrochloride by utilizing ethyl cellulose and polyvinyl pyrrolidone (film formers), dibutyl phthalate (plasticizer), menthol, cineole and propylene glycol (penetration enhancers). In vitro permeation evaluation showed higher drug flux across the skin model in the case of films containing cineole as a penetration enhancer compared to films containing menthol. The study also demonstrated no change in drug release rate with changes in concentration of enhancers [29]. Narishetty et al. investigated how the transdermal permeation of zidovudine across the rat skin gets modulated in the presence of terpenes like cineole, carvone, a-terpineol, pulegone, menthone and menthol. Transdermal system of zidovudine was developed using 66.6%w/v solution of ethanol in water as vehicle. The results obtained from the study showed that all the terpenes enhanced the flux of drug across the skin with cineole producing the most significant effect and carvone the least effect. However, no impact of terpene presence was observed on the partition pattern of drug between SC and they did not alter both the partition coefficient and thermal activity; it is stated that the underlying strategy for zidovudine enhancement of permeation by terpenoids was alteration of the characteristics of the barrier function [37].

Narishetty et al. studied how menthol and 1,8-cineole modulate the structural arrangement of lipids within the SC of human cadaver skin and permeation of zidovudine across it. Ethanol solution (66.6%) was used as vehicle in the study to administer zidovudine and 5% w/v concentration of both the permeation enhancers (menthol and 1,8-cineole) used. DSC and attenuated total reflectance–Fourier transform infrared spectroscopy (ATR-FTIR) analysis of the skin were conducted to observe the changes that occur after the application of zidovudine formulation. The results indicated that both menthol and 1,8-cineole facilitated the enhancement in the permeation of zidovudine permeability by altering the structural organization of lipids present within the SC. This reduces the complexity of lipoidal network present in the SC and results in fluidization of skin due to which drug can permeate more easily. It is stated that both the terpenoids disrupt the interlamellar hydrogen bonds by interacting with the polar heads of the lipids [47].

Nerolidol

Nerolidol has been explored as a penetration enhancer for the transdermal administration of various drugs. Cornell et al. investigated the penetration-enhancing activity of twelve different sesquiterpenes using the model drug, 5-fluorouracil. They reported that pre-treatment of skin model with sesquiterpenes enhanced the permeation of drug across the skin. The results showed that out of all the sesquiterpenes, nerolidol has the most potent permeation enhancement activity and it enhanced the pseudo-steady state of flux of model drug up to 20 times. Permeation-enhancing capacity of ethanol was also evaluated, and it was found that it increased the flux of drug up to 13 times. When both ethanol and sesquiterpenes were used together, they showed additive effects. Thus, they concluded that sesquiterpenes can effectively enhance the drug permeation across the skin [28].

El kattan et al. prepared hydroxypropyl cellulose gel formulations of four drugs such as nicardipine hydrochloride, hydrocortisone, carbamazepine and tamoxifen. They used four different types of terpenes as permeation enhancers (nerolidol, D-limonene, thymol and fenchone) for developing these gel formulations. Permeation study results showed that nerolidol has the most potent penetration enhancement activity in the case of all the four model drugs selected in the study for gel preparations. All the terpenes mentioned were much more efficient in improving the penetration of the medications that are hydrophilic, but not hydrophobic [48]. Krishnaiah et al. developed a hydroxypropyl cellulose-based membrane-moderated transdermal therapeutic system (TTS) of selegiline hydrochloride using three different terpenoids: anethole, carvone and nerolidol. In vitro permeation studies showed that nerolidol enhanced the permeation of drug to a higher extent as compared to other terpenoids across the rat skin. Nerolidol-containing system increased the permeation of drug up to 3.2 times when compared with controlled system used for the experimentation [49]. Permeation improvement activity of nerolidol is directly linked to its amphiphilic nature, which allows it to get oriented appropriately inside the lipid bilayer lamellae, leading to the disruption of the lipid bilayer [28].

Farnesol

Nokhodchi et al. investigated the permeation-enhancing effects of menthone, nerolidol, carvone and farnesol. They developed solutions of diclofenac sodium using a solvent system composed of ethanol, glycerine and phosphate buffer in 60:10:30 ratios. They utilized all the four terpenes in five different concentrations (0.25, 0.5, 1, 1.5 and 2.5%, v/v). In vitro permeation study results demonstrated that at the highest concentration nerolidol facilitated greater permeation of drug. However, at the lowest concentration, i.e. 0.25% v/v farnesol increased the drug permeation more than other enhancers used in the study [27].

Geraniol

Godwin et al. investigated how the transcutaneous permeation of three drugs (triamcinolone acetonide, hydrocortisone and caffeine) was impacted by utilizing eleven different monoterpenes. Drugs were dissolved in propylene glycol solution and then applied to the animal model skin pretreated with the enhancer solutions. The results of the experiment showed that only permeation of caffeine increased significantly due to the monoterpenes. Geraniol increased the permeation of caffeine about 16-fold, highest than any other terpenes used in the study. In the case of triamcinolone acetonide and hydrocortisone, α-terpineol increased their permeation by 2.5-fold and fivefold, respectively [26]. Hanif et al. reported that the presence of tetrahydrogeraniol as an enhancer in 5-fluorouracil gels increased the permeation of 5-fluorouracil very significantly. The results showed that maximum increment was observed when tetrahydrogeraniol was used in concentration of 8% [50].

Carvone

Krishnaiah et al. reported the formulation of a transdermal system containing hydroxypropyl cellulose gel of nicardipine hydrochloride. Carvone (8% w/w) was also integrated in the gel as an enhancer. In the in vitro experimentation, it was found that the permeation of nicardipine hydrochloride through the rat skin model increased significantly in the presence of carvone. This increase in permeation leads to an increase in bioavailability of drug by threefold from the transdermal system of nicardipine hydrochloride [25]. Gao et al. investigated the penetration enhancement ability of four cyclic terpenes (thymol, menthol, 1,8-cineole and carvone) in conjunction with ethanol, for the transdermal administration of tamoxifen over the porcine epithelium. The results showed that in the presence of carvone more permeation of tamoxifen was achieved than in comparison with the presence of menthol, thymol and cineole [51].

Terpinolene

Monti et al. investigated the influence of different terpenes on the permeation of dapiprazole base across the hairless mouse skin model. They developed various formulations of the drug using both liquid and semisolid vehicles. Experimental results showed that the presence of 1-limonene, α-bisabolol and terpinolene resulted in the enhancement of permeation of drug across the skin

up to 73 times when liquid vehicle is used. However, when semisolid vehicles were used in the formulations, permeation rises but not to that extent when liquid vehicles used. Out of all the terpenes, used terpinolene facilitated the highest skin permeation of dapiprazole when liquid vehicles were used. In the case of semisolid vehicles, limonene is perhaps the most effective permeation booster [24].

Recent applications of terpenes in pharmaceutical formulations

Natural permeation enhancers are widely used in improving the drug delivery across the transdermal barrier. They pervade into the skin and reversibly decline the hindrance or opposition offered due to the barrier function of skin. Diclofenac potassium hydrogels were prepared using geraniol, thymol and l-menthol, as iontophoretic productivity enhancers for improving transdermal delivery of the drug. The study revealed that a combination of constant voltage iontophoresis and enhancers like geraniol or l-menthol can successfully enhance the transdermal delivery of diclofenac potassium. Further, the combination of iontophoresis and penetration enhancers reduced the patch size and increased the patient compliance [52].

An antidiabetic transdermal patch of glimepiride was developed by using different concentrations of permeation enhancers like isopropyl myristate, tween 20, eucalyptus oil, Span 80 and limonene. The study affirmed that the use of permeation enhancers increased the release of glimepiride and enhanced its skin permeation. Isopropyl myristate-containing patches were reported to show the highest drug penetration across the skin [53]. In vitro permeation studies were performed for vesicles of temoporfin formulated by using soya lecithin. The vesicles also contain 1% blend of terpenes (citral, d-limonene and cineole) and ethanol. The results affirmed that the temoporfin vesicles that contain terpenes permeated markedly across the SC. However, vesicles without terpenes were unable to permeate across the SC [54].

A.K. Jain et al. performed ex vivo studies to determine the impact of different terpenes on the penetration of imipramine hydrochloride across the rodent skin. Imipramine hydrochloride dissolved in a solvent system with terpene showed a higher rate of permeation across the skin as compared to drug dissolved in a solvent system without terpene. The results confirmed the efficiency of terpenes to enhance the rate of permeation of the drug. The study concluded that cineole, terpineol, menthol and menthone have the potential for enhancing the permeability of polar and water-soluble drug [38]. Kahraman et al. formulated nanomicelles of tacrolimus monohydrate with terpenes for enhancing the delivery of tacrolimus monohydrate to the deeper sections of the skin. The skin permeation study concluded that nanomicelles containing terpinolene significantly increased the rate of permeation of the drug across the skin compared to commercial formulation and nanomicelles without terpene. Thus, nanomicelles with terpenes can be highly beneficial for improving drug delivery across the skin [55].

Transdermal organogel of haloperidol with limonene was formulated for controlled drug release. Selection of limonene was based on the results of in vitro studies carried out for linalool, limonene and cineole in propylene glycol. Limonene proved to be a promising enhancer as it enhanced skin permeability, reduced the lag time and aids to in vitro therapeutic delivery of haloperidol [44]. Liu et al. investigated microemulsion-based drug delivery system consisting of terpenes for the transdermal delivery of curcumin. It was affirmed that curcumin's rate of permeation increased up to 30–44 times in the case of limonene-containing microemulsions as compared to the microemulsions containing terpineol and 1,8-cineole. Microemulsion containing limonene enhanced transdermal delivery of curcumin by lowering the SC's diffusional barrier and viscosity. It was inferred from the research that microemulsion containing limonene is an efficient system for delivery of curcumin transdermally [56]. In another study, an o/w microemulsion system containing terpenes was developed for enhanced transdermal delivery of ketoprofen. Limonene-containing microemulsion of ketoprofen augmented the rate of penetration of ketoprofen up to 3 times as compared to the control group. Limonene proved to be the best enhancer out of all the other terpenes used, i.e. menthol, cineole and camphor, for formulating microemulsions of ketoprofen [57].

Narishetty et al. (2004) studied the effect of different terpenes on the permeation rate of zidovudine, which is an anti-HIV drug. Zidovudine is a polar molecule with poor transdermal permeability. Ex vivo permeation studies were conducted using different terpenes, namely menthol, cineole, a-terpineol, menthone, carvone and pulegone, on a rodent skin. It was observed that all the terpenes augmented the permeability of zidovudine when compared to the vehicle alone. Cineole was the promising in increasing the permeation rate as compared to other terpenes [37]. Rizwan et al. (2008) studied the impact of different terpenes, and a diterpene called forskolin, on skin permeation of valsartan. Automated transdermal diffusion cells sampling system was used for the permeation studies through rodent skin and human dead body skin. It was observed that cineole was the most effective in enhancing the diffusion of valsartan across rat and human skin. Therefore, it has a great potential to be used as an enhancer for the transdermal delivery of valsartan. Further, it was also concluded that forskolin enhances the permeation of valsartan across the skin through lipid

Table 3 Application of terpenes in penetration enhancement

Terpene and reference	Drugs	Mechanism
Carvone [47, 59–61]	Zidovudine	Fluidization of lipids
	Nicardipine HCl	Extraction of lipids
	Nimodipine	Breaking of lipid bilayer and SC lipid extraction partially
	Diclofenac sodium	Increased drug diffusion and drug partitioning
	Genistein	Disruption of lipid bilayer
1,8-Cineole [62–64]	Ondansetron hydrochloride	Fluidization of lipids
	Valsartan	SC lipid extraction and denaturation of keratin
	Propranolol hydrochloride	SC lipid extraction, disruption and keratin denaturation
Farnesol [65]	Propranolol hydrochloride	Increased drug diffusion through intercellular lipids
Geraniol [65, 66]	Propranolol hydrochloride	Increased drug diffusion
	Terbinafine	Disruption of SC lipid bilayer
Limonene [61, 62, 67]	Genistein	Disruption of SC lipid bilayer
	Ondansetron hydrochloride	
	Haloperidol	
Linalool [68]	Lomerizine dihydrochloride	Increased fluidity and disruption of lipid bilayer structure
	Geniposide	Interrupting the lipid organization and extraction of SC lipids
Menthol [61, 62, 67, 68]	Genistein	Stratum Corneum lipid bilayer disruption
	Lomerizine dihydrochloride	Increased fluidity and disruption of lipid bilayer structure
	Valsartan	SC lipid extraction and breaking up of hydrogen bonds
Nerolidol [66, 69, 70]	Terbinafine	Disruption of lipid bilayer
	Hydrocortisone	Hydrophobic lipid arrangement disruption
	Ondansetron hydrochloride	–
Terpinen-4-ol [71]	Geniposide	SC lipid agitation and extraction
	Puerarin	

bilayer disruption and extraction of SC [58]. Some of the recent applications of terpenes are discussed in Table 3.

Invasomes

Vesicular systems like conventional liposomes and novel vesicular carriers like niosomes, transferosomes, ethosomes, invasomes, flexosomes, vesosomes, ufasomes, polymerosomes, etc., were developed and tested for their suitability as a drug carrier for transdermal drug delivery [72]. Conventional liposomes developed for transdermal drug delivery showed limited penetration power and can have access only to upper skin layers [72]. Thus, they can provide only localized therapeutic benefits restricted to the upper skin layers only [72]. Novel vesicular systems with suitable elasticity have been developed in order to increase the drug permeability [73]. These novel vesicular systems involve the utilization of a penetration enhancer in their formulation for enhancing the permeation across the skin [74]. Niosomes are elastic vesicular carriers developed by using a combination of cholesterol with nonionic surfactants, and they were reported to be suitable for transdermal drug delivery [75]. Ethosomes are vesicular systems developed by using phospholipids, alcohol (ethanol and isopropyl alcohol) and water [76]. They show more rapid and significant transdermal permeation in comparison with conventional liposomes [77]. Transferosomes are elastic vesicular drug carriers composed of phospholipids (phosphatidylcholine) along with an edge activator (polysorbate or sodium cholate) [78].

Transferosomes are highly adaptive and stress responsive carrier systems which squeeze themselves along the intercellular sealing lipids of the SC to penetrate across the skin [3, 79, 80]. Flexosomes can be simply defined as liposomes with flexible membrane [81]. They are formulated by employing a combination of phospholipids with an edge activator (tween 80) [82]. Vesosomes can be defined as a multicompartment drug delivery carrier in which a smaller liposome was encapsulated within a larger liposome [83]. Ufasomes also termed as unsaturated fatty acid vesicles are suspensions of close lipid bilayers [84]. Fatty acids combined with their ionized species (soap) were utilized to form these lipid bilayers. Cholesterol, buffers and lipoxygenase are other important constituents of ufasomes [84]. The concentration of cholesterol and lipoxygenase along with the presence of divalent cations and the pH range is very crucial for the formation of stable ufasomes [84]. The fatty acids

within the ufasomes align themselves in such a manner that their carboxyl group remains in contact with aqueous medium whereas the hydrocarbon tails are pointed towards the interior matrix of the lipid bilayer membrane [84]. Polymerosomes are a category of vesicles manufactured from synthetic block copolymers of amphiphilic nature [85]. They are similar to other vesicles in their configuration having an aqueous core surrounded by bilayer of polymers instead of lipids [85]. The hydrophilic components of polymeric bilayer were directed towards the external environment, whereas the hydrophobic component is directed towards the interior of the polymeric bilayer [85].

Invasomes are novel vesicular drug carriers having structural similarity with liposomes, i.e. bilayer membrane arranged within a spherical configuration so as to form a central aqueous core surrounded by a lipoidal bilayer membrane [86]. The basic difference between invasomes and liposomes lies within their composition. Liposomes are simply composed of phospholipids and cholesterol, have rigid membrane and can penetrate very less in skin [87]. Invasomes are composed of phospholipids (soya phosphatidylcholine, lysophosphatidylcholine), terpenes and ethanol [86–89]. Invasomes have flexible membrane, and this flexibility was majorly contributed by lysophosphatidylcholine, which act as an edge activator [90]. Soya phosphatidylcholine was the primary bilayer membrane forming ingredient [91]. Invasomes can penetrate much deeper into the skin in comparison with liposomes, and this enhanced penetration capability was conferred by terpenes and ethanol [92]. Addition of terpenes and ethanol also contributes to improving the fluidity/flexibility of the bilayer membrane [86].

Penetration enhancement by invasomes and impact of formulation components on properties of invasomes

Improved penetration of invasomes across the *stratum corneum* of skin was primarily based on the presence of ethanol and terpenes in their bilayer matrix. Mechanism of penetration enhancement by ethanol was based on its interaction with the polar lipids present within intercellular spaces between the keratinocytes of the SC [86, 93]. The interaction produces structural transformation within the lipophilic (keratinized) layer of SC. The transformation results in the lowering of the lipid transition temperature [86]. The reduction in transition temperature causes fluidization of tightly packed lipids of SC, which ultimately disturbs the continuity of SC [86]. Ethanol also contributes to the stabilization of the invasomes as it generates negative charges on the surface of the vesicles, which reduces the aggregation of vesicles due to electrostatic repulsion [94].

Terpenes act as potent penetration enhancers due to their ability to break down the nearby lipoidal bilayer matrix of the SC [86]. Terpenes actually break the hydrogen bonds between the molecules present in the lipoidal matrix of SC and thus alter the packing of SC [93, 95]. In addition, terpenes were also reported to enhance the diffusion, permeability across the SC and the fluidity of the lipid bilayer matrix of invasomes [95, 96]. Thus, the capability of the terpenes and ethanol to induce deformation of vesicles by improving flexibility/ fluidity of vesicle's bilayer membrane and disruption of integrity of SC by interacting with its hydrophilic components are the mechanisms responsible behind the enhanced permeation of invasomes across the skin [97]. The topical administration of invasomal formulation was followed by the disintegration of large invasome vesicles present in the formulation [98]. Disintegration causes the release of invasomal components: phospholipids, ethanol and terpenes, and these free components produce fluidity in the lipoidal matrix of SC [98]. Invasomes having small vesicle size do not undergo disintegration and permeate through the upper layers of SC in intact form to reach the inner regions of SC [98]. The vesicles reach inner regions either by means of transfollicular route or by travelling through narrow hydrophilic channels present into SC's intercellular region [99]. It was also reported that small-size invasome vesicles can also penetrate deeper into the SC via the channel like areas present in the deep layers of SC [100, 101]. Thus, after application on the skin a substantial amount of invasomes gets disintegrated and only invasomes with small vesicle size will remain intact and penetrate into the inner regions of SC.

The phosphatidylcholine component of phospholipids also confers penetration-enhancing ability to the phospholipids [102]. The penetration enhancement action of phosphatidylcholine as indicated by various studies was mainly attributed to its head group (choline) [87]. However, phosphatidylcholine with unsaturated acyl chains was also reported to have a role in improved penetration power of phospholipids [95, 103]. Combination of phospholipids with terpenes and ethanol produces a synergistic effect on the penetration capability of invasomes which ultimately leads to the enhanced permeation of drug across the skin [10]. Many studies reported that phospholipids released after the disintegration of invasomes application on skin penetrate deep into the intercellular matrix and get mixed with the lipid bilayer of SC which changes its structural integrity, thus making it easier for active moieties to permeate deep into the skin [16, 95, 103, 104]. Improved permeation of both hydrophilic and lipophilic active moieties was reported when invasomes were utilized as drug delivery carriers for their topical administration. Microcavities may also be formed

in the matrix of SC as the components released after disintegration of invasomes change its integrity [86]. These microcavities allow higher diffusion and deeper penetration of remaining intact invasomes into the inner regions of SC, and it also enables enhancement of spreading coefficient of drugs delivered via invasomes [86]. Thus, topical drug delivery by invasomes involves a series of processes, which includes disintegration of a large number of invasomes by fusion with SC and disruption of structural organization of SC by components released by disintegration of invasomes [88, 98]. This is followed by penetration of small-size invasomes into deeper regions of SC and other skin layers either through follicles, hydrophilic channels present in SC or either through microcavities formed due to disruption of SC [86, 98, 99].

The success of invasomes depends on their penetration power which in turn depends on their capability to induce fluidity in the structural framework of SC at the local level to disrupt its function as well as on the fluidity, deformability and size of the invasomes itself. Major portion of drug from invasomes gets released after their disintegration on the skin surface and fusion with the SC matrix [86, 98]. This freed drug then gets incorporated into the lipoidal matrix of *SC* and reaches the deeper layers of skin. Some portion of the drug reaches the pilosebaceous glands along with small-size invasomes via transfollicular route and gets partitioned into the sebum fluid from where it was further distributed [99]. The rest of the drug permeates directly into the deeper layer of SC along with invasomes, which penetrates directly into deeper region of skin via hydrophilic channels present in the SC matrix [99]. All the three components of invasomes vesicle matrix, i.e. phospholipids, terpenes and ethanol, also boost the partitioning of drug substance into the intercellular lipid bilayer membrane of SC (Fig. 2.) [88].

Methods of formation of invasomes

Two methods of preparation were commonly employed for formulating invasomes. The first one is mechanical dispersion method and the second is film hydration method.

Mechanical dispersion method

In mechanical dispersion method of invasome formulation, the first step involved was the preparation of ethanolic solution of phospholipids. It was done by dissolving phospholipids in ethanol [105]. Active moiety (s) and

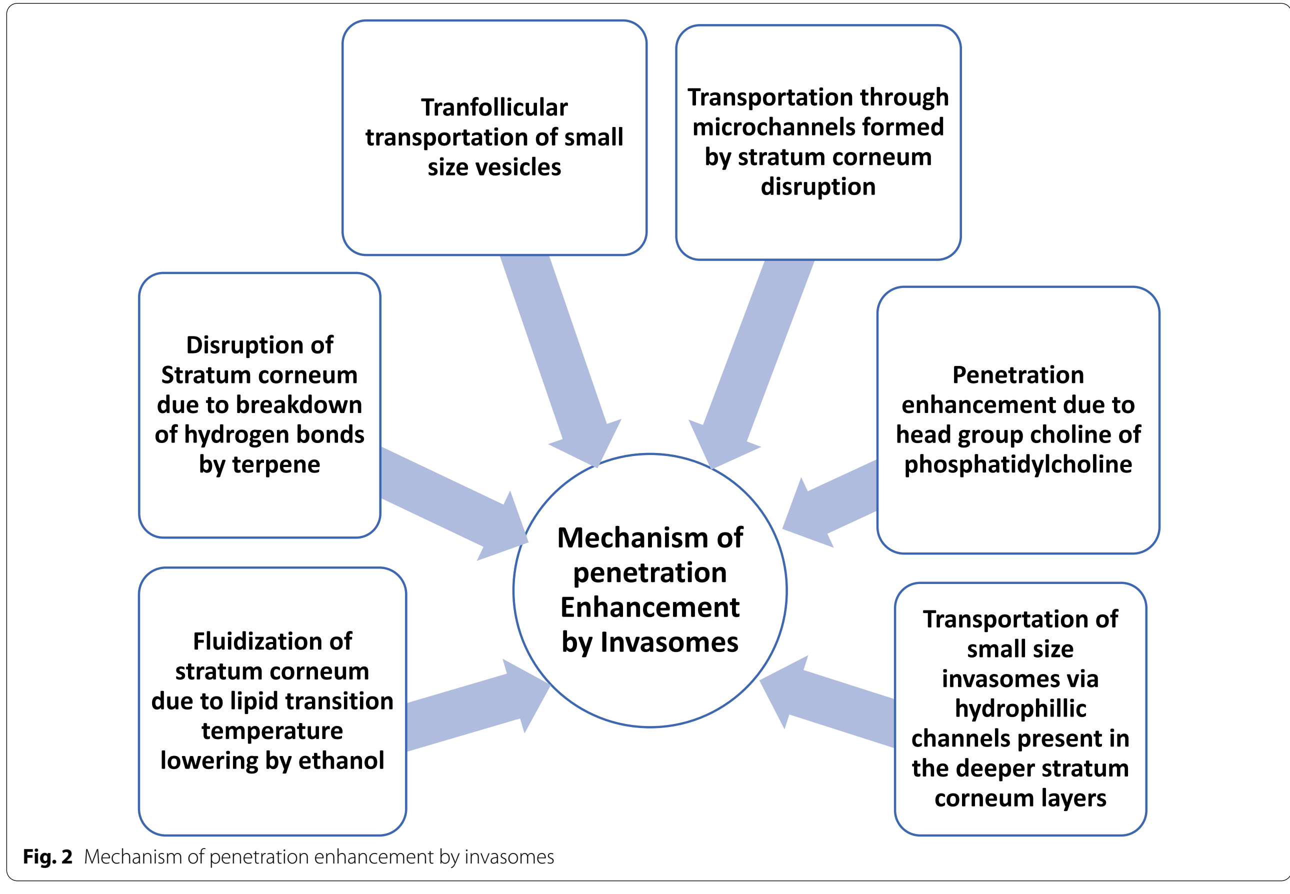

Fig. 2 Mechanism of penetration enhancement by invasomes

terpene/terpene mixture were then added to this solution. The ethanolic solution of phospholipids containing active moiety (s) and terpenes was then vortexed for a time period of 5 min followed by sonication for 5 min [105, 106]. Vortex mixing followed by sonication produces a final homogeneous solution to which either phosphate buffer (7.4) or phosphate buffer saline or any other suitable solvent was introduced by using a syringe [107, 108]. The addition of buffer/saline/solvent to the final solution was carried out under continuous vortexing which results in the formation of multilamellar vesicles or invasomes within the solution [107, 108]. Finally, the obtained solution of multilamellar invasomes was extruded through polycarbonate membranes of varying pore sizes under high pressure. Repetition of extrusion process was carried out several times in order to obtain invasomes with uniform dimensions [108, 109].

Film hydration technique

Another commonly reported method for production of invasomes was the film hydration technique. The initial step of film hydration technique includes preparation of a solution of phospholipids with ethanol. Then, a 2:1 v/v solution of methanol with chloroform was prepared. Both the solutions were mixed and then evaporated in a rotary flash evaporator for 2 h at a temperature of 50 °C and pressure ranging from 500 to 1 mbar, respectively [91, 110, 111]. The process leads to the formation and deposition of a thin film on the inner walls of flask in which the solution is kept during evaporation process. The film was then kept at 2 h at a pressure of 1mBar and then flushed with nitrogen [92, 111]. The deposited film was then hydrated with phosphate buffer saline. Hydration of film can also be carried out by utilizing a solution composed of ethanol, terpenes and phosphate buffer. Hydration of the film was carried out for a duration of 30 min, and then the hydrated film was cooled up to the room temperature. Finally, addition of terpene or terpene mixture was done, which leads to the formation of the invasomes [111, 112]. The solution of invasomes was then subjected to vortex shaking and then followed by ultrasonication [86, 111, 112]. The last step of the film hydration technique also requires repeated extrusion of invasome solution through polycarbonate membranes of different pore sizes just as mentioned in the mechanical dispersion method to obtain the vesicles of desired characteristics [113].

Characteristics and characterization of invasomes

Vesicle size

Determination of vesicle size of invasomes has been reported by using the techniques like photon correlation spectroscopy or dynamic light scattering. Amnuaikit et al. developed invasomes and transferosomes of phenylethyl resorcinol and compared their characteristics and performance against the conventional liposomal formulation of phenylethyl resorcinol. All the vesicular drug delivery systems were developed for topical administration of phenylethyl resorcinol. The vesicle size determination of all the three types of vesicles was done by zetasizer utilizing the principle of dynamic light scattering. The vesicle size of prepared invasomes was reported from 208.10 ± 12.00 to 819.30 ± 101.90 nm, and the polydispersity index was reported less than 0.3, indicating higher degree of homogeneity in terms of size [114]. Haag et al. developed invasomes of TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxyl), a nitroxide free radical by mechanical dispersion method. They performed both in vivo and ex vivo experiments in order to determine the antioxidative capacity of skin. They evaluated the vesicle size by photo correlation spectroscopy and reported that the mean size of prepared invasomes was 86 nm. The polydispersity index of invasomes was found to be 0.13 [115].

Vesicle shape

Utilization of several advanced microscopic techniques like scanning electron microscopy (SEM), transmission electron microscopy (TEM), cryo-TEM, etc., has been reported in the literature for determining the vesicle shapes of closed bilayer structure like invasomes. Prashanti et al. developed invasomes loaded with finasteride for transdermal delivery via iontophoresis technique. They evaluated the vesicle shape of the developed invasomes by SEM and reported that vesicles have spherical shape with unilamellar configuration [116]. Ntimenou et al. formulated liposomes, transferosomes and invasomes of calcein and carboxyfluorescein. They evaluated the physicochemical characteristics of all the three types of formulated vesicles and compared them. They utilized cryo-TEM for evaluating the shape of developed vesicles and reported that all the vesicles including invasomes have spherical shape. Formulated invasomes were largely composed of small unilamellar vesicles [117]. Qadri et al. formulated isradipine-loaded invasomes for transdermal application for treatment of hypertension. They utilized TEM for determining the shape of invasomes and reported that the invasomes have spherical shape along with smooth surface morphology [91].

Zeta potential

Zeta potential is considered to be an essential parameter for determining the stability of invasomes, and zetasizer is used for its measurement. Several studies have attributed the presence of negative zeta potential on invasome as the main reason for enhanced penetration rate [118, 119]. Shalaby et al. formulated liposomes, ethosomes

and invasomes of both mannitol and corticosterone and evaluated their skin penetration capability. They studied the distribution pattern of both the drugs in human skin after being released from the different vesicular carriers. Evaluation of all the three vesicular formulations revealed that all of them possess a negative zeta potential. Liposomes have the smallest and ethosomes have the highest negative zeta potential value, respectively. Invasomes also showed significant negative zeta potential values. The study reported that ethanol increases the negative zeta potential of liposomes. They also reported that ethosomes showed high negative surface charge because of utilization of negatively charged phospholipids in their composition [120]. Ammar et al. formulated vardenafil hydrochloride (VRD)-loaded ethosome-derived invasomes for transdermal application as a treatment strategy for pulmonary arterial hypertension. They initially developed ethosomes of vardenafil hydrochloride with three different concentrations of phosphatidylcholine and ethanol. They evaluated the ethosomes, selected the best ethosomal formulation and transformed it into invasomes by incorporating terpenes (limonene and cineole) into the vesicle membrane of ethosomes. They utilized three different concentrations of limonene and cineole as well as a 1:1 mixture of both the terpenes to develop invasomes. All the invasomes formulations were reported to have negative zeta potential and also have higher value in comparison with ethosomes. They concluded that incorporated terpenes in combination with ethanol act synergistically to increase the value of zeta potential of invasomes [121].

Drug entrapment efficiency

Estimation of drug entrapment efficiency is very important for drug delivery systems like invasomes. Ahmed et al. prepared avanafil-loaded invasomes and selected the optimized invasomal formulation to be developed as a transdermal film in order to treat erectile dysfunction. They calculated the drug entrapment efficiency of the invasomes utilizing centrifugation technique. They centrifuged the samples of the formulated invasomes for 45 min at 20,000 rpm and collected the supernatant fluid. The supernatant is then filtered, diluted and analysed by HPLC for determining the concentration of unentrapped avanafil [122, 123]. The findings of the study showed that the entrapment efficiency of the invasomes gets increased as the percentage of phospholipids utilized in their composition increases. This enhancement of entrapment efficiency of avanafil was attributed to its lipophilic nature of the drug due to which it can get incorporated within the lipoidal phase of invasomes. Thus, an increase in the phospholipid composition increases the chances of the avanafil incorporation within the lipid components of invasomes [122]. Imam et al. formulated risperidone-loaded soft membrane lipid vesicles and determined their entrapment efficiency. The method used for determination of drug concentration entrapped within the vesicles involves the utilization of TritonX-100 in 0.1% concentration as an agent to disrupt the vesicle membrane. Then, the solution of vesicle with TritonX-100 was filtered and the collected liquid was diluted and analysed for the drug concentration by HPLC technique. The highest drug entrapment was reported in the case of vesicles formulated with low ethanol and medium phospholipid concentration. Vesicles with high ethanol concentration were reported to have low entrapment efficiency. Disruption of vesicle membrane due to the presence of high concentration of ethanol, which results in leaching out of drug from the vesicle, was proposed as the possible reason for low entrapment efficiency [124].

Drug content

Spectrophotometry and HPLC were the common techniques used for the estimation of drug content of the invasomal formulation [111, 125]. Kamran et al*.* formulated nano-invasomal gel of olmesartan medoxomil via film hydration technique for transdermal application. They determined the drug content of the nano-invasomal formulation through spectroscopic method and reported good content uniformity. They analysed the sample of formulation at 257 nm by the UV–Vis spectrophotometric method [125, 126].

Skin permeation studies

Efficiency of invasomes to cross the skin barrier was estimated via skin permeation studies. Several methods or procedures have been reported in the literature to determine the skin penetration capability of invasomes. Trauer et al. formulated liposomes and invasomes loaded with hydrophilic and lipophilic dyes. Impact of massage and occlusion on the penetration capabilities of both the vesicular carriers under the *ex vivo* conditions was evaluated in the study. They used Franz diffusion cells to study the skin permeation and taken human breast and abdominal skin as membrane. They applied massage on the skin mounted on the Franz diffusion cell, and then with the help of confocal laser scanning microscopy estimated the depth to which both the liposomes and invasomes penetrate within the follicles. The study showed significant enhancement of penetration of both liposomes and invasomes upon application of massage. However, if occlusion was applied enhanced penetration was observed only in the case of liposomes and invasomal penetration power was diminished. Thus, it can be concluded that massage can enhance both liposomal and invasomal penetration capability, whereas in the case of occlusion the

penetration depends on the type of formulation and composition of the respective vesicular formulation [127]. Apart from human breast and female abdominal skin, animal models like Wistar rat skin, porcine skin, rabbit skin, etc., have also been used as membrane to study the skin permeation capabilities of invasomes [125, 128, 129]. The mechanism behind the deeper penetration of invasomes into the skin is attributed to their ability to interact with the lipids present within the skin. This interaction causes the tight lipoidal junctions to loosen up which alters the tight framework of SC, ultimately leading to enhanced permeation [97].

Stability studies

The major parameters taken into consideration while carrying out the stability studies in case of invasomal preparations are vesicle size, shape, polydispersity index and zeta potential. Size and polydispersity index were with dynamic light scattering technique. Microscopy techniques SEM and TEM were used for assessment of vesicle shape, and zeta potential was measured by zetasizer. Prashanti et al. performed stability studies for finasteride-loaded invasomes. They performed stability studies at two different temperatures 4 ± 1 °C and 25 ± 1 °C for a time period of 120 days. The test samples under the consideration were re-evaluated for vesicle size, shape, surface charge, polydispersity and entrapment efficiency after the stated time period. The results showed that the formulation sample stored at 4 ± 1 °C does not showed significant changes in size, shape, entrapment efficiency and zeta potential. However, the invasomes stored at the 25 ± 1 °C were reported to undergone significant variations. A reduction in zeta potential and entrapment efficiency along with increment in the vesicle size was reported in the case of invasomes stored at 25 ± 1 °C. Aggregation and fusion of vesicles caused an increase in vesicle size and lesser entrapment. Vesicle aggregation and fusion occurred due to the lowering of zeta potential as low zeta potential facilitates lesser electrostatic repulsion between the vesicles allowing them to aggregate. A reduction in surface charge and aggregation of vesicles leads to decreased entrapment of the finasteride. Thus, the study concludes that at 4 °C the invasomal formulation remains quite stable, whereas significant instability was reported in the case of invasomes stored at 25 °C for a period of 120 days [116].

Kalpana et al. formulated tolterodine tartrate-loaded invasomes and evaluated their stability behaviour under two different temperature conditions. They stored a sample of invasomes at 4–8 °C and another sample at 30 °C for a time period of 2 months. They measured the entrapment efficiency of the samples under consideration after the prescribed time period and reported a decrease in entrapment efficiency for both sample sets. A slight decrease in entrapment efficiency in the case of invasomes stored at 4 ± 2 °C and aggregation and fusion of invasome was reported as the mechanism behind this. However, a significant reduction in per cent entrapped drug was recorded for invasome samples stored at 30 °C. The effect of temperature on gel to liquid transition of lipid bilayers along with chemical degradation of phospholipids of vesicle carrier was reported as possible scientific explanation behind this deterioration [129]. Thus, from the study it was concluded that storage of invasomal formulations at refrigeration temperature range can ensure more stability and storage at elevated temperature causes significant deteriorating impact on stability of invasomal product. Dragicevic-Curic et al. performed stability study on temoporfin-loaded invasomes by storing them at two different temperatures 4 °C and 23 °C. They measured particle size and polydispersity index at fixed time intervals of both the samples in order to access the stability behaviour of formulated invasomes under two different temperature conditions. They reported a significant increase in particle size and polydispersity index in the case of invasomes stored at 23 °C after 6 months. However, for invasomes stored at 4 °C no significant difference in particle size and polydispersity index was recorded. The increase in particle size results from aggregation or fusion of invasomes indicating physical instability [54].

Therapeutic application of invasomes

Invasomes for hypertension treatment

Hypertension is a chronic medical condition and is one of the most common risk factors associated with various cardiac vascular diseases. Many pharmacological categories of anti-hypertensives are available for treatment of hypertension. However, these anti-hypertensive agents have associated issues like low permeability, solubility, bioavailability, undesirable side effects, etc. These issues can be countered to some extent via proper selection of drug delivery system and route of administration. Invasomes have also been investigated for the transdermal administration of anti-hypertensive drugs. Kamran et al. formulated a topical gel composed of nano-invasomes of olmesartan medoxomil which is a BCS class II drug, and its oral bioavailability was reported to be 28.6% when administered in the form of tablet [125, 130]. In vivo pharmacokinetic study was carried out on Wistar rats, which showed that the concentration maximum (C_{max}), in the case of tablet and nano-invasomal gel, was reached after 2.0 ± 0.22 h and 8 ± 0.41 h of administration, respectively. The relative bioavailability, i.e. bioavailability after topical application of nano-invasomes gel with respect to oral bioavailability, was found to be 115.60%.

The biological half-life of olmesartan medoxomil was reported to increase up to 135% after topical application in comparison with half-life after oral administration. Thus, the study concludes that administration of olmesartan medoxomil via transdermal route in the form of nano-invasomal gels improved the bioavailability which may lead to a reduction in dosing frequency of drug. Therefore, transdermal administration can be a better alternative to oral administration [125].

Ammar et al. formulated ethosomes of vardenafil hydrochloride and then transformed the best ethosomal formulation into invasomes by incorporating terpenes (limonene and cineole) within the membrane of ethosomes. They estimated the pharmacokinetic behaviour of the formulated invasomes after transdermal application by utilizing software-based physiologically based pharmacokinetic (PBPK) modelling. They used oral aqueous dispersion of vardenafil hydrochloride as control for this experiment. Pharmacokinetic performances of both transdermal invasomal formulation and oral aqueous dispersion were estimated for both adult and geriatric patients. PBPK modelling revealed that mean C_{max} values for aqueous dispersion and transdermal invasomes were 4.8 μg/L and 2.3 μg/L, respectively. The median time maximum (T_{max}) values were found to be 0.7 h and 2.8 h, respectively, for oral dispersion and invasomes, respectively. The plasma concentration–time curve of oral aqueous dispersion of vardenafil hydrochloride for both adults and geriatrics cases showed sharp peak-like pattern, i.e. fast attainment of C_{max}. However, in the case of transdermal invasomes the plasma concentration–time curve showed a steady-state condition, i.e. slow attainment of C_{max}, and then followed a steady-state plasma concentration of drug for both adults and geriatrics. The fast attainment of C_{max} in case of oral aqueous dispersion is due to rapid absorption after oral administration, whereas in case of invasomes slow attainment of C_{max} and delayed T_{max} is due to the barrier function of SC. The relative bioavailability was obtained by comparing the mean area under curve (AUC) values taken up to 24 h for invasomes and oral aqueous dispersion of drug, and its value was found to be 169.65% [121]. Thus, the study concludes that transdermal administration of vardenafil hydrochloride using invasomes as drug delivery carriers can be a potential approach to increase its bioavailability [121].

Invasomes for acne treatment

Acne is a chronic inflammatory disease of pilosebaceous unit. Androgens are believed to play an important role in the onset of acnes [131]. Primarily, the skin of face, neck, chest and back is mostly affected by this inflammatory condition [131]. Process of development of acne lesions can be divided into four different stages. Acne development starts with the release of inflammation mediators (CD_4, and macrophages infiltrate the pilosebaceous region and increase its vasculature). This is followed by formation of comedones due to alteration in production of keratin layer. The third stage is characterized by increment in sebum production, a process controlled by androgens. The final stage is the colonization of follicles by *Propionibacterium acnes* [132]. Several treatment strategies like oral antibiotics, topical formulation of retinoids, benzoyl peroxide and antibiotics were utilized for treatment of acne but have limited success [133–135]. Isotretinoin given via oral administration was effective for treatment of severe acne condition but reported to cause teratogenicity [136, 137]. Invasomes have also been investigated by researchers for efficient drug delivery via topical route for the treatment of acne.

El-Nabarawi et al. utilized film hydration technique for the production of dapsone-loaded invasomes. Dapsone has anti-inflammatory activity which has been proved to be beneficial in acne treatment as reported by various scientific literature studies. Four different terpenes such as limonene, cineole, fenchone and citral were used in various concentrations to produce different sets of invasomes. They evaluated the formulated invasomes and determined the impact of concentration of terpenes on the properties of invasomes. The best invasomal formulation was selected for in vivo study. Wistar rat animal model was utilized in in vivo study to determine the penetration power of the formulated invasomes and their capability of deliver the dapsone into the deeper regions of skin. They compared the in vivo results obtained for the dapsone-loaded invasomes against the results obtained for alcoholic solution of dapsone. Results of in vivo experimentation reported the presence of higher concentration of dapsone within the deep skin layers when administered via invasomes in comparison with results obtained for alcoholic solution of dapsone. The respective concentrations of dapsone within the skin were found to be 4.11 mcg/cm^2 and 1.71 mcg/cm^2 after topical application of invasomes and alcoholic solution of dapsone, respectively. The AUC graph plotted for data taken up to 10 h showed a twofold greater AUC for invasomes in comparison with dapsone solution. Thus, the study revealed that invasomes can deliver dapsone efficiently into the deeper regions of skin and therefore may prove to be a more potent treatment strategy for acne [112]. Han et al. developed an invasome-based topical antiacne formulation containing crude extracts of *Ocimum basilicum.* They proposed from their study that invasomes based topical formulation of crude extracts are highly efficient and stable. Therefore, invasomes offered advancement in the drug delivery strategy for

acne treatment as well as ensured the antiacne activity of extracts of *Ocimum basilicum* [138].

Invasomes for cancer treatment

Cancer is one of the most challenging conditions to deal in modern medical science. Many therapeutic strategies involving the use of several chemotherapeutic agents are available for treatment of cancer. However, still many of these strategies have limited success rate and also various serious adverse effects were associated with the utilization of these strategies and chemotherapeutic agents. Therefore, it becomes necessary to develop or reach out to newer therapeutic strategies and test them for their applicability in cancer treatment. Invasomes, novel deformable vesicular systems, were also evaluated by researchers for their capability to deliver anticancer agents.

Vidya et al. developed a UV spectrometric method for performing in vitro evaluation of the invasomes of anastrozole and also carried out the validation of the method. Spectrophotometric properties of anastrozole were evaluated in different solvents such as water, phosphate buffers (pH 6.8 and 7.0) and alcohol. Pure drug showed the best absorption characteristics in phosphate buffer 7.0, and absorption maximum for astranazole was reported to be at 210 nm. They formulated the invasomes of anastrozole via film hydration method and carried out the analysis of all the invasome samples at 210 nm as per the ICH guidelines. All the results were found to be in acceptable limits. The method allows reproducible quantification of anastrozole present in the invasome samples in between the range of acceptance limit, i.e. 98–102% [139]. Vidya et al. formulated anastrozole (aromatase inhibitor)-loaded invasomes by film hydration method for the treatment of breast cancer in post-menopausal women. Invasomes were prepared by using phospholipon 80H, fenchone and ethanol. The prepared invasomes were evaluated for shape, size, zeta potential and entrapment efficiency. On the basis of results of these evaluations, best invasome formulation was selected and incorporated within the sodium carboxymethylcellulose gel to formulate a suitable invasome gel. Male Wistar rat skin was used to perform ex vivo diffusion study and skin deposition studies for the formulated invasomes. The results showed that invasomes having 4% concentration of terpenes in their composition have the highest entrapment efficiency, enhanced permeation profile and also showed significantly high drug concentration accumulation within the skin. Invasome gel was prepared with a blend of sodium carboxymethylcellulose, propanol and propylene glycol to which distilled water was added to make the final weight of the gel base up to 10 g. Invasomes of anastrozole, 10 mg in weight, were added to the gel base with continuous stirring. The gel was then evaluated for drug content, pH, homogeneity, spreadability, extrudability, viscosity, diffusion release pattern, skin irritation, stability and for its anticancer activity on human malignant breast adenocarcinoma [Michigan Cancer Foundation (MCF)-7] cell line. The results obtained from the evaluation of invasome gel were compared with the results obtained for the control gel as well as with the results obtained for two nano-invasomal gel. The first gel was simply prepared by mixing 10 mg anastrozole with 2.5% fenchone concentration. The second gel was prepared with 4% concentration of fenchone along with 10 mg anastrozole.

The results of evaluation of the various gels taken for study showed that invasomal gel formulation of anastrozole has higher capability to permeate across the skin in comparison with the control gel and nano-invasomal gels. Significantly higher deposition of drug within the skin was reported in case of invasomal gel formulation of anastrozole in comparison with control gel and nano-invasomal gels. The concentration of drug deposited within the skin for all the formulations is as follows: 149.2 μg/cm^2 (invasome gel), 11.38 μg/cm^2 (control gel), 31.39 μg/cm^2 (nano-invasome gel 4%) and 24.33 μg/cm^2 (nano-invasome gel 2.5%), respectively. The data represent a 13-fold increase in drug deposition within the skin after application of invasomes gel as compared to control gel. Cell line studies were conducted on MCF-7 cell lines using two different doses—2.5 μl/ml and 5 μl/ml—of all the formulations. Observations were recorded every 24 h up to 3 days for cytotoxic effect exerted by the formulations on the cells. The result revealed that significant cytotoxicity was observed against MCF-7 cell lines at the dose of 5 μl/ml of invasomal gel at 72 h. Skin irritation study was performed on depilated rabbit skin, and no signs of irritation, oedema and erythema were observed after 72 h of study. Therefore, it was concluded from the study that delivery of anastrozole using invasomes as drug delivery systems increased the drug penetration and drug deposition within the skin. The developed invasomal also exhibited significant cytotoxicity against MCF-7 cell lines and thus can be used as a potential treatment alternative for breast cancer in post-menopausal women [140].

Invasomes for erectile dysfunction

Erectile dysfunction is a medical condition effecting generally males of age above 40 years [141]. It is defined as the inability to attain and maintain sufficient erection of penis for satisfactory sexual intercourse [142]. Several factors have been identified and reported in the scientific literature studies as the possible cause of erectile dysfunction. On the basis of the factors involved, erectile

dysfunction was categorized into three different types: psychogenic, organic and mixed psychogenic and organic [141]. Psychogenic erectile dysfunction can result from various psychological factors. Performance anxiety (fear of failure during sexual intercourse) is one of the prominent psychological reasons for erectile dysfunction [143]. Psychogenic erectile dysfunction constitutes a significant portion of total cases of erectile dysfunction. Organic erectile dysfunction may result from numerous factors which involve neuronal factors and diseases (like Parkinson's disease and Alzheimer's disease), hormonal disturbances, changes in blood flow in penile muscles due to arterial malfunctioning and drug induced (antiandrogens, anti-hypertensives, nicotine, alcohol, etc.) [144–146]. Mixed psychogenic and organic type of erectile dysfunction results from involvement of both kinds of factors. Erectile dysfunction has a worldwide prevalence, and by the year 2025 approximately 322 million people will be suffering from this medical condition [147]. Several treatment strategies have been investigated and applied by the researchers and biomedical scientists to treat erectile dysfunction. Invasomes have also been investigated for their applicability in the treatment of erectile dysfunction. Ahmed et al. formulated invasomes of avanafil using Box–Behnken experimental design [122]. Successful drug delivery of avanafil was countered by several drug-related and body-related challenges like poor water solubility, significant first pass metabolism, and diminished absorption in the presence of food [148–150]. They evaluated the prepared invasomes for entrapment efficiency, size and shape of invasomes. They estimated the impact of concentrations of phospholipids, ethanol, terpenes and the type of terpene used in the formulation on the shape, size and entrapment efficiency of invasomes. The size of the invasomes was determined by dynamic light scattering technique, and the result revealed that most of the invasomal formulations prepared have polydispersity index values less than 0.3. This indicated the formation of monodisperse and homogenous invasome formulations. The entrapment efficiency of the formulated invasomes was found to be ranging from $83.12 \pm 5.61\%$ to $97.67 \pm 4.38\%$. The study showed that invasomes having high concentration of phospholipids and terpenes have higher entrapment efficiency. On the basis of the results obtained after the evaluation of formulated invasomes, the best optimized formulation (vesicle size 109.92 nm and entrapment efficiency 96.98%) was selected for further processing to be developed as transdermal films. The selected invasomal formulation was simply incorporated into the hydroxypropyl methyl cellulose-based transdermal film to develop avanafil-loaded invasome-based transdermal films. The invasomes-based films were then subjected to the ex vivo and in vivo experimentation. The ex vivo permeation study was performed using Franz diffusion cells, and abdominal Wistar rat skin was used as the membrane. The transdermal film of plain avanafil was used as a control in this experiment. The result obtained for invasome-loaded film was compared with the result obtained for plain transdermal film of avanafil. The experiment showed that amount of drug permeated from the avanafil-loaded invasomes-based film was enhanced 2.514 times as compared to plain avanafil-loaded film. High penetration capability of invasomes due to their composition as well as their nano-size range results in enhanced permeation of avanafil in comparison with plain avanafil film [122]. The in vivo experimentation was also carried out in male Wistar rat animal model. The in vivo performance of avanafil-loaded invasomes-based transdermal film was compared with the performance of the plain avanafil transdermal film and oral avanafil suspension at a dose of 30 mg/kg dose. The analysis of plasma concentration–time graphs of all the formulations showed that in comparison with the oral suspension of avanafil and plain avanafil transdermal film the invasomal transdermal film of avanafil has significantly larger area under curve. The relative bioavailability of invasomal film with respect to the oral suspension and plain transdermal film was found to be 148.5% and 451.44%, respectively, i.e. nearly 1.5 times and 4 times increase in bioavailability [122]. Higher bioavailability in comparison with oral suspension may be due to avoidance of first pass metabolism. In comparison with plain transdermal film, higher bioavailability profile of invasomal transdermal film of avanafil may be due to ability of invasomes to penetrate deeper into the skin. Thus, from the study it can be concluded that bioavailability of avanafil can be enhanced significantly when administered through transdermal route using invasomes as a drug delivery tool [122].

Invasomes for antioxidant therapy

Free radicals are produced within the human body as by-products of various metabolic reactions. These radicals perform role of mediators in various physiological reactions as well as signalling molecules [151]. However, these free radicals (reactive oxygen, nitrogen and chlorine species) have a high potential to cause severe cellular damages [151]. Antioxidant defence system was responsible to control the level of these free radicals in the body environment. Some components of these antioxidant defence system are generated with the cell via endogenous pathways, and some are obtained from meal like vitamins (E and C) and micronutrients (copper, zinc and magnesium). Many naturally occurring plant components like citric acid from citrus fruits and ferulic acid obtained from rice, wheat and oats are very essential antioxidants required for body cells. Terpenoids, oxygenated

derivatives of terpenes like thymol were also reported to have potent antioxidant property [152]. In modern therapeutics, many drug delivery systems including invasomes have been developed to administer antioxidants.

Shah et al. formulated liposomes, invasomes and LeciPlex of idebenone, an active pharmaceutical ingredient having antioxidant/anticancer activity and azelaic acid, another API possessing antiacne activity. Two different categories of leciplex were formulated for both drugs first one with cetyltrimethylammonium bromide (CTAB) and second one with didodecyldimethylammonium bromide (DDAB). They characterized liposomes, leciplex and invasomes of both the APIs for shape, size, zeta potential and entrapment efficiency. The particle size analysis done by dynamic light scattering technique showed that in case of idebenone, invasomes have the smallest particle size with polydispersity index of less than 0.1. In case of azelaic acid also invasomes have the smallest particle size. Zeta potential of leciplex of both the drugs was reported to be greater than + 60 mV. Liposomes of idebenone and azelaic acid have zeta potential values of − 1.6 mV and + 3.6 mV. However, invasomes of both the drugs have zeta potential values around − 13 mV. Positive values of zeta potential of leciplexes indicate colloidal stability of formulation. High negative zeta potential values of invasomes were due to phosphatidic acid and phosphatidylinositol in their composition. All the formulations of both drugs showed excellent entrapment efficiency having values greater than 90%. Penetration power of all the vesicular formulations of both the drugs was determined by performing ex vivo permeation study using fluorescent dye (dil). The ex vivo experimentation was conducted by using Franz diffusion cell, and skin of human female was used in experimentation as membrane. Results of ex vivo permeation study revealed that idebenone leciplex and azelaic acid-loaded invasomes are capable of penetrating deeper into skin as compared to other formulated vesicles. In vitro cytotoxicity study was performed on B16F10 melanoma cell lines and in vitro antimicrobial activity was performed on *Propionibacterium acne*, respectively. Results of the studies revealed that LeciPlex of idebenone exerted more potent cytotoxic activity as compared to the respective liposomes and invasomes of idebenone. In case of azelaic acid, DDAB leciplex showed the highest antimicrobial activity. In vivo study was performed for azelaic acid-loaded vesicles in female Wistar rats, and it showed that invasomal formulation of azelaic acid has the most potent antiacne activity. Thus, from the study it was concluded that the developed vesicular formulations can efficiently deliver both the drugs idebenone and azelaic acid via transdermal route [96].

Chen et al. formulated four different types of vesicular drug delivery systems for ferulic acid, viz. liposomes, Tween 80-based deformable liposomes, ethosomes and invasomes. They evaluated all the developed formulations for particle size, surface charge, shape, entrapment efficiency, in vitro skin penetration power and also studied the ability of formulations to get deposited deep into the skin deposition. Rotary evaporation method was used for preparation of all the formulations. Particle size analysis showed that liposomes of ferulic acid have the largest particle size among all the formulations. Polydispersity index for all the formulations was found to be less than 0.2, indicating mono-dispersity and homogeneity within the formulations. Zeta potential values of all the formulations were found to be negative except liposomes which have positive values. Cryo-transmission electron microscopy revealed that in case all the four formulations most of the vesicles are unilamellar. The reported particle size of invasomal formulation of ferulic acid and the value of polydispersity index were 129.1 ± 1.3 nm and 0.112 ± 0.007, respectively. In vitro skin permeation and skin deposition study was conducted on human female human abdominal skin obtained from plastic surgery by using Franz diffusion cell. Results showed that ethosomes permeate deeper into the skin as compared to other formulations; this may be due to high ethanol concentration in the vesicle membrane. All vesicle formulations with flexible membrane have more penetration efficiency when compared to conventional liposomes having rigid membrane. It is also confirmed from the study that skin deposition of ferulic acid was found to be highest in the case where ethosomes are used as the drug delivery agent. All the formulations followed zero-order kinetics of drug release. Thus, the study concluded that if drug is needed to be deposited on the upper skin layers, then liposomes are the best formulations, but if penetration and deposition into deeper regions of skin were required then vesicles with flexible membranes were more suitable [128].

Invasomes for psoriasis

Psoriasis is a chronic inflammatory skin condition or cutaneous disease characterized by abnormal and excessive differentiation of keratinocytes along with the formation of erythematous and papulosquamous lesions [153]. Plaques, pustular, guttate, flexural and erythrodermic psoriasis are the five different types of psoriasis reported in the scientific literature studies [154]. This classification was primarily based on the appearance and features of the lesion developed over the skin. The physiological reason behind the onset of psoriasis was activation of T lymphocytes in the epidermal and dermal regions. Predominant T lymphocyte CD8 + activation in epidermis and CD4 + activation in dermis was reported in the case of psoriasis [153]. T lymphocyte activation starts with

the binding of T cells with the antigens present on major histocompatibility complex (I and II). The activation of T cells was then followed by proliferation after which they get entered into the systemic circulation [153]. The activated T cells then initiate the immunological process by starting the secretion of interleukin (IL)-1, tumour necrosis factor (TNF)-α, and interferon (IFN)-γ [153, 155]. Release of these biochemicals starts the formation of Th2 cytokines (interleukins, IL-4, IL-10 and IL-11) [155]. The whole biological phenomena finally result in the generation of psoriasis plaque due to excessive proliferation of keratinocytes, changes in vascularity and migration of inflammatory cells towards the affected skin [156].

Dragicevic-Curic et al. formulated invasomal dispersions containing temoporfin (hydrophobic photosensitizer, useful in treatment of skin diseases like basal cell carcinoma and psoriasis) 0.15% w/v, ethanol 3.3% w/v and either a blend of terpenes (terpene mix 1, 2, 3 and 4) or a single terpene (cineole, citral and d-limonene) in 1% w/v concentrations [114]. All the components were dissolved in an ethanolic phospholipid solution (75:25 w/w solution of phospholipid/ethanol). 10% w/v concentration of this ethanolic phospholipid solution was used in invasome formulation. All the formulated solutions were then vortexed for 5 min to obtain the clear invasome solutions. Finally, phosphate buffer solution pH 7.4 was added to all the invasome solutions in order to adjust the final volume and achieve a 100% w/v composition for all the dispersions. All the dispersions were then extruded through polycarbonate membranes of different pore sizes (400, 200, 100 and 50 nm). They also formulated three sets of conventional liposomes of temoporfin (0.15% w/v) to compare the performance profile of invasomes temoporfin with that of liposomes [54]. The first set was prepared with the drug only and second with drug and ethanol (3.3% w/v) [54]. The third set of liposomes were prepared with using ethanol (3.3% w/v) and mixture of terpenes (cineole/citral/d-limonene) [54].

Particle size analysis, polydispersity index and surface charge study results showed that invasomes containing cineole have the lowest particle size of 105.4 ± 0.2 nm [116]. Invasomes containing terpene mix 3 (cineole/citral/d-limonene = 0.17:0.17:0.66 v/v) have the largest particle size of 169.3 ± 1.2 nm [55]. Liposomes formulated with 3.3% w/v ethanol have the lowest particle size of 82.7 ± 0.3 nm among all the formulations [55]. The lowest polydispersity index value of 0.066 ± 0.008 was reported for cineole-containing invasomes, and the highest value was reported for invasomes with terpene mix 3. Liposomes without ethanol have a particle size of 126.1 ± 0.4 nm and a PDI value of 0.127 ± 0.005 [54]. Invasomes formulated with cineole, citral and d-limonene mostly have unilamellar vesicles; however, oligolamellar vesicles were also present in formulation prepared with cineole. The vesicles were mostly of spherical shape, and also deformed vesicles were also present in invasomal formulations. Stability studies revealed that invasomes stored at 4 °C showed a slight increase in particle size and polydispersity index, whereas invasomes when stored at room temperature showed a significant increase in particle size and polydispersity index. This showed that invasomes are more stable at 4 °C than at room temperature. However, the drug content analysis reported that no significant differences in drug content were observed for the formulations when stored at 4 °C and at room temperature for a time period of 12 months. In vitro penetration studies were conducted with Franz diffusion cells using human female abdominal skin. The best skin penetration profile was reported for the invasomes composed with 1% w/v cineole. This means that the highest skin permeation and deposition of temoporfin within the SC and deeper layers of skin were achieved with invasomes having 1% cineole in their composition. The invasomes formulated with a single terpene were more efficient drug delivery systems in comparison with invasome formulated with mixture of various terpenes for topical administration of temoporfin. Most of the invasomes were found to be more effective carrier systems in comparison with liposomes. However, liposomes having 3.3% w/v ethanol in their composition were found to be more efficient in penetrating the skin and deliver the temoporfin into deeper skin layers than many invasome formulations. Thus, from the study it can be concluded that topical invasome formulations can deliver temoporfin efficiently deep into the skin and therefore can be highly beneficial for treatment of skin diseases like basal cell carcinoma, psoriasis, acne, etc. [114].

Invasomes for alopecia treatment

Alopecia is a very common and chronic inflammatory dermatological disorder, which affects hair follicles [157]. The patients suffering from alopecia may lose hairs either from some regions of scalp or from the whole scalp or from the whole body. Accordingly, alopecia was categorized into four types: androgenetic alopecia (male pattern alopecia), alopecia areata (hair loss from scalp in small circular patches), alopecia totalis (loss of hairs from whole scalp) and alopecia universalis (whole body hair loss) [157]. Although alopecia is not a painful medical condition and does not pose a serious threat to life, it severely impacts the psychology of the affected person. The aetiology of alopecia was not very well defined, but scientific literature studies have provided evidence reporting the involvement of androgens, specially dihydrotestosterone in development of androgenetic alopecia [157, 158]. Invasion of hair follicles by T cells (CD4+and

CD8+) was reported as the reason behind the occurrence of alopecia areata [159]. In modern medical science, still the treatment of alopecia is mainly dependent on the two United States Food and Drug Administration (USFDA)-approved drugs—minoxidil and finasteride—but both drugs have considerable side effects [160]. Herbal or plant-based formulations are popular among masses, but these products are composed of several ingredients. Therefore, it becomes difficult to identify the exact component behind the antialopecia activity due to which rationality of such products always comes under question. Thus, newer drugs as well as treatment strategies are required for improvement and development in antialopecia therapy. Invasomes have also been formulated and investigated for their application in antialopecia therapy.

Mura et al. formulated three different sets of elastic vesicles of minoxidil with three different penetration enhancers (Labrasol®, Transcutol® and cineole) [161]. One formulation without penetration enhancer (minoxidil liposomes) was also made and used as control in the experiment. The prepared elastic vesicles were then subjected to evaluations like morphology, size distribution, entrapment efficiency, zeta potential, deformability and in vitro penetration study. Vesicles prepared with Labrasol® showed the highest drug content (38.40 ± 1.90 mg/ml), the largest particle size (202 ± 3 nm) and the maximum negative zeta potential (− 58 ± 2mv). The cineole-containing vesicles showed the highest entrapment efficiency (71.3 ± 3.5%). Prepared vesicles were extruded through polycarbonate membranes having a pore diameter of 50 nm. The extrusion was carried out to determine the deformability of vesicles, and the size of vesicles was again determined after extrusion. Complete passage of the elastic vesicles through the membrane pores was reported, whereas only a limited amount of control liposomes was able to pass through the membrane. Re-estimation of particle size after extrusion revealed that the size of elastic vesicles remained nearly unchanged; however, control liposomes showed a reduction in size. This indicates higher deformability of elastic vesicles than control liposomes. In vitro skin permeation study for all the vesicles was carried out with the skin of newborn Goland–Pietrain hybrid pigs, and Franz diffusion cells were used to conduct the study. Results reported that elastic vesicles prepared with cineole and Labrasol® enabled nearly three times higher deposition of minoxidil within the skin layers in comparison with control liposomes [161]. Transcutol®-containing vesicles showed a slightly higher deposition of minoxidil as compared to control liposomes. Direct correlation between deformability of vesicles and concentration of drug deposited in the skin was indicated by the in vitro drug deposition estimation, i.e. higher deformability leads to higher drug deposition. Mura et al. also concluded that the elastic vesicles were able to form depots within the skin layers from where minoxidil was slowly released. This increases the retention of minoxidil within the skin layers, therefore improving its cutaneous bioavailability.

Mura et al. also carried out in vitro permeation study with the pig skin pretreated with the empty vesicles containing only penetration enhancers but devoid of drug. They also performed permeation study with pig skin for commercial solution of minoxidil and for the solutions of Labrasol®, Transcutol®, and cineole containing minoxidil. They compared results of permeation study of elastic vesicles with the results of solutions. It was reported that in case of solution of penetration enhancers containing minoxidil the permeation of minoxidil improved in comparison with the commercial solution of minoxidil. In case of vesicular formulations, also the vesicles containing penetration enhancers in their composition showed higher permeation of minoxidil against control liposomes. Vesicles containing cineole showed maximum drug permeation [161]. Thus, from the study it was concluded that elastic vesicles delivered higher concentration of minoxidil in comparison with liposomes and improved the cutaneous bioavailability of minoxidil. Therefore, deformable vesicles formulated with suitable penetration enhancers can be an efficient carrier system for cutaneous administration of minoxidil [161].

Prashanti et al. formulated invasomes of finasteride (5-α reductase inhibitor) for transdermal delivery via iontophoresis [116]. They used three terpenes (limonene, carvone and nerolidol; 0.5%, 1.5% and 1%) for the formulation and developed nine invasomal formulations. They tested all the formulations for lamellarity, size, shape, zeta potential, entrapment efficiency and in vivo permeation. The prepared formulations mostly have unilamellar vesicle membrane wall and have spherical shape. The size of the formulated invasomes varied from 4.54 ± 0.30 µm to 13.00 ± 0.20 µm. Zeta potential analysis showed negative surface charge for all the formulations. All the formulations showed significant enhancement in permeation of finasteride in comparison with the aqueous solution of finasteride used as control in in vitro permeation experiment. Male Wistar rat skin was used for the experiment. Invasome formulation containing limonene (0.5%) showed the highest permeation enhancement. Optimized invasome formulation was then again subjected to the permeation study using iontophoresis technique. The result showed that application of iontophoresis technique enhanced the permeation of finasteride by 25.83 times as compared to aqueous solution of finasteride used as control [116].

The optimized invasomal formulation was then further developed or processed in the form of gel which was used for in vivo study. Rabbits were used as animal model for the in vivo study, and oral suspension of finasteride was used control for the experiment. Results of in vivo experimentation were plotted as plasma concentration vs time curve, and it showed that area under the curve (AUC) obtained for transdermal invasome gel of finasteride was 3.04 times greater than AUC of oral suspension of finasteride. T_{max} for both oral suspension and transdermal invasomal gel was reported to be of 2 h. C_{max} achieved with oral suspension and transdermal invasomal gel was 840.23 ± 15.46 ng/ml and 656.53 ± 25.03 ng/ml, respectively. Transdermal invasomal gel of finasteride showed relative bioavailability of 303% against the oral suspension [118]. Histopathological studies were conducted on the rat skin. Mild inflammation and thickening of epidermis were observed in skin treated with transdermal invasome gel of finasteride. Stability studies were performed for optimized invasome formulation of finasteride at 4 °C ± 1 °C and 25 °C ± 1 °C for 120 days [12]. It showed that the formulation remained more stable at 4 °C ± 1 °C and minimal changes in characteristic size, shape, zeta potential as well as entrapment efficiency. However, size, shape, zeta potential and entrapment efficiency of optimized invasomes formulation sample stored at 25 °C ± 1 °C changed significantly. Thus, the study concluded that finasteride could be effectively delivered through transdermal route with application of iontophoresis technique [118].

Conclusion

Terpenes are the natural permeation enhancers or sorption promoters which are obtained from plants as secondary metabolites. They are generally regarded as safe (GRAS) by the Food and Drug Administration and are being used since ancient times for their valuable therapeutic potential. Due to nontoxic and non-irritant property, terpenes are considered to be highly competent category of permeation enhancers for several drugs. Terpenes such as menthol, limonene and cineole can enhance the rate of permeation across the SC for both hydrophilic and lipophilic drugs and thus play a vital role in developing novel transdermal delivery systems with high efficiency and increased rate of permeation. Researchers have developed various novel vesicular systems in the past few decades, and one of such system is the invasome, which utilizes terpenes as a formulation component. The invasomes have been developed to be used as a carrier system for various drugs that are utilized to treat several diseases. Several advantages were offered as a drug delivery system by invasomes in comparison with conventional vesicular systems. The most prominent advantage is higher permeation and the second one is deeper penetration into the lower layers of skin which is attributed to the terpenes present in the invasomes which changes the structural integrity of SC, thus making the permeation of the invasomes easier. Ethanol is also present as a component in the invasomes, which makes the vesicles more flexible; thus, they easily enter into the deeper layer through the channels or pores present in the upper skin layers. These advantages associated with invasomes make them a suitable drug delivery carrier for enhancing the transdermal/percutaneous absorption of drugs.

Abbreviations

FDA: Food and Drug Administration; SC: *Stratum corneum*; GRAS: Generally recognized as safe; FTIR: Fourier transform infrared spectroscopy; ATR-FTIR: Attenuated total reflectance–Fourier transform infrared spectroscopy; DSC: Differential scanning calorimetry; SEM: Scanning electron microscopy; TEM: Transmission electron microscope; PBPK: Physiologically based pharmacokinetic modelling; C_{max}: Concentration maximum; T_{max}: Time maximum; AUC : Area under curve; CTAB: Cetyltrimethylammonium bromide; DDAB: Didodecyldimethylammonium bromide.

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Author contributions

BK contributed to conceptualization, methodology, writing—original draft, writing—review and editing, and visualization; MP and RA contributed to writing—original draft and writing—review and editing. PKS supervised and reviewed the study. All authors gave their individual critical revision and final approval of the version to be submitted.

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Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

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The authors declare that they have no competing interests.

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